Virginia Department of Environmental Quality

Draft 2012 Water Quality Integrated Report

Public Comment-Response Document

Comments received

March 26, 2012 to April 24, 2012

Table of Contents

Comments from EPA Region III..... 1
DEQ Response..... 3

Comments from Hampton Roads Sanitation District..... 7
DEQ Response..... 9

Comments from the Center for Biological Diversity..... 10
DEQ Response..... 17

Comments from the National Committee for the New River..... 21
DEQ Response..... 23

Comments from the Loudoun Watershed Watch..... 24
DEQ Response..... 27

Comments from the Shenandoah Riverkeeper..... 29
DEQ Response..... 34

Appendix I: Publications Enclosed with Comments from the Center for Biological Diversity

Appendix II: Additional Comments for the Shenandoah River

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
REGION III
1650 Arch Street
Philadelphia, Pennsylvania 19103-2029

April 18, 2012

SUBJECT: EPA comments on Virginia's Draft 2012 305(b)/303(d) Water Quality Integrated Report

FROM: Gregory Voigt, Office of Standards, Assessment and TMDLs (OSAT), United States Environmental Protection Agency, Region 3 (EPA)

TO: John M. Kennedy, Office of Water Monitoring and Assessment, Virginia Department of Environmental Quality (DEQ)

THRU: Larry Merrill, Associate Director, OSAT
EPA Region 3

Thank you for the opportunity to review and comment on Virginia's Draft 2012 305(b)/303(d) Water Quality Integrated Report (IR). In addition to the specific comments below, EPA would like to have a conference call with DEQ staff to discuss the 2012 listing status of the mainstem Shenandoah River.

Comments:

1. On the 2010 IR, A15E-01-BAC is identified as 'Pohick Creek' (p.3.3a-3), but on the draft 2012 IR, A15E-01-BAC is identified as 'Gunston Cove' (Appendix 3-2).
2. For Austin Run (A28R-01-BAC), the 2010 IR indicates an original list date of 2004, but the draft 2012 IR indicates an original list date of 2012 (see Appendix 1a-5).
3. For Bridges Creek (A31E-11-BAC), the 2010 IR indicates an original list date of 2004, but the draft 2012 IR indicates an original list date of 2012 (see Appendix 1a-5).
4. A34E-01-SF2: Should the waterbody name be 'The Glebe - PARTIAL DELIST'?
5. What happened to the A34E-26-SF (The Glebe) listing from the 2010 IR?
6. What happened to the G05R-06-PH (Grassy Swamp Creek) listing from the 2010 IR?
7. For Mill Creek (G08R-02-BAC), the 2010 IR indicates an original list date of 2004, but the draft 2012 IR indicates an original list date of 2012 (see Appendix 1a-20).
8. Appendix 1a-26 indicates Totier Creek Reservoir (H17L-01-DO) was initially listed in 2004, but Appendix 5 (p.429) indicates the impairment is new for 2012.
9. Should the initial list date for Deep Run (H39R-19-DO) be 2004? I could not find it on previous lists.
10. What happened to the I07R-01-BAC (Dunlap Creek) listing from the 2010 IR?
11. For Little Patterson Creek (I22R-04-BAC), the 2010 IR indicates an original list date of 2004, but the draft 2012 IR indicates an original list date of 2012 (see Appendix 1a-32).
12. What happened to the E25R-01-DO (Mud Creek) listing from the 2010 IR?
13. What happened to the E26E-20-BAC (Broad Creek) listing from the 2010 IR?
14. For Waqua Creek (K17R-02-BAC), the 2010 IR indicates an original list date of 2002, but the draft 2012 IR indicates an original list date of 2012 (see Appendix 1a-53).
15. Should the initial list date for Nottoway River - Upper (K30R-02-BAC) be 2008? I could not find it on any previous list.
16. Should the initial list date for Bear Creek (O03R-02-BAC) be 2010? I could not find it on the 2010 list.

17. Should the initial list date for Staley Creek (O03R-03-BAC) be 2010? I could not find it on the 2010 list.
18. Should the initial list date for Powell River (P17R-08-BEN) be 2010? I could not find it on the 2010 list.
19. Should the initial list date for Roaring Fork (P17R-09-BEN) be 2010? I could not find it on the 2010 list.
20. Why was Big Cherry Reservoir (P18L-01-PH) moved from category 4C on the 2010 IR to category 5C on the draft 2012 IR?
21. Should the initial list date for South Fork Powell River (P18R-03-BAC) be 2010? I could not find it on the 2010 list.
22. Should the initial list dates for Dismal Creek (Q05R-01-BAC) be 2006 and 2010? I could not find it on previous lists.
23. Should the initial list date for Home Creek (Q08R-02-BEN) be 2010? I could not find it on the 2010 list.
24. What happened to the Q12R-03-BAC (Bartlick Creek) listing from the 2010 IR?
25. Should the initial list date for Pound River (Q13R-03-BEN) be 2006? I could not find it on previous lists.
26. Should the initial list date for North Fork Pound River Tributaries (Q13R-08-BEN) be 2010? I could not find it on the 2010 list.
27. Should the initial list date for North Fork Pound River (Q13R-09-BEN) be 2010? I could not find it on the 2010 list.
28. Appendix 3-63: Should the waterbody name for VAP-C01E_BAR02A08 be Barrett Creek instead of Great Wicomico River?
29. Should the initial list date for Reason Creek (C01E-57-SF) be 2010? I could not find it on the 2010 list.
30. Should the initial list date for Lanes Creek (C04E-52-SF) be 2002? I could not find it on previous lists.
31. Should the initial list date for Starling Creek - Lower (C10E-13-SF) be 2008?
32. Should the initial list date for Fisher Cove (C13E-23-SF) be 2008? I could not find it on previous lists.
33. A TMDL was approved by EPA for Mattawoman Creek – Upper (C14E-13-SF) on 5/6/2010. This is a CD segment and should not be listed in Category 5.
34. A TMDL was established by EPA for Chesapeake Bay Segment CB7PH (CB7PH-EBEN-BAY) on 12/29/10. This segment should not be listed in Category 5.
35. Appendix 3-98: Should the cause group code associated with the e. coli delisting for Lake Anna/Gold Mine Creek (VAN-F07L_GMC01A02) be F07L-01-BAC instead of F06R-03-BAC?
36. Why was Pamunkey River (F13E-01-BAC) moved from category 4C on the 2010 IR to category 5C on the draft 2012 IR?
37. Should the initial list date for Aberdeen Creek (F26E-02-SF) be 2002? I could not find it on previous lists.
38. What happened to the F26E-22-SF (Hockley Creek) listing from the 2010 IR?
39. What happened to the F26E-26-SF (Purtan and Leigh Creeks) listing from the 2010 IR?
40. Should the initial listing date for Laurel Creek (N21R-06-BAC) be 2004, not 2012?
41. There are several waters in Category 4A of the draft 2012 IR for which EPA has no record of a TMDL being completed. EPA Region III TMDL staff will work with DEQ TMDL staff to resolve these discrepancies prior to DEQ's submittal of the final 2012 IR.

DEQ's Response to EPA Comments:

1. On the 2010 IR, A15E-01-BAC is identified as 'Pohick Creek' (p.3.3a-3), but on the draft 2012 IR, A15E-01-BAC is identified as 'Gunston Cove' (Appendix 3-2). The draft 2012 IR has the correct waterbody name for A15E-01-BAC. In the 2010 IR, A15E-01-BAC should have been identified as Gunston Cove on p. 3.3a-3, not as Pohick Creek. Gunston Cove is formed by the confluence of Pohick Creek and Accotink Creek.
2. For Austin Run (A28R-01-BAC), the 2010 IR indicates an original list date of 2004, but the draft 2012 IR indicates an original list date of 2012 (see Appendix 1a-5). The cycle first listed has been corrected to 2004. 2012 was the first cycle in which the listing was based upon *E. coli* instead of fecal coliform.
3. For Bridges Creek (A31E-11-BAC), the 2010 IR indicates an original list date of 2004, but the draft 2012 IR indicates an original list date of 2012 (see Appendix 1a-5). The 2004 impairment was for fecal coliform. The impairment converted to enterococci in the 2012 cycle. Therefore the initial list date is 2012, but the due date remains 2016 because of the original bacterial impairment.
4. A34E-01-SF2: Should the waterbody name be 'The Glebe - PARTIAL DELIST'? Name corrected.
5. What happened to the A34E-26-SF (The Glebe) listing from the 2010 IR? The impairment was delisted. There was a typo in the Delist comment for VAP-A34E_GLB02A00 (A34E-36-SF instead of A34E-26-SF). The typo has been corrected.
6. What happened to the G05R-06-PH (Grassy Swamp Creek) listing from the 2010 IR? The impairment was delisted. There was a typo in the Delist comment for VAP-G05R_GRC01A04 (G02R-06-PH instead of G05R-06-PH). The typo has been corrected.
7. For Mill Creek (G08R-02-BAC), the 2010 IR indicates an original list date of 2004, but the draft 2012 IR indicates an original list date of 2012 (see Appendix 1a-20). The 2004 impairment was for fecal coliform. The impairment converted to E. coli in the 2012 cycle. Therefore the initial list date is 2012, but the due date remains 2016 because of the original bacterial impairment.
8. Appendix 1a-26 indicates Totier Creek Reservoir (H17L-01-DO) was initially listed in 2004, but Appendix 5 (p.429) indicates the impairment is new for 2012. Totier Creek Reservoir was originally listed for a DO impairment in the Hypolimnion in the 2004 cycle. All DO violations occurred in the Hypolimnion. With the adoption of the Lake Nutrient Standards and the DO WQS only applying in the Epilimnion, Totier Creek Reservoir was de-listed as impaired in the 2008 cycle based on the new Lake Nutrient Standards. In the 2012 cycle, it was determined that Totier Creek Reservoir was impaired for DO based on 5 violations of the DO WQS in the Epilimnion. The original listing date in ADB was INCORRECTLY identified as 2004. I have updated ADB to correctly reflect that the DO listing is in 2012, not 2004.
9. Should the initial list date for Deep Run (H39R-19-DO) be 2004? I could not find it on previous lists. No. The segment was delisted in 2006 and impaired again in 2012. The initial list date has been corrected to 2012.
10. What happened to the I07R-01-BAC (Dunlap Creek) listing from the 2010 IR? Dunlap Creek is delisted with the 2012 IR as recorded in the 2012 ADB and delist package. These initially 2006 Listed waters are fully delisted with the 2012 IR. Bacteria (E.coli) excursions of the 235 cfu/100 ml instantaneous criterion are zero of 14 observations with an exceedance rate of 0.0% at station 2-DNP001.98 (Route 603 Bridge at Earlehurst). The 2006 initial 303(d) Listing is based on two of nine E.coli excursions. Exceeding values were 320 and 400 cfu/100 ml. The bacteria (E.coli) 303(d) Listing continued through the 2010 Cycle.
11. For Little Patterson Creek (I22R-04-BAC), the 2010 IR indicates an original list date of 2004, but the draft 2012 IR indicates an original list date of 2012 (see Appendix 1a-32). As recorded in the 2012 ADB and Impaired Waters Fact Sheet :
    2-LIP001.00- Escherchia Coli (E.coli) replaces the former fecal coliform impairment with the 2012 assessment. Five of 12 Escherichia coli (E.coli) samples exceed the 235 cfu/100 ml instantaneous criterion within the 2012 Data Window. Exceeding values range from 250 to 1300

cfu/100 ml. These waters were initially listed in 2004 where FC exceeded the former 400 cfu/100 ml WQS instantaneous criterion in two of nine samples. The two exceedences are 2800 (2001) and 2100 cfu/100 ml (2001). In both the 2006 and 2008 assessments FC exceeds in two of 12 samples with the same excursions as in previous cycles. No additional data extended into the 2008 or 2010 data windows. The TMDL due date remains 2016 as the original listing occurred in 2004 for fecal coliform bacteria and not escherichia coli; a 2012 initial listing.

12. What happened to the E25R-01-DO (Mud Creek) listing from the 2010 IR? The impairing station was determined to be tidally influenced and is now located in VAP-E25E_MUC0A04. As this segment is part of the Chesapeake Bay WQS segmentation, it is included under the RPPMH-DO-BAY listing instead.
13. What happened to the E26E-20-BAC (Broad Creek) listing from the 2010 IR? The impairment was delisted. There was a typo in the Delist comment for VAP-E26E_BRD01A00, -BRD02A00, -BRD03A00, and -BRD04A00 (E26E-20 instead of E26E-20-BAC). The typo has been corrected.
14. For Waqua Creek (K17R-02-BAC), the 2010 IR indicates an original list date of 2002, but the draft 2012 IR indicates an original list date of 2012 (see Appendix 1a-53). The 2002 impairment was for fecal coliform. The impairment converted to E. coli in the 2012 cycle. Therefore the initial list date is 2012, but the due date remains 2014 because of the original bacterial impairment.
15. Should the initial list date for Nottoway River - Upper (K30R-02-BAC) be 2008? I could not find it on any previous list. Initial listing 2008 3 viol/25 obs. Delist 2010 E.coli-Recreation Use 3 viol/36 obs (**Final 2010: 3.5 - 137**). The Recreation Use is impaired for Ecoli with 5 viol / 36 obs at 5ANTW0015.99 (**Draft 2012: App 5 - p932**)
16. Should the initial list date for Bear Creek (O03R-02-BAC) be 2010? I could not find it on the 2010 list. The 2010 CGC was O03R-01-BAC for this listing. A portion of the segments in this CGC had TMDL studies completed and became 4A. This one remains 5A; therefore, it needs a new CGC.
17. Should the initial list date for Staley Creek (O03R-03-BAC) be 2010? I could not find it on the 2010 list. The 2010 CGC was O03R-01-BAC for this listing. A portion of the segments in this CGC had TMDL studies completed and became 4A. This one remains 5A; therefore, it needs a new CGC.
18. Should the initial list date for Powell River (P17R-08-BEN) be 2010? I could not find it on the 2010 list. The 2010 CGC was P17R-01-BEN for this listing. A portion of the segments in this CGC had TMDL studies completed and became 4A. This one remains 5A; therefore, it needs a new CGC.
19. Should the initial list date for Roaring Fork (P17R-09-BEN) be 2010? I could not find it on the 2010 list. The 2010 CGC was P17R-02-BEN for this listing. A portion of the segments in this CGC had TMDL studies completed and became 4A. This one remains 5A; therefore, it needs a new CGC.
20. Why was Big Cherry Reservoir (P18L-01-PH) moved from category 4C on the 2010 IR to category 5C on the draft 2012 IR? The original impairment was attributed to natural causes, 4C. Big Cherry now has pollutant caused impairment (P18L-01-HG), 5A. Because mercury was discovered in edible fish fillets, VDH established a Fish Consumption Advisory which resulted in Category 5A for Fish Consumption Use. The overall category is now 5C.
21. Should the initial list date for South Fork Powell River (P18R-03-BAC) be 2010? I could not find it on the 2010 list. The 2010 CGC was P18R-01-BAC for this listing. A portion of the segments in this CGC had TMDL studies completed and became 4A. This one remains 5A; therefore, it needs a new CGC.
22. Should the initial list dates for Dismal Creek (Q05R-01-BAC) be 2006 and 2010? I could not find it on previous lists. The 2010 CGC was Q04R-01-BAC for this listing. Dismal Creek was not included in the Levisa TMDL study, so was given a unique CGC.
23. Should the initial list date for Home Creek (Q08R-02-BEN) be 2010? I could not find it on the 2010 list. The 2010 CGC was Q04R-01-BEN for this listing. Home Creek was not included in the Levisa TMDL study, so was given a unique CGC.

24. What happened to the Q12R-03-BAC (Bartlick Creek) listing from the 2010 IR? It became Q12R-01-BAC and was included with a nearby tributary with similar land uses.
25. Should the initial list date for Pound River (Q13R-03-BEN) be 2006? I could not find it on previous lists. The 2010 CGC was Q13R-02-BEN for this listing. A portion of the segments in this CGC had TMDL studies completed and became 4A. This one remains 5A; therefore, it needs a new CGC.
26. Should the initial list date for North Fork Pound River Tributaries (Q13R-08-BEN) be 2010? I could not find it on the 2010 list. The 2010 CGC was Q13R-02-BEN for this listing. A portion of the segments in this CGC had TMDL studies completed and became 4A. This one remains 5A; therefore, it needs a new CGC.
27. Should the initial list date for North Fork Pound River (Q13R-09-BEN) be 2010? I could not find it on the 2010 list. The 2010 CGC was Q13R-02-BEN for this listing. A portion of the segments in this CGC had TMDL studies completed and became 4A. This one remains 5A; therefore, it needs a new CGC.
28. Appendix 3-63: Should the waterbody name for VAP-C01E_BAR02A08 be Barrett Creek instead of Great Wicomico River? Yes, the name has been corrected.
29. Should the initial list date for Reason Creek (C01E-57-SF) be 2010? I could not find it on the 2010 list. Yes. In 2010, the Reason Creek shellfish impairment was mistakenly listed under C01E-16-SF. The fact sheet number was corrected this cycle.
30. Should the initial list date for Lanes Creek (C04E-52-SF) be 2002? I could not find it on previous lists. No. The segment was delisted in 2006 and impaired again in 2012. The initial list date has been corrected to 2012.
31. Should the initial list date for Starling Creek - Lower (C10E-13-SF) be 2008? Previous 2008 impairment is delisted based on DSS change of shellfish condemnation category from Condemned to Seasonally Condemned. The Shellfishing Use is fully supported with observed effect due to the DSS Seasonally Condemned shellfish direct harvesting condemnation # 075-118 M1 (effective 20081105). Delist 2010 fecal coliform (**Final 2010: 3.5 - 165**). The Shellfishing Use is impaired based on the DSS shellfish harvesting condemnation & seasonal # 075-118 A (effective 20091023). (**Draft 2012: App 5 - p1223**).
32. Should the initial list date for Fisher Cove (C13E-23-SF) be 2008? I could not find it on previous lists. Partial delist 2010 – Fecal coliform [First listed 2008] (**Final 2010: 3.5 - 168**). The Shellfishing Use is Fully Supported due to the Open DSS shellfish harvesting area # 084-043 (effective 20080805). Proposed for delisting previous 2008 IR DSS shellfish direct harvesting condemnation # 084-043 C (effective 20050908). Added in 2008 IR shellfish closures. Not covered under TMDL. The Shellfishing Use is impaired based on the DSS Condemnation # 084-043 C (effective date 20101216). Not covered under the TMDL. (**Draft 2012: App 5 - p1240**).
33. A TMDL was approved by EPA for Mattawoman Creek – Upper (C14E-13-SF) on 5/6/2010. This is a CD segment and should not be listed in Category 5. TMDL is EPA Approved 5/6/2010; SWCB Approved 9/30/2012.
34. A TMDL was established by EPA for Chesapeake Bay Segment CB7PH (CB7PH-EBEN-BAY) on 12/29/10. This segment should not be listed in Category 5. The EBEN (benthic impairment) cause is not addressed by the Bay TMDL.
35. Appendix 3-98: Should the cause group code associated with the e. coli delisting for Lake Anna/Gold Mine Creek (VAN-F07L_GMC01A02) be F07L-01-BAC instead of F06R-03-BAC? The cause group code for the delisting of Lake Anna/Gold Mine Creek segment has been updated to F07L-01-BAC.
36. Why was Pamunkey River (F13E-01-BAC) moved from category 4C on the 2010 IR to category 5C on the draft 2012 IR? It was moved from Category 4A to Category 5A because it was realized that it should not have been considered a "nested" impairment due to the presence of a municipal WWTP that was not addressed in the downstream TMDL.
37. Should the initial list date for Aberdeen Creek (F26E-02-SF) be 2002? I could not find it on previous lists. Yes. It was previously part of F26E-15-SF. However, the TMDL "York River: Gloucester Point to Jones Creek", which was approved by the EPA on 7/30/2007, only addressed the 1998 portion of the condemnation. This downstream expansion is Category 5B. As the condemnation first expanded in the 2002 cycle, the TMDL will be due in 2014.

38. What happened to the F26E-22-SF (Hockley Creek) listing from the 2010 IR? Partial Delist 2012 (**Draft 2012: App 3 - 102, 103, 104, 105**). During the 2010 cycle, a 5.8816 mi2 mile portion of the York River, plus Hockley Creek and several tributaries were impaired of the Shellfish Use due to the non-administratively condemned portion of 049-004A, 8/25/2005. The impairment was addressed in the report "Bacteria Total Maximum Daily Load (TMDL) Development for the Upper York River, the Lower Pamunkey River, and the Lower Mattaponi River (Tidal) Watersheds" which was completed during the 2012 cycle and was approved by the EPA on 7/28/2010. However, during the 2012 cycle, condemnation A. 7/15/2010 shrank considerably and is now fully contained with the administratively condemned area. The tributaries are now open, Hockley Creek remains closed as 049-004C. The majority of the mainstem York River is now open for harvest; however, one portion remains closed as part of the Hockley Creek condemnation, and another is now seasonally condemned under 048-128M1, 7/15/2010. The open and seasonally condemned areas will be partially delisted (Category 2C); the condemned areas will be considered Category 4A.
39. What happened to the F26E-26-SF (Purtan and Leigh Creeks) listing from the 2010 IR? The impairment was delisted. There was a typo in the Delist comment for VAT-F26E_PTN01A08 (F26E-12-SF instead of F26E-26-SF). The typo has been corrected.
40. Should the initial listing date for Laurel Creek (N21R-06-BAC) be 2004, not 2012? The original listing for fecal coliform is a 2004 Listing. E.coli replaced fecal coliform with the 2012 assessment; thus a 2012 initial listing date. The TMDL Due Date remains as 2016 based on the original FC 2004 Listing.
41. There are several waters in Category 4A of the draft 2012 IR for which EPA has no record of a TMDL being completed. EPA Region III TMDL staff will work with DEQ TMDL staff to resolve these discrepancies prior to DEQ's submittal of the final 2012 IR. Please see Excel files accompanying this document.

Cleaning wastewater every day for a better Bay.

April 27, 2012

By e-mail

John M. Kennedy
DEQ Office of Water Monitoring and Assessment
P.O. Box 1105
Richmond, Virginia 23218-1105
John.Kennedy@deq.virginia.gov

RE: Comments on Draft 2012 305(b)/303(d) Water Quality Assessment Integrated Report

Dear Mr. Kennedy,

The Hampton Roads Sanitation District appreciates the opportunity to comment on the above referenced document and is encouraged to see enhancements in the assessment process that have the potential to more accurately characterize the quality of Virginia's waters. In general, the trend monitoring depicted a positive outlook and is a testament to the successes of Clean Water Act implementation at the state level. Though the coastal monitoring completed in conjunction with EPA could not be used to assess against standards, the general characterization of our coastal waters was helpful and informative. The report and its content improve with each assessment year despite the challenging economic environment. As DEQ continues to improve the assessment process, HRSD would like to offer comment on issues with VPDES regulatory implications.

Analytical Methodology

The VPDES permit program requires that only 40CFR Part 136 analytical procedures be used to analyze samples when such a method is available. The assessment program does not have a similar requirement. Both the ambient monitoring program and the VPDES program determine the potential for impact in state waters. Though all data used for supporting and listing purposes is collected using an EPA accepted and DEQ approved method, this does not address the concern that the use of varying methodologies between the two programs could result in conflict and undermine the credibility of the listings and subsequent TMDLs. In the previous response to comments, DEQ indicated that there has been no known incidence of conflict. Though there may have been no conflict to date, DEQ must be aware of this concern and strive to achieve unanimity between the two programs when possible.

Tissue Screening Values

HRSD requests that Tissue Screening Values (TSV) that are not linked directly to Water Quality Standards be subject to the Administrative Procedure Act (APA) process since these values do have regulatory impact. The Virginia Department of Health (VDH) utilizes its own screening values to guide the decision to post a fish consumption advisory. These VDH screening values are not subject to the APA process. As an example, the DEQ screening

Commissioners: Vishnu K. Lakdawala, PhD, Chairman; B. Anne Davis, Vice-Chairman; Frederick N. Elofson, CPA; Gerald S. Johnson; Michael E. Glenn; Arthur C. Bredemeyer; Maurice P. Lynch, PhD; I. Vincent Behm, Jr.
www.hrsd.com

value for PCBs in fish tissue is 54 mg/kg. The DEQ screening value has a direct linkage to the Water Quality Standard which is subject to review and approval by stakeholders. However, VDH issues fish consumption advisories based on a screening value of 50 mg/kg PCBs in fish tissue. This screening value has NOT been subject to the APA process. In addition, though lead and arsenic standards do not exist for fish tissue, VDH currently has the discretion to issue advisories based on the results of the fish tissue analyses. Such advisories on fish consumption require the state to identify the waterbody as impaired. The regulatory community is not provided with the opportunity to comment on the VDH tissue screening values or their derivation. While some VDH derived TSVs are listed in the Water Quality Monitoring Guidance but there isn't enough information available on the derivation of these screening values to provide meaningful comment.

Tributyltin Impairments

The tributyltin water quality standard for saltwater was revised in the last triennial review. The waters of Hampton Roads and the Elizabeth River were delisted for this impairment in the previous Water Quality Assessment Integrated Report (2010). The current report, however, contains carry-over information in section 7.4, page 327 which still identifies these waters as impaired.

Thank you for the opportunity to comment. The report is an invaluable tool in characterizing the waters of the state and in identifying waters in need of improvement.

Respectfully,

Jamie S. Heisig-Mitchell
Chief of Technical Services
Hampton Roads Sanitation District
1436 Air Rail Avenue
Virginia Beach, VA 23455
757-460-4220
jmitchell@hrsd.com

DEQ's Response to Hamptom Roads Sanitation District (HRSD)

DEQ will continue to emphasize the importance of coordination amongst all water quality management planning programs--watershed monitoring and assessment, TMDLs, and Virginia Pollution Discharge Elimination System permitting. Not only do stakeholders benefit when these programs work in concert, but the agency also has a vested interest in minimizing conflicts arising from different program requirements.

Per Virginia Regulations 1 VAC 30-46, all analyte data submitted to DEQ for regulatory purposes, including both permit-mandated reporting and 303(d) listing, must be from a NELAC-accredited laboratory. This ensures that only data of high quality are used. Because laboratory certification and auditing are the responsibilities of the Division of Consolidated Laboratory Services (DCLS), concerns about inconsistencies in analytical procedures should be directed to DCLS.

The Water Quality Monitoring, Information, and Restoration Act (WQMIRA) authorizes DEQ to make 303(d) listing decisions based on recommendations from the Department of Health (VDH). VDH public health assessments and fish consumption advisories are not subject to the requirements of the Administrative Process Act (APA). This exemption from formal rulemaking is recognition that protection of public health is of utmost importance to the Commonwealth. HRSD is correct that VDH has the discretion to issue advisories on lead and arsenic, but to date it has not done so and it is not anticipated it will any time soon.

Thanks for your thorough review of the report. The information about tributyltin and the Elizabeth River is outdated and will be corrected for the final version.

Sent via US and electronic mail

April 24, 2012

John M. Kennedy
DEQ Office of Water Monitoring and Assessment
P.O. Box 1105
Richmond, Virginia 23218-1105
John.Kennedy@deq.virginia.gov

Re: Comments on Virginia's Draft 2012 Integrated Report

On behalf of the Center for Biological Diversity, I am writing to urge DEQ to include Virginia's coastal and Chesapeake Bay waters on the list of threatened or impaired waters due to ocean acidification. Virginia has taken an important step forward in its water quality assessment by analyzing biological indicators and adding waters to its draft 2012 303(d) list due to those biological assessments. The nearshore survey effort is extremely impressive. Nonetheless, the state should also take action now to address waters that are threatened or impaired by the contribution of ocean acidification. Since Virginia's water quality standard for pH is insufficient to protect aquatic life from the threat of ocean acidification, DEQ must evaluate the threat to aquatic life by ocean acidification. As stated in the Center's prior letters and data, Virigina's coastal and estuarine waters are threatened and impaired by acidification, those data are incorporated here by reference. This letter summarizes some of the most recent and relevant data and information that should warrant inclusion of coastal and bay waters on the 303d list.

The oceans are becoming more acidic faster than they have in the past 300 million years, a period that includes four mass extinctions (Honisch et al. 2012). Researchers looked at the geologic record and found that that the Paleocene-Eocene Thermal Maximum was the closest event to what we're experiencing now by adding carbon dioxide to the oceans. At that time, the climate warmed by about 5˚C, yet the rate of carbon dioxide increase was only about 10% of what the earth is experiencing now. This means that the ocean chemistry changes that are occurring due to ocean acidification are unprecedented and are cause for concern that the oceans may face mass extinctions of many species that we care about including Chesapeake Bay's shellfish. Friedrich et al. concluded that anthropogenic ocean acidification already exceeds the natural variability on regional scales and is detectable in many of the world's oceans, including Atlantic regions (Friedrich et al. 2012). Observed trends over the last couple of decades off Bermuda indicate that aragonite saturation has declined -0.04 per decade—exceeding the last glacial termination by orders of magnitude (Id.).

Areas of the Chesapeake Bay have already been lost for oyster harvesting due, in part, to ocean acidification (Finchham 2012). The problems in the Chesapeake Bay are analogous to oyster die-offs in the Pacific Northwest that have now definitively been linked to ocean acidification (Barton 2012). Barton et al. have now definitively linked the devastating oyster mortalities in Washington and Oregon to ocean acidification. Specifically, the study found that oyster larvae had difficulty with growth and production in waters with elevated CO_2 . In response to reported shellfish hatchery problems in the Pacific Northwest, Barton et al. reported the results of their observations from the Whiskey Creek Hatchery on Netarts Bay, Oregon in the summer of 2009 (Barton et al. 2012). Notably, this study analyzed calcifying organism responses in the naturally fluctuating ambient-water CO_2 chemistry of the Pacific Northwest's coastal upwelling system, a study reaching beyond the laboratory to actual observation. Larval production and mid-stage growth (~120 um to ~150 um) of the oyster, *Crassostrea gigas*, were both significantly negatively correlated with the aragonite saturation state of waters in which larval oysters were spawned and reared for the first 48 hours of life. The study demonstrated that during times of low aragonite saturation, larval oysters experienced adverse effects. Although the impact of the exposure was not immediate, the delayed reaction caused a significant decline in growth for mid-sized oyster larvae and reduced overall production. The findings corroborate other laboratory studies that show that many marine species, especially at the larval stage, are adversely affected by ocean acidification.

As described in Waldbusser et al. 2010, acidification is impacting the Chesapeake Bay even faster than the open ocean. Already, calcification of juvenile oysters is compromised by acidification (G.G. Waldbusser et al. 2010). Oyster populations are already at historically low levels, and an examination of 23 years of water quality data concluded that significant trends in acidity will have impacts on juvenile oyster growth and survival (*Id.*). The study found that average pH in some tributaries corresponded to those that caused reduced calcification in oysters (*Id.*). It also noted a trend toward reduced calcification for juvenile oysters (*Id.*). Higher temperatures and CO2 additively decreased calcification rates. Although carbon dioxide from atmospheric sources is not the only factor leading to changes in acidity in the Chesapeake Bay, according to the scientists the possibility of a shifting baseline "should not be ignored" and "even gradual decreases in baseline pH could alter estuarine carbonate dynamics in important ways sooner than open ocean environments (*Id.*). The Clean Water Act requires identifying these waters as impaired, or at minimum threatened, for any contribution that would cause violation of the water quality standards—which include the designated uses for fishing, the growth and propagation of fish, other aquatic life, and wildlife, shellfish propagation; actual or potential areas for the harvesting of oysters, soft-shell clams, hard-shell clams, and brackish water clams (COMAR 26.08.02.02).

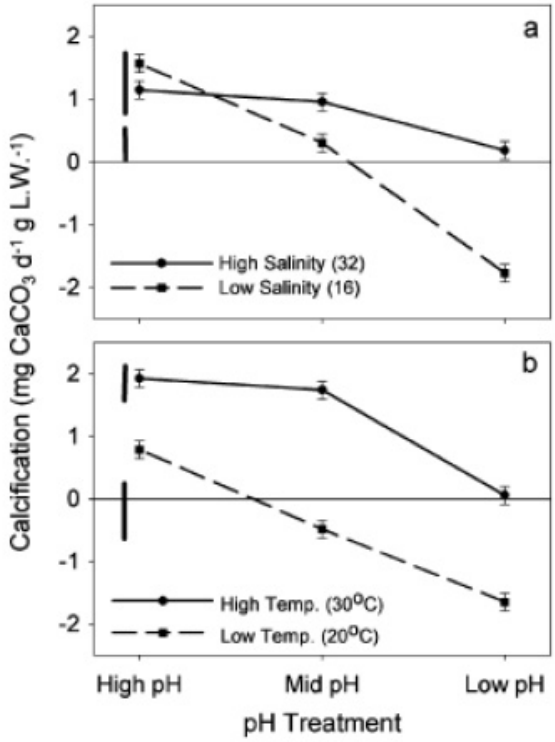


Fig. 1. Biocalcification rates of C. virginica for treatment combinations of a salinity by pH and b temperature by pH estimated by least squares means from each two-way ANOVA. Units of calcification are milligram of calcium carbonate per gram oyster whole live weight per day. Treatment levels of salinity, temperature, and pH are listed in Table 1. Error bars are standard errors of the least squares means estimates. Non-significant differences among estimated least squares means by pairwise comparisons with Bonferroni correction are noted by values falling within the black vertical bars next to the left y-axis at α=0.05

Recent science continues to substantiate findings that ocean acidification harms calcifying animals. Studies of Northwest Atlantic bivalves demonstrate that changes in ocean acidification and temperature can have significant negative consequences for these coastal animals (Talmage and Gobler 2011). The experiment looked at preindustrial, present, and future carbon dioxide levels and found significant changes at each level, especially for larval life stages. Specifically, Talmage and Gobler concluded that eastern oyster and bay scallop were sensitive to ocean acidification, while ocean acidification and temperature together impaired the survival, growth, development, and lipid synthesis of hard clams and bay scallops.

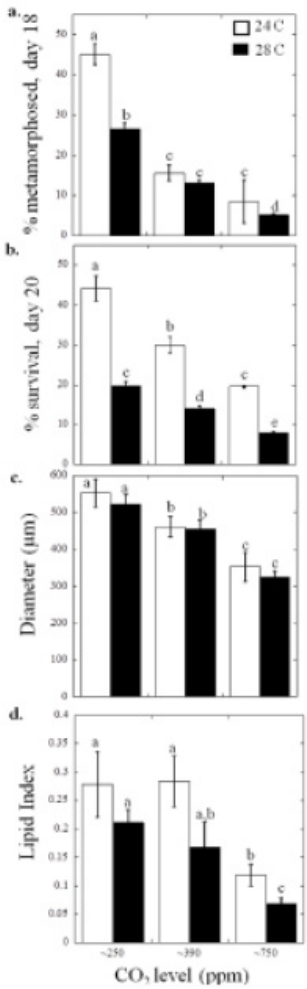


Figure 2. Performance of Mercenaria mercenaria larvae grown under three levels of CO_2, approximately 250, 390, and 750 ppm, and two temperatures 246C (white bars) and 286C (black bars; see Table 1 for carbonate chemistry). a. Percent metamorphosed of individuals 18 days post- fertilization, b. Percent larval survival (20 days post-fertilization), c. Diameters of larvae (20 days post-fertilization), and d. Lipid index (lipid area/total area) (20 days postfertilization).
Error bars represent standard deviation of replicated vessels per treatment (n = 4 per treatment), and for Tukey multiple comparisons, p#0.05. Statistical results were based on arcsine square root transformations of the % data for a. and b.
doi:10.1371/journal.pone.0026941.g001

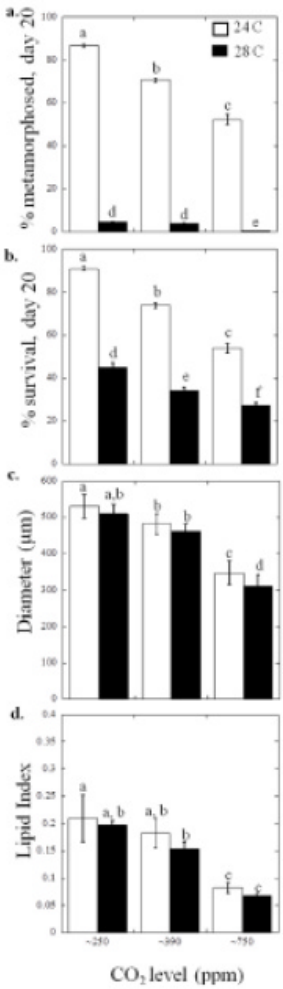


Figure 3. Performance of Argopecten irradians larvae grown under three levels of CO_2, approximately 250, 390, and 750 ppm, and two temperatures 246C (white bars) and 286C (black bars; see Table 1 for carbonate chemistry). a. Percent metamorphosed of individuals 18 days post- fertilization, b. Percent larval survival (20 days post-fertilization), c. Diameters of larvae (20 days post-fertilization), and d. Lipid index (lipid area/total area) (20 days postfertilization).
Error bars represent standard deviation of replicated vessels per treatment (n = 4 per treatment), and for Tukey multiple comparisons, p#0.05. Statistical results were based on arcsine square root transformations of the % data for a. and b.
doi:10.1371/journal.pone.0026941.g002

Waldbusser et al. conducted a study of eastern oyster under 4 levels of pH that encompass a range typical of the mesohaline waters of the Chesapeake Bay (7.2–7.9 on the NBS scale) (George G. Waldbusser, Steenson, and Green 2011). They found that in as little as 2 weeks under various pH levels, shells began to dissolve even in waters that were not corrosive 7.9 pH (see

figure 4). The treatments were not atypical for estuarine waters in the Chesapeake Bay and demonstrate that shell dissolution increases with declining pH especially for fresh shells.

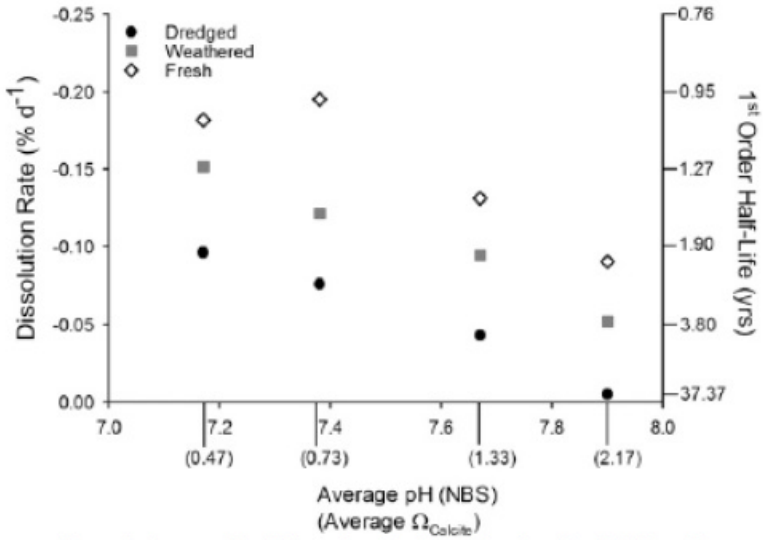


Figure 4. Average dissolution rates of individual treatments related to pH. The saturation state for calcite is presented below the pH values for reference. On the right y-axis are the first-order half-life values computed from the rate constants measured during the experiments. It is important to note the measurements are instantaneous rate constants for a short window of time, roughly 2 wk (see text).

A special issue of the Chesapeake Quarterly from the Maryland Sea Grant College focuses on the studies that are being done of the Chesapeake Bay and the impacts of ocean acidification on the Bay's aquatic life (enclosed). DEQ is urged to consider this data as well as other emerging information about ocean acidification.

New science also confirms negative consequences of ocean acidification on Atlantic herring, Atlantic cod, and *Menidia beryllina* a common Atlantic estuarine fish. In Atlantic cod, exposure to CO2 resulted in severe to lethal tissue damage in many internal organs, with the degree of damage increasing with CO2 concentration (Frommel et al. 2011). Larval survival and length of *M. beryllina* unambiguously decreased with increased carbon dioxide treatments (Baumann, Talmage, and Gobler 2011). Eggs exposed to high levels also had a higher rate of malformations with larvae having curved bodies. Increased carbon dioxide in the water also negatively affected Atlantic herring larvae (Franke and Clemmesen 2011). Slower growing larvae are more vulnerable to predation and decreased feeding success (Baumann, Talmage, and Gobler 2011; Franke and Clemmesen 2011). Larval survival is key to recruitment, thus ocean acidification has the potential to act as an additional source of natural mortality, affecting populations of already exploited fish stocks (Frommel et al. 2011).

In addition to the letters presented by the Center for Biological Diversity, Virginia has a duty to examine monitoring data from the Chesapeake Bay as well as its other coastal waters to evaluate them for water quality problems due to ocean acidification.

Conclusion

In summary, Virginia has an obligation to list its coastal and Chesapeake Bay waters as threatened or impaired under section 303(d) of the Clean Water Act. The Chesapeake Bay studies as well as other information included here and in prior letters demonstrates that biological functions of calcification are already being impaired by ocean acidification as a contributing factor. Ocean acidification is a threat to seawater quality, and the Clean Water Act requires the state to identify waters that are threatened or impaired.

Additionally, Virginia should incorporate the latest scientific information about ocean acidification into a revision of the marine pH water quality standard and develop additional water quality standards and assessments to address ocean acidification.

Sincerely,

/s/ Miyoko Sakashita

Miyoko Sakashita
miyoko@biologicaldiversity.org

Enclosed

Barton, Alan; Hales, B; Waldbusser, G; Langdon, C.; Feely, R.A. 2012. "The Pacific oyster, Crassostrea gigas, shows negative correlation to naturally elevated carbon dioxide levels: Implications for near-term ocean acidification effects." Limnol. Oceanography, 57(3) 2012, 698-710. doi: 10.4319/lo.2012.57.3.0698

Baumann, Hannes, S.C. Talmage, and C.J. Gobler. 2011. "Reduced early life growth and survival in a fish in direct response to increased carbon dioxide." *Nature Climate Change* (December): 6-9. doi:10.1038/NCLIMATE1291.

Chesapeake Quarterly. 2012. Acid Test for the Great Shellfish Bay?

Franke, A., and C. Clemmesen. 2011. "Effect of ocean acidification on early life stages of Atlantic herring (Clupea harengus L.)." *Biogeosciences* 8 (12) (December 15): 3697-3707. doi:10.5194/bg-8-3697-2011.

Friedrich, T., A. Timmermann, A. Abe-Ouchi, N. R. Bates, M. O. Chikamoto, M. J. Church, J. E. Dore, et al. 2012. "Detecting regional anthropogenic trends in ocean acidification against natural variability." *Nature Climate Change* 2 (2) (January 22): 1-5. doi:10.1038/nclimate1372.

Frommel, Andrea Y., Rommel Maneja, David Lowe, Arne M. Malzahn, Audrey J. Geffen, Arild Folkvord, Uwe Piatkowski, Thorsten B. H. Reusch, and Catriona Clemmesen. 2011. "Severe tissue damage in Atlantic cod larvae under increasing ocean acidification." *Nature Climate Change* 2 (1) (December 11): 1-5. doi:10.1038/nclimate1324.

Honisch, B., a. Ridgwell, D. N. Schmidt, E. Thomas, S. J. Gibbs, a. Sluijs, R. Zeebe, et al. 2012. "The Geological Record of Ocean Acidification." *Science* 335 (6072) (March 1): 1058-1063. doi:10.1126/science.1208277.

Talmage, Stephanie C., and Christopher J. Gobler. 2011. "Effects of Elevated Temperature and Carbon Dioxide on the Growth and Survival of Larvae and Juveniles of Three Species of Northwest Atlantic Bivalves." Ed. Brian Gratwicke. *PLoS ONE* 6 (10) (October 31): e26941. doi:10.1371/journal.pone.0026941.

Waldbusser, G.G., E.P. Voigt, Heather Bergschneider, M.A. Green, and R.I.E. Newell. 2010. "Biocalcification in the eastern oyster (Crassostrea virginica) in relation to long-term trends in Chesapeake Bay pH." *Estuaries and Coasts* 34 (2) (May 29): 1–11. doi:10.1007/s12237-010-9307-0.

Waldbusser, George G., Ryan a. Steenson, and Mark a. Green. 2011. "Oyster Shell Dissolution Rates in Estuarine Waters: Effects of pH and Shell Legacy." *Journal of Shellfish Research* 30 (3) (December): 659-669. doi:10.2983/035.030.0308.

DEQ's Response to the Center for Biological Diversity

DEQ appreciates the attention the Center for Biological Diversity (CBD) has brought to the issue of ocean acidification. The direct effect of atmospheric CO_2 levels on water quality is an emerging concern, one that promises to challenge environmental regulation and management for years to come. As CBD's literature review illustrates, the Chesapeake Bay is at the forefront of estuarine research. The enormous body of data collected in the waters of the Bay provides a solid foundation for understanding the physiochemical and biological dynamics inherent to all coastal ecosystems. DEQ is grateful for its long-term partnership with the Bay research community, which has informed the decision-making behind the Bay TMDL and other regulatory tools being implemented for the protection of Virginia's sensitive coastal ecosystems.

While DEQ agrees that the issue of ocean acidification deserves further attention, a review of available monitoring datasets does not reveal evidence of systemic violations of Virginia's water quality standards (pH 6.0 – 9.0). DEQ analyzed more than 700,000 observations, spanning six years (from January 2005 to December 2010) and characterizing a range of environmental conditions, including both tidal tributaries and the Bay mainstem. In addition to the bimonthly vertical profile datasets generated by DEQ and researchers at Old Dominion University, a large number of high-frequency continuous monitoring datasets were included in this analysis. Continuous monitoring is much more sensitive than discrete sampling at detecting violations of water quality standards. It also allows for precise characterization of diurnal, tidal, and seasonal physiochemical dynamics. The monitors are deployed and maintained by the Virginia Institute for Marine Science, which has served as a role model for long-term continuous monitoring of estuarine waters for almost a decade. Many of the continuous and discrete monitoring datasets used in DEQ's analysis can be downloaded from the Virginia Estuarine and Coastal Observatory System website http://www.vecos.org and the Chesapeake Information Management System http://www.chesapeakebay.net. A summary of these data is shown in Figures 1 and 2.

The majority of continuous measurements (99%) was at or above pH 7.2, and no continuous monitors detected long periods of time (longer than two consecutive 24-hour periods) in which pH was sustained below 7.2. The time-series generated by the continuous monitors reveal considerable diurnal and seasonal variability in pH. At the single-depth monitors, the mean diurnal pH range at the surface was 0.4 ± 0.2, possibly reflecting the variable nature of metabolic processes associated with highly productive estuarine waters (Hofmann et al., 2011). Dynamics in pH also appear to vary spatially, with mesohaline waters exhibiting larger swings than polyhaline waters. These findings support the argument made by Hofmann et al. 2011 that more high-frequency datasets from a diverse set of coastal ecosystems are needed to better understand the natural dynamics of pH. Conclusions based on extrapolations from oceanic grab samples are likely inappropriate for determining impairment in estuarine waters.

While violations of Virginia's pH standard are evident in both continuous and discrete monitoring data, most are of the standard's upper limit (pH = 9.0) rather than the lower (pH = 6.0). Six violations of the

lower limit (see Figure 2) were observed on a minor tributary of the Rappahannock River that is currently listed as impaired. DEQ suspects pH is depressed there due to the creek's low slope and flow—two characteristics which promote acidic and hypoxic conditions independent of anthropogenic influences. The depressed pH is more pronounced at upstream sections of this tributary, further indicating the lowered pH is caused by localized conditions characteristic of freshwater swamps and not by generalized ocean acidification. The three other mesohaline creeks that are currently on the 303(d) list for low pH have similar features. If natural conditions and nutrient enrichment are ruled out as drivers, targeted TMDLs will be scheduled for these waters. Either way they will continue to be monitored and assessed.

Nutrient enrichment is implicated as a major source of pollution in the Chesapeake Bay, and it is addressed by the Bay TMDL. Since eutrophication can both depress (Cai et al., 2011) and elevate pH (Waldbusser et al. 2011), it is expected that successful implementation of the Bay TMDL will promote healthy pH levels throughout the Bay and its tributaries. But as Waldbusser et al. (2011) suggests, the dynamics inherent to estuarine waters complicate predictions even under the best circumstances.

CBD's literature review highlights the importance of pH on aquatic life. Evaluation of the integrity of the benthic community is a major part of the assessment of the aquatic life use. Thirty-seven percent of Virginia's meso- and polyhaline waters have degraded benthic communities. It is believed that chronic hypoxia and sediment contamination are the major stressors impacting benthic fauna in the Bay, but stressor identification analysis will be performed as part of the TMDL development process. While much work is needed to achieve full attainment of the Bay's aquatic life use, Virginia is heartened by the growing reefs of the eastern oyster in the Lynnhaven River and the recent uptick in blue crab abundance revealed in the 2012 Winter Dredge Survey. These recent trends indicate that management activities are having a positive effect on the Bay's health.

While Virginia's off-shore environment has not been monitored as extensively as the Bay, violations of pH in these waters have not been documented by DEQ since the inception of its water quality monitoring program.

DEQ uses both empirical and modeled monitoring data, held to specific quality requirements, to justify listing and delisting decisions. Different lines of evidence may be considered to substantiate a judgment of water quality impairment, but DEQ is ultimately required to base this decision upon documented violations of Virginia's water quality standards, which are promulgated only after scientific peer-review and procedures of formal rule-making. DEQ accepts non-agency data on a continual basis, but data must have been submitted by March 2011 to have been included in the 2012 Integrated Report. While the experimental work cited by CBD is informative, it does not meet the criteria DEQ requires for monitoring data used in assessments. Thus, DEQ has no justification for listing its estuarine and coastal waters for ocean acidification at this time.

Virginia currently has no standard for "allowable" variability in pH. Such a criterion is needed to establish whether a waterbody has experienced a detrimental shift in aquatic life conditions. As required by federal regulation, DEQ amends water quality standards in a process called Triennial

Review. Recommendations should be made to the agency during this time so that they can be considered through formal rule-making. Only after standards are adopted by the State Water Control Board and approved by EPA can they be used in water quality assessments. Virginia's next Triennial Review is anticipated to begin February 2013.

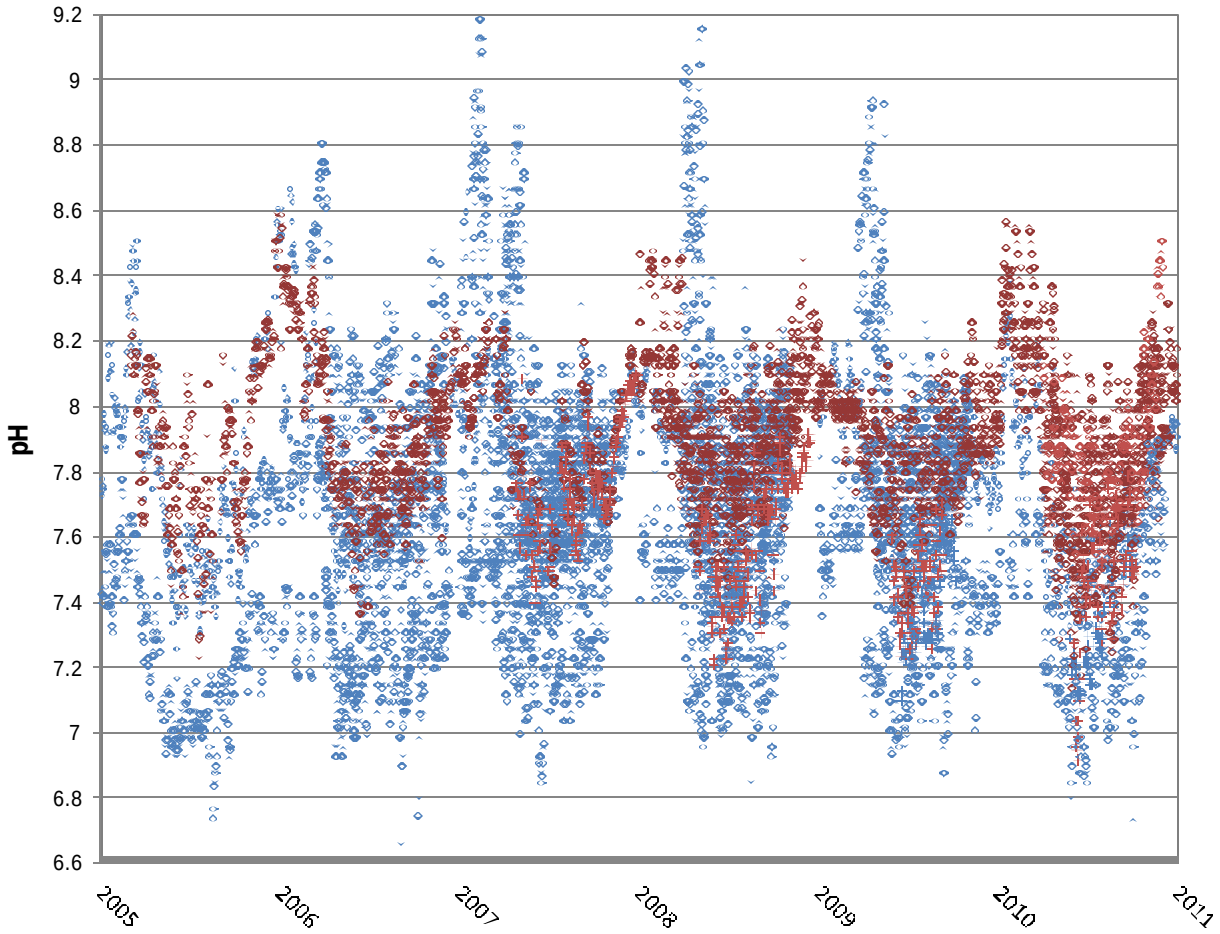


Figure 1. Daily minimum pH values recorded at 20 continuous monitors deployed in mesohaline (blue) and polyhaline (red) waters of the Chesapeake Bay (n=14,177). Most data were collected in shallow near-shore environments at the water surface. Some data, indicated by the cross-shaped markers, were collected by two Vertical Profilers deployed in deeper waters (> 10 meters). More information about continuous monitoring in the Chesapeake Bay can be found at http://www.vecos.org.

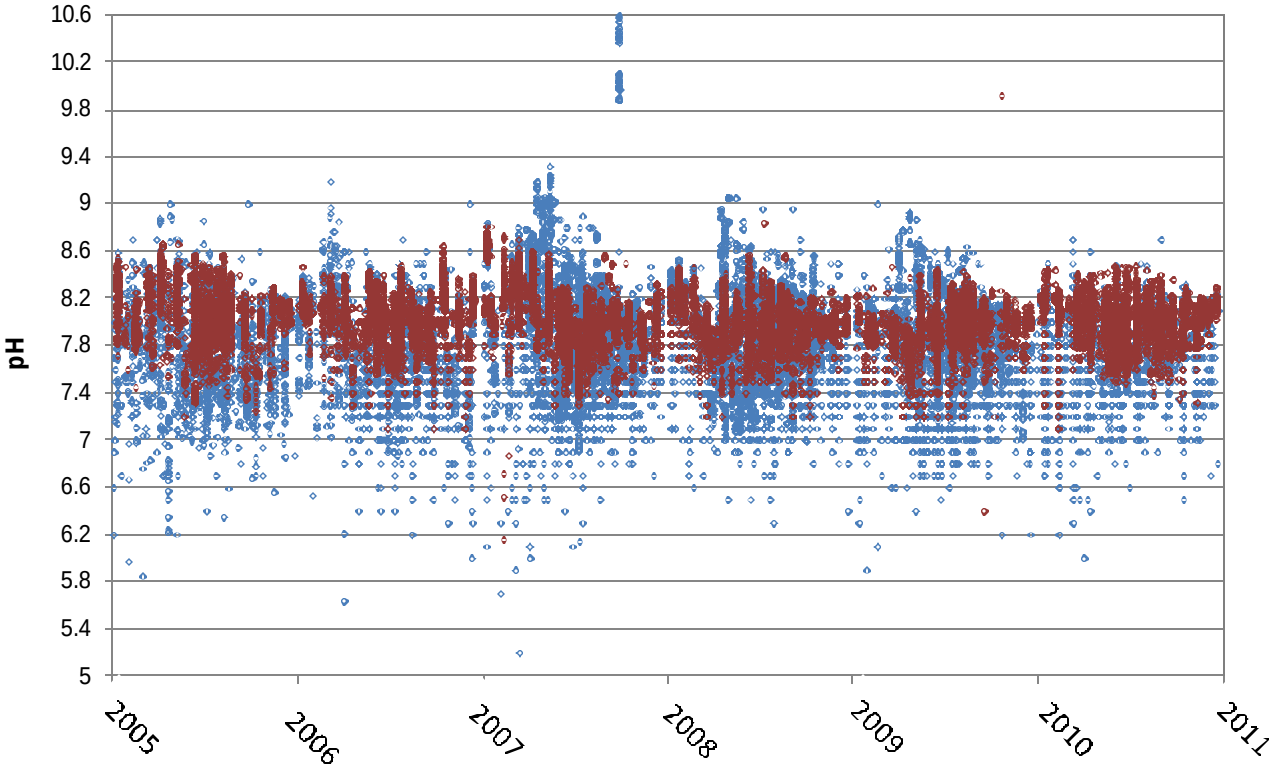


Figure 2. Discrete measurements of pH taken at 378 monitoring stations in mesohaline (blue) and polyhaline (red) waters of the Chesapeake Bay (n = 59,599).

Literature Cited

Cai WJ, Hu X, Huang WJ, Murrell M, Lehrter J., et al. (2011) Acidification of subsurface coastal waters enhanced by eutrophication. Nature Geoscience 4, 766-770.

Hofmann GE, Smith JE, Johnson KS, Send U, Levin LA, et al. (2011) High-frequency dynamics of ocean pH: A multi-ecosystem comparison. PloS ONE 6(12).

Waldbusser GG, Voigt EP, Bergschneider H, Green MA, Newell RI (2011) Biocalcification in the eastern oyster (*Crassostrea virginica)* in relation to long-term trends in Chesapeake Bay pH. Estuaries and Coasts 34, 221-231.

BOARD OF DIRECTORS

Henry Doss, President

Bob Lovett, Vice President

Bob Kelly, Treasurer

Martha Stephenson, Secretary

Jonathan Halsey

Brantley Ivey

Fred Jordan

Dixie Leonard

John Pine

Lorrie Sprague

Dave Wallace

Anna Ziegler

STAFF

Chris Arvidson, Assistant Director

Brad Baskette, Land Protection Assistant

Lynn Caldwell, Restoration Director

Carol Coulter, Director of Operations

Laura Green, Administrative Assistant

Ben Lucas, Land Protection Coordinator

George Santucci, Executive Director

Jessica Stearns Water Quality Assistant

Courtney Wait, Advocacy Coordinator

April 27, 2012

John M. Kennedy
DEQ Office of Water Monitoring and Assessment
P.O. Box 1105
Richmond, Virginia 23218-1105

RE: DEQ Draft 2012 305(b)/303(d) Water Quality Assessment Integrated Report

Dear Mr. Kennedy,

This letter represents comments and concerns from the National Committee for the New River (NCNR) regarding VA DEQ's 2012 Draft 303(d) list of impaired waters as required by the Clean Water Act.

NCNR's mission is to ensure the protection of the health and scenic beauty of the New River, a national treasure, pursuant to state and federal water regulations. With regard to these regulations, we understand the importance of the 303(d) list and appreciate the future development of TMDL's for these impairments. A listing of impaired, while illustrating current water quality issues, provides the added benefit of developing plans that identify pollution sources and determine maximum pollution levels not to be exceeded in the future. As part of our goal and our mission to restore and protect these streams, NCNR is prepared to do all we can to meet these goals, and ensure the goals are met by the Department of Environmental Quality.

In this 2012 draft list, 21 segments – a total of 160.93 stream miles – are newly listed as impaired in Virginia's New River watershed. Of the 21 newly impaired segments, 14 are caused by E. coli and 5 by temperature. This seems to be a trend for the New River watershed as of the 72 segments listed on the "Waters needing TMDL Study" list, 45 of those stream/river sections are impaired due to E.coli while another 12 sections impaired by temperature. The "Fact Sheets for Impaired (Category 5) Waters in 2012" explains many of the sources for the bacteria and temperature impairments in the New River watershed to be agricultural uses based on livestock and lack of adequate riparian buffers. As an organization we stress the importance of healthy riparian buffers and wish to see priority given to these stream areas and floodways. It would be beneficial to water quality improvements to see state funding go towards other agencies that can proactively assist the farming community so that their practices were not impairing the waters of their neighbors.

POST OFFICE BOX 1480 WEST JEFFERSON, NORTH CAROLINA 28694 336-846-6267 FAX 336-846-6433
WWW.NCNR.ORG INFO@NCNR.ORG

BOARD OF DIRECTORS

Henry Doss, President

Bob Lovett, Vice President

Bob Kelly, Treasurer

Martha Stephenson, Secretary

Jonathan Halsey

Brantley Ivey

Fred Jordan

Dixie Leonard

John Pine

Lorrie Sprague

Dave Wallace

Anna Ziegler

STAFF

Chris Arvidson, Assistant Director

Brad Baskette, Land Protection Assistant

Lynn Caldwell, Restoration Director

Carol Coulter, Director of Operations

Laura Green, Administrative Assistant

Ben Lucas, Land Protection Coordinator

George Santucci, Executive Director

Jessica Stearns Water Quality Assistant

Courtney Wait, Advocacy Coordinator

Another recommendation for improving the health of our waterways that are impaired due to human waste, like septic systems and other decentralized systems, would be to prioritize them for further study. In the interest of human health, sources that contribute to un-treated human sewage should be identified, and then landowners contacted to know best how to correct the problem. Similarly, this "source" for impaired waters should have agencies and local government that can assist the people causing the "source" to find ways to reduce that pollutant contribution.

Additionally we suggest that more monitoring sites be established to determine actual causes and sources of pollution. Defining these factors will be specifically beneficial for the establishment of a sound planning method for pollution reduction in waters of the New River. This could also be accomplished with a more effective use of citizen monitoring programs. National Committee for the New River has trained volunteers throughout the watershed that could be utilized better for more detailed bacteria and temperature monitoring.

Thank you for the opportunity to comment on the DEQ Draft 2012 305(b)/303(d) Water Quality Assessment Integrated Report.

Sincerely,

George Santucci
Executive Director

POST OFFICE BOX 1480 WEST JEFFERSON, NORTH CAROLINA 28694 336-846-6267 FAX 336-846-6433
WWW.NCNR.ORG INFO@NCNR.ORG

DEQ's Response to the National Committee for the New River

NCNR may be interested in two programs administered by the Department of Conservation and Recreation (DCR). The Virginia Agricultural BMP Cost-Share Assistance Program provides funding to private individuals, organizations, and businesses that install select best management practices (BMPs) on agricultural lands. Some conservation practices that are covered include streamside buffers, stream exclusion fencing, and use of cover crops. There is also funding support for residential BMPs (mainly focused on on-site sewerage improvements) in targeted watersheds—such as the watersheds of Mill and Dodd Creeks. In addition to cost-share, DCR runs the Virginia Agricultural BMP Tax Credit Program. Agricultural producers with an approved conservation plan can take a credit against state income tax of 25 percent of the first $70,000 spent on agricultural BMPs. There are also tax credits to encourage farmers to use conservation equipment. The Skyline Soil and Water Conservation District should be contacted for more information about both of these programs. Assistance programs are also provided by USDA Farm Service Agency and Natural Resources Conservation Service, Virginia Cooperative Extension, Virginia Department of Game and Inland Fisheries, and the Virginia Department of Forestry.

DEQ values its partnerships with citizen monitoring groups such as the NCNR, which has submitted data for 34 monitoring stations and over 300 sampling events in the New River. Your data aids DEQ in listing/delisting decisions and helps to direct resources to locations needing follow-up monitoring. DEQ appreciates your commitment to the New River and hopes to continue working with you in the future.

David Ward
Loudoun Watershed Watch
38659 Bolington Rd
Lovettsville, VA 20180
April 26, 2012

John M. Kennedy
DEQ Office of Water Monitoring and Assessment
P.O. Box 1105
Richmond, Virginia 23218-1105

Sent via e-mail to John.Kennedy@deq.virginia.gov

Subject: Comments on **Draft 2012 305(b)/303(d) Water Quality Assessment Integrated Report**

Loudoun Watershed Watch is pleased to submit comment on the DRAFT 2012 Water Quality Assessment Integrated Report appearing at http://www.deq.virginia.gov/Programs/Water/WaterQualityInformationTMDLs/WaterQualityAssessments/2012305b303dIntegratedReport.aspx

Specifically, we wish to comment on the "Delisting" in Loudoun County appearing in Appendix B (http://www.deq.virginia.gov/Portals/0/DEQ/Water/WaterQualityAssessments/IntegratedReport/2012/ir12_Appendix3_Waters_Identified_for_Delist.pdf)

There are four proposed delistings for recreational use (bacteria) in Loudoun County. We do not believe that VA DEQ should delist these streams for the following reasons:

1. Several samples in 2006 failed to pass Quality Assurance. The results for the four events that year suggest that the 10% threshold could have been exceeded for the 5-year data window.

2. Examining data beyond the data window indicate that the period 2005 to 2010 is a temporary low and that recent 2011 and 2012 results suggest that future data windows will show impairments. There is a strong likelihood that these segments will need to be "relisted" in 2014.

3. Given the very limited number of samples collected, the variability is significant and that one sample can easily change the results when evaluating the 10.5% threshold. We suggest caution in delisting simply because, say 10.3% is less than 10.5%. Recall that 10.5 percent was selected as a number greater than 10 percent and one could strictly speaking have selected 10.000001% as the threshold above 10 percent.

The text for the four delistings is copied below with specific comments on each river segment.

Potomac and Shenandoah River Basins

```
VAN-A01R_PIA01A00   Piney Run  3.51 Miles
Delisting Summary: Recreation
DELIST 2012 - E. coli - A01R-01-BAC, VAN-A01R-01, 00023 (CFL 1998 as fecal coliform)
During the 2010 cycle, this segment was assessed as not supporting the recreation use because
of excursions from the maximum E. coli bacteria criterion (6 of 31 samples - 19.4%) recorded
at DEQ's ambient water quality monitoring station 1aPIA001.80 at the Route 671 bridge
crossing. Additional bacteria monitoring has been conducted and found that a total of 3 of 29
samples (10.3%) exceed the maximum E. coli bacteria criterion. It has been determined that
this segment should be delisted for E. coli based upon an acceptable exceedance rate.
```

In 2006 four samples failed QC. Of these there were two exceedances. If the two failed exceedances were to be counted, then 5 (3 passed, 2 failed QC) of the 34 (29 passed, 4 failed QC) would results in 14.7%. It is argued that delisting is premature.

```
VAN-A07R_BEC01A00  Beaverdam Creek  6.33 Miles
Delisting Summary:  Recreation
DELIST 2012 - E. coli - A07R-01-BAC, VAN-A07R-01, 00065 (CFL 1998 - fecal coliform)
During the 2010 cycle, this segment was assessed as not supporting the recreation use because
of excursions from the maximum E. coli bacteria criterion (8 of 26 samples - 30.8%) recorded
at DEQ's ambient water quality monitoring station 1aBEC004.76 at the Route 734 bridge.
Additional bacteria monitoring has been conducted and found that a total of 2 of 24 samples
(8.3%) exceed the maximum E. coli bacteria criterion. It has been determined that this segment
should be delisted for E. coli based upon an acceptable exceedance rate.
```

In 2006 four samples failed QC. Of these there were two exceedances. If the two failed exceedances were to be counted then 4 (2 passed, 2 failed QC) of the 28 (24 passed, 4 failed QC) would results in 4/28=14.2%. It is argued that delisting is premature.

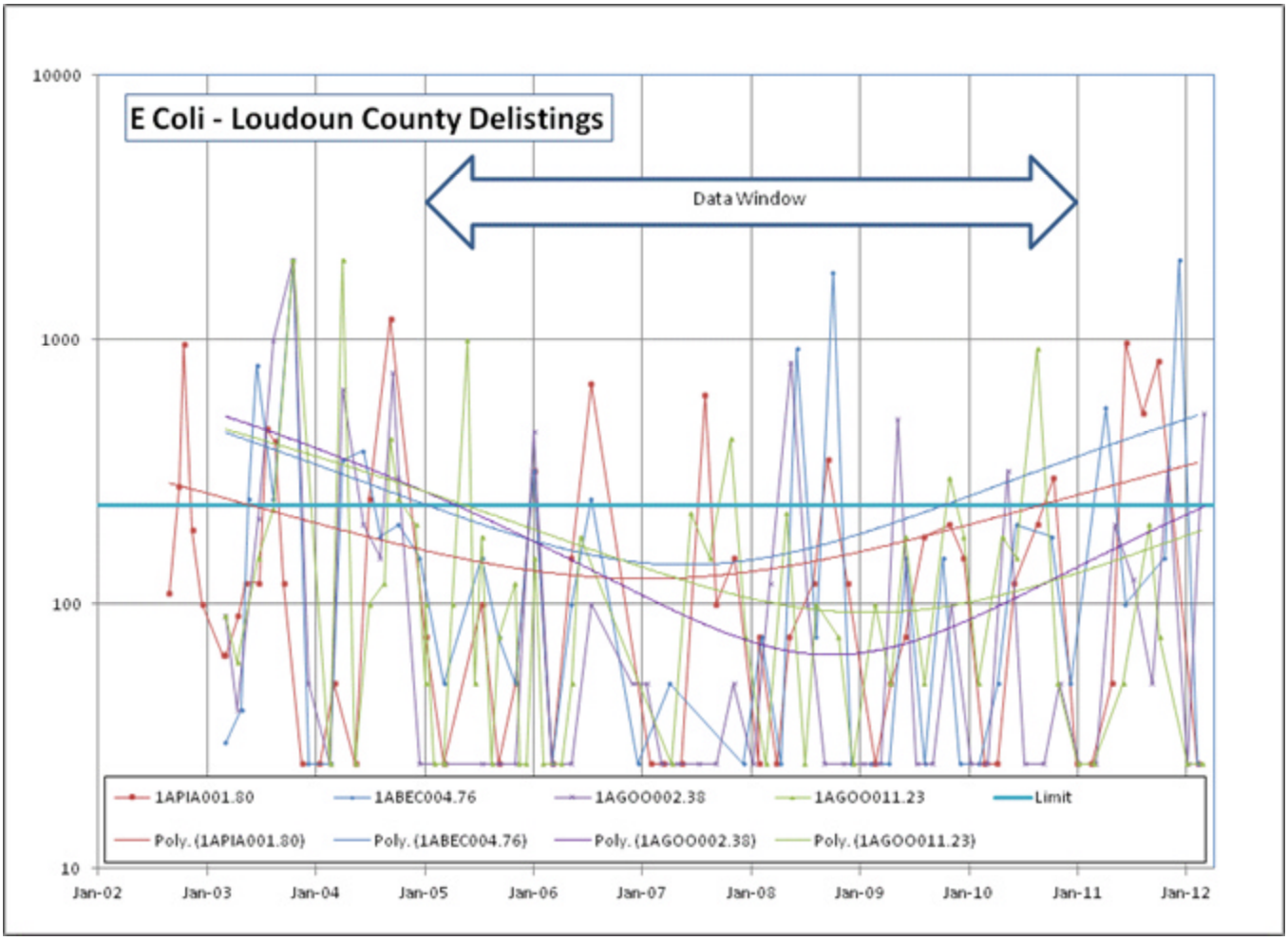
```
 VAN-A08R_GOO01A00  Goose Creek  4.76 Miles
Delisting Summary:  Recreation
PARTIAL DELIST 2012 - E. coli - A08R-01-BAC, 00062 (CFL 2006)
During the 2010 cycle, this segment was assessed as not supporting the recreation use because
of excursions from the maximum E. coli bacteria criterion (6 of 30 samples - 20.0%) recorded
at DEQ's ambient water quality monitoring station 1aGOO002.38 at the Route 7 bridge.
Additional bacteria monitoring has been conducted and found that a total of 3 of 29 samples
(10.3%) exceed the maximum E. coli bacteria criterion. It has been determined that this
segment should be delisted for E. coli based upon an acceptable exceedance rate.
```

In 2006 four samples failed QC. Of these there was one exceedances. If the one failed exceedances were to be counted then 4 (3 passed, 1 failed QC) of the of 34 (29 passed, 4 failed QC) would results in 4/34=11.7% It is argued that delisting is premature.

```
VAN-A08R_GOO03A02  Goose Creek  2.52 Miles
Delisting Summary: Recreation
DELIST 2012 - E. coli - A08R-06-BAC, 60011 (CFL 2006)
During the 2010 cycle, this segment was assessed as not supporting the recreation use because
of excursions from the maximum E. coli bacteria criterion (6 of 41 samples - 14.6%) recorded
at DEQ's ambient water quality monitoring station 1aGOO011.23 at the Route 621 bridge.
Additional bacteria monitoring has been conducted and found that a total of 4 of 39 samples
(10.3%) exceed the maximum E. coli bacteria criterion. It has been determined that this
segment should be delisted for E. coli based upon an acceptable exceedance rate.
```

During the data window (2005-2010) there were no samples that failed QC. During 2006 there were not exceedances, although two measurements did exceed 125 CU/100 ml. While the percentage of 10.3 is calculated from 39 samples, this is just under the limit of 10.5% (a legal interpretation of a number greater than 10). It is argued that delisting is premature.

When data from 2002 to 2012 were examined and a second-order polynomial curve fit (including those with failed QC), each site demonstrated a declines from 2002 to 2006 and then an upward trend such that by mid-2011, levels were approaching 2002 conditions.



We appreciate the opportunity to comment on the water quality assessment and look forward to your response.

Sincerely,

David Ward
Loudoun Watershed Watch

DEQ's Response to Loudoun Watershed Watch

DEQ appreciates the review and comments submitted by Loudoun Watershed Watch on the Draft 2012 Water Quality Assessment Integrated Report regarding the delistings noted in Appendix B.

The water quality assessment utilizes all of the QA/QC approved data collected at DEQ water quality stations within a 6 year period. For the 2012 Integrated Report, this 6 year window of data is January 1, 2005 through December 1, 2010. Data collected within this window that failed to pass QA/QC were not considered or analyzed for the assessment of water quality.

Delisting comments:

1. Piney Run – VAN-A01R_PIA01A00
   In the 2012 IR data window from January 1, 2005 through December 31, 2010, there were 29 valid bacteria samples collected and assessed. The four invalid bacteria samples collected in 2006 were not used in the assessment of Piney Run in the 2008 or 2010 Integrated Reports, and were not used in the assessment of Piney Run in the Draft 2012 Integrated Report.

2. Beaverdam Creek – VAN-A07R_BEC01A00
   In the 2012 IR data window from January 1, 2005 through December 31, 2010, there were 24 valid bacteria samples collected and assessed. The four invalid bacteria samples collected in 2006 were not used in the assessment of Beaverdam Creek in the 2008 or 2010 Integrated Reports, and were not used in the assessment of Beaverdam Creek in the Draft 2012 Integrated Report. The most recent data collected within the assessment data window (2009 and 2010) show no exceedances of the bacteria criteria in the 11 bacteria samples collected.

3. Goose Creek – VAN-A08R_GOO01A00
   In the 2012 IR data window from January 1, 2005 through December 31, 2010, there were 29 valid bacteria samples collected and assessed. The four invalid bacteria samples collected in 2006 were not used in the assessment of Goose Creek in the 2008 or 2010 Integrated Reports, and were not used in the assessment of Goose Creek in the Draft 2012 Integrated Report.

4. Goose Creek – VAN-A08R_GOO03A02
   In the 2012 IR data window from January 1, 2005 through December 31, 2010, there were 39 valid bacteria samples collected and assessed. There were two bacteria samples collected in 2006 that exceeded 125 CU/100 ml, but were well below the single sample maximum of 235 cfu/100 ml.

The 2012 Water Quality Assessment Guidance Manual (Memo No. 11-2007) contains the assessment procedures and methods for the development and reporting of the 2012 Integrated Report. Rule 1 states that "at least two exceedances and >10.5% of the total samples is required before a water is listed as impaired" for the conventional parameters including dissolved oxygen, nutrients, pH, bacteria, and temperature (except in tidal waters). More specifically to the bacteria parameters, Rule 3 states that "where data are not sufficient to calculate a monthly geometric mean, at least two exceedances and >10.5% of the total samples taken during the assessment period exceeding the instantaneous maximum bacteria standard for primary contact recreation is impaired."

These stations will continue to be monitored and assessed, if the data warrants, these stream segments may be listed again. Many stream segments are near the threshold of impaired/not impaired and it is not uncommon for these stream segments to be placed on and removed from the impaired waters list over several assessment cycles.

Shenandoah Riverkeeper
P.O. Box 405
Boyce, VA 22620
540.837.1479
keeper@shenandoahriverkeeper.org
www.shenandoahriverkeeper.org

April 23, 2012

Via e-mail (John.Kennedy@deq.virginia.gov)
John M. Kennedy
Office of Water Monitoring and Assessment
Virginia Department of Environmental Quality
P.O. Box 1105
Richmond, VA 23218-1105

RE: Comments on 2012 305(b)/303(d) Water Quality Assessment Integrated Report

Dear Mr. Kennedy,

Please accept the following comments from the Shenandoah Riverkeeper regarding Virginia's draft 2012 305(b)/303(d) Water Quality Assessment Integrated Report. These comments were prepared with advice and assistance from the Environmental Law and Conservation Clinic at the University of Virginia School of Law.

Shenandoah Riverkeeper is a program of the Potomac Riverkeeper Inc., a 501(c)(3) non-profit corporation. Shenandoah Riverkeeper's mission is to use citizen action and enforcement to protect and restore water quality in the Shenandoah River Watershed for people, fish, and aquatic life. Shenandoah Riverkeeper and Potomac Riverkeeper are grass-roots organizations with over 2,000 combined members. These members use the Shenandoah and Potomac Rivers to swim, fish, boat, and recreate. Some are landowners along the river, and many use the river for business uses and as drinking water.

We request that the North Fork, South Fork, and Main Stem of the Shenandoah River be added to the list of Category 5 impaired waters due to significant algae blooms, which violate Virginia's nuisance aquatic plant life standard and interfere with the public's recreational use of these waters. Although portions of these waters are already listed for a variety of impairments, the state should add the algae blooms as an additional cause of impairment.

Most of the Shenandoah system currently suffers from significant algae blooms through much of the year. These blooms, by themselves, violate Virginia's water quality standards. The General Criteria explicitly provide:

> State waters, including wetlands shall be free from substances attributable to sewage, industrial waste, or other waste in concentrations, amounts, or combinations which contravene established standards or *interfere directly or indirectly with designated uses* of such water or which are inimical or harmful to human, animal, plant, or aquatic life.
>
> Specific substances to be controlled include, but are not limited to . . . substances which *nourish undesirable or nuisance aquatic plant life*.[1] (emphasis added.)

As will be described more fully below, algae blooms that occur in the Shenandoah system clearly amount to "undesirable or nuisance aquatic plant life," which alone violates the general criteria. In addition, these blooms seriously impair recreational uses of these rivers. The Commonwealth's water quality standards make such recreational use a designated use of all state waters,[2] and the impairment of that use constitutes violations of water quality standards.

[1] 9 VAC 25-260-20(A).
[2] 9 VAC 25-260-10 (A).

I. The Nature and Extent of the Algae Blooms Constitute Nuisance Aquatic Vegetation and Impair Recreational Use of the Rivers.

In my role at Shenandoah Riverkeeper, but also as a professional fishing guide for nearly a decade and recreational user for two decades, I have witnessed the severe algae blooms that hinder recreational use of the rivers. In my observations, the algae can and do bloom at nearly any time of year and excessive nutrients are always available in the system to fuel algae growth. It is quite rare in fact, to float a stretch of river and not see portions of the water column or the river bottom that have very significant growth of one form or another nearly year round. With the availability of nutrients at all times, it seems that a handful of different algae species responds when other water conditions are conducive to the growth of that particular algae. The variables seem to include: a) sunlight angle b) water clarity c) presence of turbidity d) temperature e) length of day f) pH g) alkalinity and h) hardness. These parameters are in constant fluctuation and contribute to the annual variability in the presence of algae.[3]

During late October of 2009, I took it upon myself to make more formal and comprehensive observations of the algae. During that month, I observed a filamentous periphytic algae in the South Fork of the Shenandoah River that covered the floor of the River from bank to bank. The particular algae bloom extended approximately fifty miles from Front Royal to Luray. In the spring of that same year, even though the river had cleared some, algae could be seen on rocks along a seventy-five mile stretch of the river from Island Ford to Front Royal. I have previously provided DEQ with photos that confirm these observations during my comments on the previous 2010 305(b)/303(d) Water Quality Assessment Integrated Report Draft, and which were part of the record during the time when EPA approved the previous draft 2010 303(D)/305(B) Water Quality Assessment Integrated Report. I would like to incorporate those comments, photos, and observations by reference in these comments.[4]

In July of 2010, I began receiving complaints from landowners and businesses regarding a major algae bloom on the South Fork. In particular, one landowner referred to the bloom as the worst algae bloom he had ever seen, and he further stated that he was no longer using the river because the algae was "so thick and smelled so bad." In that same month, I had a concerned mother contact me, because after her child swam in the river his swim trunks smelled like sewage. The woman happened also to be a reporter for the Winchester Star, and shortly thereafter an article in the paper highlighted algae problem. That article can be found at: http://www.winchesterstar.com/articles/printable/358092.

The algae bloom in July of 2010 also impacted local businesses. Multiple outfitters, including Downriver Canoe Company, Front Royal Canoe Company, and Shenandoah River Outfitters reported to me negative impacts from the algae blooms. In some cases, they reported that customers had complained about diminished enjoyment of the river due to the severe algal growth. Fishermen also complained that the algae in the Shenandoah River ruined their recreational experience. In particular they were frustrated, because the algae would foul their hooks making it impossible to fish large segments of the river. They were also disgusted by the stench of the algae. These complaints, and others, were also detailed in our comments regarding the 2010 305(b)/303(d) Water Quality Assessment Integrated Report.

Since the algae blooms of 2009 and 2010, I have continued to monitor the algae blooms on the River, and it is clear that they still impair recreational use and are a nuisance to those who use the River. The annual pattern has not changed and we continue to see periodic riverwide planktonic blooms after river rises, and then large periods of various periphytic and floating algae during the rest of the year.

During the spring and summer of 2011 for example, the Main Stem Shenandoah, through its entire reach exhibited the worst planktonic bloom I have seen to date. The water literally was the color of thick green paint for over three months. Fish were sluggish and wildlife activity around the river was down to nearly zero. This did not clear up until the sun angle and temperatures lowered in September. The river was completely unsuitable for recreational use. Also generally, we continue to see a handful of other algae during large parts of the summer and fall months that covered the bottom of the river and/or the surfaces over large swaths of river. In repeated attempts to use the river last summer, I was turned off by either a heavy carpeting of filamentous algae covering the bottom of the river, or chunks and mats of algae floating around on the surface.

---

[3] Summers, James, *Assessment of Filamentous Algae in the Greenbrier River And Other West Virginia Streams* (West Virginia DEP DWWM, December 17, 2008).

[4] Letter from Jeff Kelble, Shenandoah Riverkeeper (Sep. 23, 2010) (on record with Shenandoah Riverkeeper).

Most recently, in order to collect information for these comments, I solicited comments from stakeholders in order to better understand how algae in the Shenandoah River has continued to impacted recreational use of the river. From the over 80 responses we received, it is clear that the algae continues to be a nuisance and violate the recreational designated use set forth in the Virginia Water Quality Standards. Those letters, which are appended to this comment and that we incorporate in our official comments, speak for themselves and detail the location and approximate dates of the observed nuisance algae by recreational river users and landowners.

DEQ should note that these letters include observations of nuisance algae blooms at points along nearly the entire lengths of the North Fork, South Fork, and Main Stem Shenandoah. DEQ should also note that the areas most often mentioned are the areas most heavily used by the public. This does not necessarily mean algae problems are either limited to or focused in these areas. Quite the contrary. During my tenure as Riverkeeper I have documented repeatedly that when certain algae bloom, they are present from the top of the North Fork and South Fork and run all the way to the confluence with the Main Stem and on down to Harper's Ferry and the terminus of the river.

This is the case with the late spring/early summer planktonic blooms. This river wide bloom is most often associated with 1) the early May bloom of a wooly green bottom covering algae, 2) the mid-summer blooms of cyanobacteria (blue-green toxic oscillotoria algae as identified by Harold Marshal of ODU) and other dark green filamentous bottom covering and star-grass coating algae, and 3) the bright green filamentous algae that blooms almost overnight in late September/early October when the native grasses die back naturally.[5] On August 27, 2010, Shenandoah Riverkeeper drew two samples of water, one from the Main Stem Shenandoah at Route 7, the other from the Rappahannock River 300 yards downstream of the Remington Route 29 Business Bridge. The water sample and algae were preserved in a red fixative that Dr. Marshal had provided to Shenandoah Riverkeeper several days prior. Among other things this is what we derived from this single simple test:

1) The algae community on the Shenandoah wase different than the Rappahannock
2) The algeas found on the Shenandoahn are indicative of high nutrient levels and warm weather (summer sample)
3) There were several species of blue green algae, aka Cyanobacteria present which are known nuisance algaes and which account for bad odors, floating mats and the production of mycrocystin which is a liver toxin

In general, the complaints we are attaching highlight the excessive algae growth that occurs during the winter and mid-summer months in the North Fork, South Fork, and Main Stem of the Shenandoah River. Virtually all of the letters complain of a planktonic algae bloom following rain events in the winter, as well as filamentous algae ("rock snot") and "bubbly" algae that covers the surface of the river in the summer months. That said, many complaints reiterate the point that although it is especially bad in the winter and summer, algae growth remains a nuisance throughout the year.

It is worth pointing out a few specific examples of how the excessive algae has made swimming, fishing, and boating both unpleasant and difficult. Colby Trow, the owner of a local fly shop and guide service, states that he has photos of the river for each month of the past ten years, and it is difficult to find a single photo that doesn't illustrate the excessive algae growth. He also describes being embarrassed when clients ask him why the river is so dirty. William Amshey, states in his letter that he avoids wet wading in the river for "fear of infection." Another citizen, Andrew Riccobono, states that he travels as far as Pennsylvania or New York to fish, because at certain times of the year his flies become "covered in green muck after every cast" while fishing in the Shenandoah. Yet another citizen, Beau Morgan, describes the algae as sometimes being so thick that "one could hardly get a canoe to glide across it." After describing his frustrations associated with trying to fish in the Shenandoah River and the foul smell that emanates from the algae, Bill Millhouser says plainly, "I just cannot use the River due to the odors and annoyance."

We are attaching a map that shows the location of the algae blooms either observed directly by Jeff Kelble or reported in the letters that we are submitting to DEQ. The map clearly demonstrates that the entire reach of North Fork, South Fork, and mainstem Shenandoah are plagued by undesirable nuisance algae blooms every year, although reports are concentrated around the most heavily used areas.

These citizen letters demonstrate the frustration felt by landowners and river uses but represent only a few examples of the many Virginia citizens whose recreational use of the Shenandoah River has been diminished due to the excessive algal

[5] E-mail from Harold G. Marshall, Professor, Old Dominion University, (Sep. 22, 2010, 2:29 PM) (Attachment I to these comments).

growth. The unnatural levels of algae in these parts of the river clearly violate the requirement in the General Criteria of the water quality standards that waters be free from substances that cause the growth of undesirable or nuisance aquatic plant life. In addition, all the Commonwealth's waters have recreation as a designated use, and the algae blooms frustrate and impair that use.

II. The Algae Blooms Violate Water Quality Standards, Even in the Absence of Numeric Criteria or Thresholds for Nutrients.

In response to our similar comments on the 2010 Integrated Report, DEQ stated that it could not list the Shenandoah River due to algal growth, because "no listing threshold exists for nutrients, algae, freshwater submerged aquatic vegetation, and undetermined potential immune suppressors in these waters." This response implies that in order to list those portions of the river, a numeric criterion would have to be set and exceeded. This position is contrary to the Clean Water Act. The narrative standards that DEQ has established are just as enforceable as a numeric standard would be, and the facts set out above demonstrate a violation of those standards. The information we are submitting today about the algae blooms in the Shenandoah system show quite plainly that pollution is fueling the growth of undesirable and nuisance aquatic plant life, and that the algae blooms are impairing recreational uses of the river, both in violation of state water quality standards.

In *PUD No. 1 of Jefferson County v. Washington Dept. of Ecology*,[6] the Supreme Court of the United States stated that "EPA has not interpreted § 303 to require States to protect designated uses exclusively through enforcement of numerical criteria."[7] The Court in that case went on to say that:

> Petitioners also appear to argue that use requirements are too open ended, and that the Act only contemplates enforcement of the more specific and objective 'criteria.' But this argument is belied by the open ended nature of the criteria themselves. . . . We think petitioners' attempt to distinguish between uses and criteria loses much of its force in light of the fact that *the Act permits enforcement of broad narrative criteria based on, for example, 'aesthetics.'*[8] (emphasis added.)

The Court further explained that the idea of *only* enforcing specific numeric criteria is ridiculous, because that would imply that States would have to "study to a level of great specificity each individual surface water to ensure the criteria applicable to that water are sufficiently detailed and individualized to fully protect that water's designated uses."[9]

The point is simple: "listing thresholds" are not necessary in order to designate the South Fork, North Fork, and Main Stem of the Shenandoah River impaired due to excess algae. The fact that the algae blooms rise to the level of "undesirable or nuisance" aquatic plant life and hinder recreational use of these waters is enough to list the waters as impaired. In our research, we have been unable to find any regulations or guidance from either DEQ or EPA that would suggest otherwise.

The various citizen letters submitted with these comments show that the algae blooms in the Shenandoah system are excessive, are aesthetically unpleasing, cause foul odors, make it frustratingly difficult and sometimes impossible to fish in the river, make it unpleasant to wade and swim in the river, and even make it difficult to navigate the river with a canoe. The general criteria, applicable to all waters of the state, require that waters "shall be free from substances attributable to sewage, industrial waste, or other waste" in amounts which interfere with designated uses. The criteria specifically provide that "substances to be controlled include . . . substances which nourish undesirable or nuisance aquatic plant life."[10] The algae blooms we have documented plainly exceed any threshold for "undesirable and nuisance aquatic plant life," and DEQ is responsible for adding that impairment to the impaired waters list.

In addition, all state waters are designated for "recreational uses, e.g., swimming and boating."[11] The significant, unpleasant, and pervasive algae blooms in the Shenandoah River straightforwardly interfere with those uses. This is an impairment of water quality standards under the Clean Water Act just as much as if a numeric criteria were violated. DEQ

---

[6] 511 U.S. 700 (1994).
[7] *Id.* at 715.
[8] *Id.* at 715-16.
[9] *Id.* at 717-18.
[10] 9 VAC 25-260-20(A).
[11] 9 VAC 25-260-10 (A).

does not need a numeric threshold to determine that these portions of the Shenandoah River violate the General Criteria set forth in the Virginia Water Quality Standards.

We ask that DEQ to add violations of the nuisance aquatic plant life and recreational use standards to the listed causes of impairment for the North Fork, South Fork and Main Stem Shenandoah River in the state's 2012 305(b)/303(d) Water Quality Assessment Integrated Report. If DEQ fails to take this step, it will be a great disservice to the citizens of Virginia who value the recreational opportunities that the Shenandoah River has historically provided. We look forward to working with both DEQ and EPA to provide any additional information you might need in connection with this issue. If you have any questions regarding this request, please contact Jeff Kelble at: jeff@shenandoahriverkeeper.org or (540) 837-1479. We appreciate your time and consideration and are grateful for the opportunity to provide comments.

Sincerely,

Jeff Kelble

CC: Melanie Davenport

Thank you for your comments on the 2012 Draft Water Quality 305(b)/303(d) Integrated Report regarding the presence of algae in the Shenandoah Basin. The Department appreciates your concern for the Shenandoah River and its tributaries and your organization's efforts to protect and preserve the uses of this important natural resource. However, DEQ believes that listing the entire stretches of the North Fork, South Fork and mainstem Shenandoah Rivers as impaired for recreational use due to the presence of "nuisance" algae is inappropriate.

Virginia's EPA-approved 2012 Water Quality Assessment Guidance states:

> **Impaired waters needing a TMDL are those waters that do not meet Water Quality Standards due to a pollutant(s).** A pollutant, as defined in 40 CFR 122.2, means any dredged spoil, solid waste, incinerator residue, filter backwash, sewage, garbage, sewage sludge, munitions, chemical wastes, biological materials, radioactive materials (except those regulated under the Atomic Energy Act of 1954, as amended (42 U.S.C. 2011 et seq.)), heat, wrecked or discarded equipment, rock, sand, cellar dirt and industrial, municipal, and agricultural waste discharged into water. (*emphasis added*)

As you noted in your comments, the 9 VAC 25-260-20(A) General Criteria provides as follows:

> State waters, including wetlands shall be free from **substances** attributable to sewage, industrial waste, or other waste in concentrations, amounts, or combinations which contravene established standards, or interfere directly or indirectly with designated uses of such water, or which are inimical or harmful to human, animal, plant, or aquatic life.
>
> Specific **substances** to be controlled include, but are not limited to . . . substances which nourish undesirable or nuisance aquatic plant life. (*emphasis added*).

Algae itself is not a pollutant to be controlled within the meaning of the General Criteria, whereas the presence of nutrients, depending on their source and impacts, may be. However, at this time DEQ has no reliable methodology for assessing what may or may not be a "nuisance" nor has it reliable data demonstrating in what amounts and under what conditions nutrients might cause such a "nuisance" and therefore prompt a listing threshold. As no listing threshold exists, there also exist no attainment goals for these uses and subsequent delisting.

As outlined in the 2012 Virginia Water Quality Assessment Guidance, recreational use assessment includes swimming and other primary and secondary contact recreation uses such as water skiing and pleasure boating. Determinations of "fully supporting" versus "impaired" are based on violations of the e-coli bacteria in freshwater and enterococci bacteria in saltwater and transition zones. Refer to the excerpt from Table 1: Designated Use Matrix from Virginia's Water Quality Assessment Guidance below.

5.	Recreation/Swimming Use	Conventional Pollutant (E. coli and enterococci bacteria) and/or VDH beach closures/advisories or other available bacteria data. Previously listed fecal coliform impairments with no additional conventional bacteria data.

Virginia's bacteria water quality standard is based on the EPA-approved human health risk criteria of an illness rate of 8 in 1,000 individuals at the instantaneous Water Quality Standard of 235 cfu/100 ml. The risk levels are based on swimming, where water frequently gets in the mouth, nose, and eyes. DEQ believes that the use of a human health risk based standard is the most appropriate to determine recreational use support. Impaired waters are defined as those with chronic, recurring or ***human health*** (*emphasis added*) related WQ Standard violations using Quality Assurance/Quality Control (QA/QC) approved ambient monitoring data, special study data and/or other programmatic in-stream data collections.

Listing the entire stretches of the North Fork, South Fork and mainstem Shenandoah Rivers based on anecdotal and subjective observations would be an abandonment of the agency's long standing use of the human health risk-based numerical criteria for recreational use. To date, no scientific cause and effect documentation has produced a water quality criterion to evaluate human health risks associated with the presence of algae. The proposal to list the entire stretches of these rivers would be based on an "aesthetic" narrative standard alone. Virginia has not listed waters solely based on narrative criteria in previous assessment cycles. While the aquatic life use can be impaired due to the general narrative criteria for benthic macroinvertebrates, it is supported through the surrogate data from the Virginia Stream Condition Index. Validated monitored data is and has been the basis for DEQ's listing of impaired waters in Virginia. This approach allows for consistency from assessment cycle to assessment cycle.

DEQ evaluated data from many and varied sources for the 2012 Integrated Report. These data are broken into two general categories for assessment purposes. The first type of data is QA/QC approved "monitored" data. These data come from the collection and analysis of chemical, biological, and/or physical samples taken by DEQ and/or other DEQ-approved data submitted during the reporting period. These data are referred to as Level III and are considered the highest quality data. The 303(d) Impaired Waters list is comprised of only QA/QC approved monitored data due to the assessment confidence associated with the QA/QC monitoring requirements. Monitored data are obtained using EPA accepted methods and DEQ approved protocols.

All non-DEQ monitoring submittals, except U.S. Geological Survey chemical data submittals, must provide a sampling and analysis protocol and all field data for review. The second type of data used in assessment is considered "evaluated" data. These physical, chemical and/or biological data are primarily obtained from sources where there is not an EPA accepted sampling protocol and/or the use of non-DEQ approved sampling and analysis protocols. These data are considered Level II and of lower quality with less confidence in their results; they are not used for listing waters as fully supporting or impaired. If these types of data indicate water quality problems, the water is characterized as having an observed effect. To date, there are no studies reliably and numerically correlating nutrient levels with

the presence of "nuisance" algae to the substantial exclusion of other causal conditions. Further, algae have no systematic method of data collection and evaluation to allow a determination of what might be categorized as a "nuisance". Observations of algae growth can only be evaluated based on a subjective, anecdotal criteria and would be classified as Level II data.

DEQ monitors and assesses as many miles of Virginia's streams and rivers as resources allow. The North Fork, South Fork and mainstem Shenandoah Rivers are no exception. These rivers are systematically monitored and assessed against all available uses that monitoring data can provide.

Algae growth has not been linked to impairments that have been identified on stretches of the Shenandoah Basin rivers and streams. Typically, excessive algae can cause low dissolved oxygen levels in affected waters. These conditions have not been documented in these waters. As nutrients and algae are natural parts of a riverine system, it is very difficult to determine the "natural" amount for a riverine eco-system and when those levels exceed what is appropriate for a "fully supported" aquatic system. Algae blooms vary according to time and season and are unpredictable. Other available data do not correlate nutrient levels to an increased algae presence. Trend data evaluated in the 2012 Integrated Report indicate either no change or declining trends for nitrogen and phosphorus levels on the North Fork and South Fork Shenandoah Rivers. See table below:

Shenandoah Basin Trend Stations: Status 1991-2010

Station	Total Nitrogen	Total Phosphorus
1BNFS000.57	Declining	No change
1BNFS010.34	Declining	No change
1BNFS070.67	No change	No change
1BNFS093.53	No change	No change
1BSSF003.56	Declining	Declining
1BSSF054.20	Declining	Declining
1BSSF100.10	Declining	Declining

Stream Codes:

NFS North Fork Shenandoah River
SSF South Fork Shenandoah River

Virginia DEQ recognizes that the presence of excess nutrients can be among the factors leading to algal growth and thus, it is sensible that waters with notable algae growth be evaluated for the presence of excess nutrients, as well as other potentially-contributing factors. DEQ is in the process of developing riverine freshwater nutrient standards which will allow the Department the ability to list streams/rivers as impaired for nutrients and by default address algal growth and bloom issues in the future. While nutrient criteria have not yet been adopted, the development of these standards has been ongoing. The Academic Advisory Committee comprised of representatives from Virginia Tech, Virginia Commonwealth University, Virginia Institute of Marine Science and the University of Virginia has completed its report to the agency and we are now reviewing their recommendations. Once these criteria have been established, they will be considered for inclusion in Virginia's Water Quality Standards through the Triennial Review Process which updates, adds and deletes standards to better reflect the most recent science, trends and knowledge of pollutant effects.

As mentioned above, the development of these standards is a very complex undertaking as it is not a simple function of setting a fixed, maximum allowable amount of nutrients, as all waters are different and varied criteria based on other factors are necessary to determine impairment. As you mentioned in your comments, different algae species respond when conditions are conducive to the growth of that particular algae. Those variables include sunlight angle, water clarity, presence of turbidity, temperature, length of day, pH, alkalinity and hardness. These parameters are in constant fluctuation and contribute to the annual variability in the presence of algae.

As with all river systems, conditions in the headwater streams and tributaries have a significant impact on conditions in the mainstem. The exchange of nutrients and sediment loads to the system occur primarily in these smaller, extensive watersheds and are transported to the mainstem. In the South Fork Shenandoah River, stressor analysis determined the cause of the benthic impairment was phosphorus entering the system upstream of the impaired reach. Through the development of the TMDL, it was determined that the needed phosphorus load reduction could not be achieved in the impaired reach as the reductions required were more than the inputs located in that reach. Reductions upstream would be necessary to restore the benthic community. Implementation of TMDL's developed for phosphorus in the upstream tributaries was determined to provide the needed phosphorus load reductions. This situation led EPA to re-classify the benthic aquatic life use impairment on the South Fork Shenandoah River from Category 5A (Impaired, requiring a TMDL) to Category 4A (Impaired, no TMDL required), based on the approved TMDL's upstream that outlined the necessary phosphorus reductions to return the aquatic life use to a fully supporting status. This study confirms that the listing of the mainstem waters in the Shenandoah Basin for the presence of algae based upon nutrients and the subsequent development of a TMDL would be ineffective in addressing the presence of algae because the nutrients present are being transported from upstream sources.

Local TMDLs, and to a lesser extent implementation plans, have been developed on 17 tributaries to the Shenandoah basin rivers which have identified sediment and nutrients as causes for benthic impairments. These TMDL studies and implementation plans identify reductions and practices required to meet these loads and are outlined below.

North Fork Shenandoah River	**South Fork Shenandoah River**
Turley Creek - Sediment	Muddy Creek - Sediment & Phosphorus
Long Meadow Run – Sediment & Nitrogen	Mossy Creek – Sediment
Smith Creek - Sediment	Mill Creek (Rockingham) - Sediment & Phosphorus
Holmans Creek - Sediment	Pleasant Run - Sediment & Phosphorus
Mill Creek - Sediment	Moffett Creek – Sediment
Toms Brook Sediment	Upper Middle River – Sediment
	Lewis Creek - Sediment, Lead & PAH's
Mainstem Shenandoah	Christians Creek – Sediment
Spout Run - Sediment	South River - Sediment & Phosphorus
	Quail Run - Ammonia & Total Residual Chlorine
	South Fork Shenandoah River - Sediment & Phosphorus

Although the designated uses to be restored and protected by these TMDLs may differ from the purpose of the impaired listing which is being sought, those that deal with nutrients and sediments are expected

to help improve the algal conditions as well. One example of how these existing and developing TMDLs can contribute to the cleanup of Shenandoah waters from the standpoint of nutrients is the South River TMDL (approved by EPA 12/9/10), which was developed to address bacteria and benthic impairments. This TMDL calls for phosphorus and sediment load reductions of 51% and 39%, respectively, in order to meet the established TMDL endpoint. Phosphorus must be reduced by 70% from cropland, pasture, transitional and residential uses; along with a 70% reduction of the phosphorus load from septic systems. An Implementation Plan (IP) was developed in October 2010 by the Dept. of Conservation and Recreation along with multiple stakeholders in the region. The IP, which covers both the South River and Christians Creek, was approved by the State Water Control Board in June 2012, and is available at this DEQ webpage:
http://www.deq.virginia.gov/Portals/0/DEQ/Water/TMDL/ImplementationPlans/southchristiansip.pdf.
The IP calls for significant pollution reduction actions with staged implementation over 20 years, employing multiple Best Management Practices across all developed land use types, such as:

- Livestock exclusion with riparian buffers
- Constructed wetlands
- Continuous no-till , conservation tillage and small grain cover crops
- Pet waste education programs
- Streambank stabilization
- Bioretention filters, sedimentation basins and other urban stormwater controls

In addition to the TMDLs and IPs focused on local waters, it is very important to recognize the development and approval of the far-reaching and comprehensive Chesapeake Bay TMDL and the Virginia Watershed Implementation Plan (WIP). The entire Shenandoah basin is included in the watershed that is subject to the Chesapeake Bay TMDL and extensive controls on the point and nonpoint source inputs of nitrogen, phosphorus and sediment are called for under the WIP, many of which are already in operation. Implementation of the WIP for Chesapeake Bay restoration will also have the coincidental benefit of improving local water quality conditions in the headwater areas. Significant municipal and industrial point sources are required to either upgrade to near state-of-the-art nutrient reduction technology or make other arrangements (e.g., nutrient credit exchange) to reduce their nutrient discharges and meet assigned nutrient waste load allocations (WLA); other smaller discharges are required to maintain "capped" loadings at their current design capacity. In 2011, the first year for compliance with Virginia's Chesapeake Bay Nutrient Discharge Watershed General Permit, the Shenandoah basin's significant dischargers achieved substantial nutrient reductions. The total nutrient loads discharged were well below their aggregate annual WLA -- Total Nitrogen (TN) was only 48% of the annual WLA and Total Phosphorus (TP) was just 67% of the annual WLA. Comparing 2011 to 2009 discharge figures, the TN load was reduced by 561,000 pounds per year; the TP load was 169,000 pounds per year lower.

For nonpoint sources, particularly agriculture, the WIP contemplates a significant expansion of the coverage of best management practices to control polluted runoff. Of the agricultural practices contained in Virginia's WIP, the most widely used practices (nutrient management, buffers, soil conservation and livestock exclusion) are expected to be at 90% or greater in 2025 in terms of coverage on all land that is suitable for receiving such practices. In addition, the WIP prescribes 75,000 tons of manure to be exported from Rockingham and Page counties to outside the Chesapeake Bay watershed annually. Beyond reductions achieved to date, within Virginia's entire Chesapeake Bay watershed, the

WIP calls for an additional 10 million pounds of reductions in nitrogen delivered to the Bay and approximately 1.8 million pounds of phosphorus reduction by 2025.

The comments you provided are constructive and we share your concerns. The Virginia DEQ recognizes that the reports included with these comments indicate that excessive algae may exist in various locations in the Shenandoah Basin.

Virginia DEQ does not have specific criteria or standards to directly address the algae issues you have identified, and while the nutrient study process underway discussed above can seem to be frustratingly slow, it is imperative that the agency develop accurate, scientifically valid and defensible tools to determine the level of nutrients that reach the point of impairment. A premature listing prior to development of these standards strains the agency's credibility and objectivity in the eyes of both the regulated community and the public at large.

We believe it is appropriate to recognize these sections of the river as having an observed effect of aquatic algae. Therefore, we propose to modify the 2012 Draft Water Quality 305(b)/303(d) Integrated Report accordingly and list these waters under VA Category 2B ("*waters are of concern to the state but no water quality standard exists for a specific pollutant, or the water exceeds a state screening value or toxicity test*") for the recreational use. This designation means that these areas will remain a priority for monitoring and assessment in the future and will be evaluated when water quality standards related to nutrients and supporting indicators are available for free flowing rivers and streams. DEQ just received (July 2012) the aforementioned report from the agency's Academic Advisory Committee entitled, A "Screening Approach" for Nutrient Criteria in Virginia. That report is now under review to see if the findings have relevance to the development of a methodology for assessing whether or not a "nuisance" aquatic plant condition exists in a waterbody.

Appendix 1

Publications Enclosed with Comments from the

Center for Biological Diversity

Limnol. Oceanogr., 57(3), 2012, 698–710
© 2012, by the Association for the Sciences of Limnology and Oceanography, Inc.
doi:10.4319/lo.2012.57.3.0698

The Pacific oyster, *Crassostrea gigas*, shows negative correlation to naturally elevated carbon dioxide levels: Implications for near-term ocean acidification effects

Alan Barton,[a] Burke Hales,[b,*] George G. Waldbusser,[b] Chris Langdon,[c] and Richard A. Feely[d]

[a] Pacific Coast Shellfish Grower's Association, Emerald Isle, North Carolina
[b] College of Oceanic and Atmospheric Sciences, Oregon State University, Corvallis, Oregon
[c] Department of Fisheries and Wildlife and Coastal Marine Experiment Station, Oregon State University, Newport, Oregon
[d] Pacific Marine Environmental Laboratory, National Oceanic and Atmospheric Administration, Seattle, Washington

Abstract

We report results from an oyster hatchery on the Oregon coast, where intake waters experienced variable carbonate chemistry (aragonite saturation state < 0.8 to > 3.2; pH < 7.6 to > 8.2) in the early summer of 2009. Both larval production and midstage growth ($\sim$ 120 to $\sim$ 150 μm) of the oyster *Crassostrea gigas* were significantly negatively correlated with the aragonite saturation state of waters in which larval oysters were spawned and reared for the first 48 h of life. The effects of the initial spawning conditions did not have a significant effect on early-stage growth (growth from D-hinge stage to $\sim$ 120 μm), suggesting a delayed effect of water chemistry on larval development.

Rising atmospheric carbon dioxide (CO_2) driven by anthropogenic emissions has resulted in the addition of over 140 Pg-C (1 Pg = 10^{15} g) to the ocean (Sabine et al. 2011). The thermodynamics of the reactions between carbon dioxide and water require this addition to cause a decline of ocean *p*H and carbonate ion concentrations ($[CO_3^=]$). For the observed change between current-day and preindustrial atmospheric CO_2, the surface oceans have lost approximately 16% of their $[CO_3^=]$ and decreased in pH by 0.1 unit, although colder surface waters are likely to have experienced a greater effect (Feely et al. 2009). Projections for the open ocean suggest that wide areas, particularly at high latitudes, could reach low enough $[CO_3^=]$ levels that dissolution of biogenic carbonate minerals is thermodynamically favored by the end of the century (Feely et al. 2009; Steinacher et al. 2009), with implications for commercially significant higher trophic levels (Aydin et al. 2005).

There is considerable spatial and temporal variability in ocean carbonate chemistry, and there is evidence that these natural variations affect marine biota, with ecological assemblages next to cold-seep high-CO_2 sources having been shown to be distinct from those nearby but less affected by the elevated CO_2 levels (Hall-Spencer et al. 2008). Coastal environments that are subject to upwelling events also experience exposure to elevated CO_2 conditions where deep water enriched by additions of respiratory CO_2 is brought up from depth to the nearshore surface by physical processes. Feely et al. (2008) showed that upwelling on the Pacific coast of central North America markedly increased corrosiveness for calcium carbonate minerals in surface nearshore waters. A small but significant amount of anthropogenic CO_2 present in the upwelled source waters provided enough additional CO_2 to cause widespread corrosiveness on the continental shelves (Feely et al. 2008). Because the source water for upwelling on the North American Pacific coast takes on the order of decades to transit from the point of subduction to the upwelling locales (Feely et al. 2008), this anthropogenic CO_2 was added to the water under a substantially lower-CO_2 atmosphere than today's, and water already en route to this location is likely carrying an increasing burden of anthropogenic CO_2. Understanding the effects of natural variations in CO_2 in these waters on the local fauna is critical for anticipating how more persistently corrosive conditions will affect marine ecosystems.

The responses of organisms to rising CO_2 are potentially numerous and include negative effects on respiration, motility, and fertility (Portner 2008). From a geochemical perspective, however, the easiest process to understand conceptually is that of solid calcium carbonate ($CaCO_{3,s}$) mineral formation. In nearly all ocean surface waters, formation of $CaCO_{3,s}$ is thermodynamically favored by the abundance of the reactants, dissolved calcium ($[Ca^{2+}]$), and carbonate ($[CO_3^=]$) ions. While oceanic $[Ca^{2+}]$ is relatively constant at high levels that are well described by conservative relationships with salinity, ocean $[CO_3^=]$ decreases as atmospheric CO_2 rises, lowering the energetic favorability of $CaCO_{3,s}$ formation. This energetic favorability is proportional to the saturation state, Ω, defined by

$$\Omega_f = \frac{[CO_3^=][Ca^{2+}]}{K_{sp,f}}$$

where the subscript f refers to the phase of the mineral being formed and $K_{sp,f}$ is the apparent thermodynamic solubility product of that phase. Precipitation is thermodynamically possible for $\Omega_f > 1$, and greater values of Ω_f correspond to greater energetic favorability for precipitation. Calcium carbonate exists in a variety of phases distinguished by crystal structure and associated contributions of contaminaxting elements. The most common phases are the low-magnesium phases calcite and aragonite and high-magnesium calcite. Aragonite is about twice as

* Corresponding author: bhales@coas.oregonstate.edu

soluble as calcite, and the solubility of high-Mg calcites increases with increased magnesium in the crystal lattice (Morse and Mackenzie 1990). Aragonite, in particular, plays an important role in the calcareous structures produced by corals, pteropods, and early life stages of larval oysters.

The $CaCO_3$ shell-forming bivalves, including oysters (Gazeau et al. 2007; Miller et al. 2009; Waldbusser et al. 2011), mussels (Gazeau et al. 2007; Thomsen et al. 2010), and clams (Green et al. 2009; Talmage and Gobler 2009; Waldbusser et al. 2010), show negative responses to lowered Ω values, even when those perturbations occur above the $\Omega = 1$ thermodynamic threshold for the minerals in question. Larval oysters appear to be particularly susceptible to the influences of ambient seawater chemistry, as they form their larval shell material out of the more soluble aragonite (Stenzel 1964) and only deposit less soluble calcite following settlement. Additionally, some studies indicate amorphous calcium carbonate (ACC) as an even more soluble mineral precursor (Weiss et al. 2002) during early stages of calcification, although other studies question the ubiquity of this finding (Mount et al. 2004; Kudo et al. 2010).

Regardless of exact calcification mechanisms, previous studies have shown that oyster larvae respond negatively to more acidic conditions. Kurihara et al. (2007) found a reduction in development success when *Crassostrea gigas* larvae were exposed to acidified conditions (pH = 7.4). Watson et al. (2009) exposed D-hinge (1-d-old) larvae of the Sydney rock oyster (*Saccostrea glomerata*) to a range of pH conditions (7.6–8.1) over a period of 10 d and showed a significant decline in the survival and growth of young larvae at lower pH. In a study starting with 5-d-old larvae, Miller et al. (2009) also showed a significant decrease in growth rate of *C. virginica* when exposed to lower pH conditions but relatively little effect on survival. Reduced larval growth of *C. virginica* in response to reduced pH was also found by Talmage and Gobler (2009), with negative effects on survival and metamorphosis also observed. Studies on adult bivalves have shown that net calcification was possible under more acidified conditions (Gazeau et al. 2007), and the ability to overcome dissolution increased with postlarval size (Waldbusser et al. 2010). Mineral polymorph, high energetic costs of early life history development, and the generally high mortality rates in larvae (Rumrill 1990) all suggest larvae will be the most susceptible developmental stage of marine bivalves.

On the North American Pacific coast, native populations of the oyster *Ostrea lurida* were unable to sustain extensive harvesting (White et al. 2009), and the commercial oyster industry there is supported primarily by cultivation of the nonnative *C. gigas*, which was introduced near the beginning of the 20th century but has limited naturally sustaining populations (Ruesink et al. 2005) because of cold in situ temperatures limiting reproduction of this species and low residence times of water and, consequently, planktonic larvae in many local estuaries (Banas et al. 2007). Therefore, the commercial oyster industry is dependent on hatcheries that rear larvae to settlement size before distributing them to the growers. In recent years, natural and hatchery larval production have been severely depressed in the Pacific Northwest, and a lack of sufficient "seed" has threatened an industry with a total economic value estimated at US$278 million as of 2009 (Pacific Coast Shellfish Growers Association 2010). Hatchery problems started in 2006, when high concentrations of the pathenogenic bacterium *Vibrio tubiashii* were observed, perhaps associated with high-nutrient, low-O_2 coastal upwelled waters (Grantham et al. 2004; Chan et al. 2008) mixing with warm late-summer bay waters (Elston et al. 2008). The Whiskey Creek Shellfish Hatchery in Netarts Bay on the northern Oregon coast (~ 45.4°N, 123.9°W) experienced high concentrations of *V. tubiashii* and difficulties in seed production (Elston et al. 2008). However, by the summer of 2008, bacterial monitoring indicated that pronounced mortality events often occurred in the absence of detectable *V. tubiashii*. On the other hand, these larval mortality events often coincided with strong coastal upwelling and the presence of seawater thermodynamically unstable with respect to aragonite in Netarts Bay.

Although the effects of ocean acidification have been forecast for nearly 30 yr (Feely and Chen 1982), the issue has only recently received community-wide interest with workshops (Fabry et al. 2009) and strategy documents (e.g., National Research Council 2010) completed in the last few years, along with development of large-scale international integrated projects (http://www.epoca-project.eu) and locally integrated observation networks (http://c-can.msi.ucsb.edu). As a result, the chemical signature and ecological effects of acidification have been under way long before adequate scientific monitoring and classical experimentation could sufficiently be put in place to resolve either the baseline or the mechanisms of ecosystem responses. Because of this known information deficiency, workshop reports (Fabry et al. 2009) have strongly recommended that the scientific community engage affected stakeholders and attempt to mine nontraditional data sources for relevant information.

In this study, we follow this recommendation by augmenting existing monitoring measurements with state-of-the-art carbonate chemistry validation to evaluate the response of *C. gigas* larvae, grown under otherwise optimal conditions at an affected commercial hatchery on the Oregon coast, to natural changes in carbonate chemistry associated with periodic seasonal upwelling during the summer of 2009.

Methods

Whiskey Creek Hatchery (WCH), a commercial shellfish hatchery and largest independent producer of oyster seed in the Pacific Northwest, is located in Netarts Bay, a small bay on the northern Oregon coast (Fig. 1). Netarts Bay is a lagoon-type estuary dominated by water inputs from the adjacent North Pacific Ocean, with equivalent mean depth and tidal amplitude (~ 2 m) and only minimal freshwater inputs via two small creeks that enter the east and south edges of the bay. The hatchery is situated on the eastern edge of the bay, about halfway between its northern and southern extents. Seawater for larval rearing is drawn

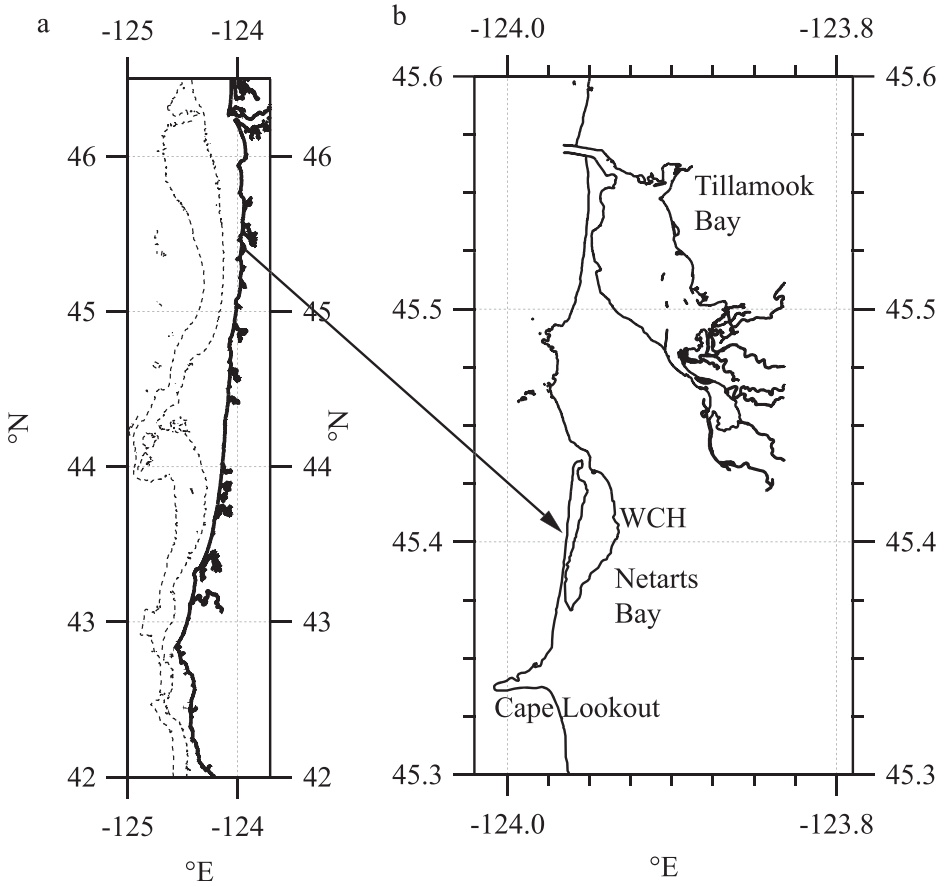


Fig. 1. (a) Map of Oregon coast from California border to the mouth of the Columbia River. Dotted contours offshore show the position of the 100- and 200-m isobaths. (b) Expanded view of Netarts Bay, where "WCH" denotes the position of the Whiskey Creek Hatchery.

directly from the bay through a submerged intake pipe about 0.5 m above the bottom at an average water depth of about 2 m. Temperature is maintained at either ~ 18°C (for broodstock) or ~ 25°C (for fertilization and larval rearing) within the hatchery, while salinity varies with natural fluctuations, in this case over a range of ~ 28–33.

The oyster production cycle within the hatchery follows general procedures for oyster hatchery production. Broodstock are obtained from sustaining native populations in Willapa Bay, Washington, oyster reserve or from the Molluscan Broodstock Program (http://hmsc.oregonstate.edu/projects/mbp/index.html) throughout the production cycle. After they are brought to the hatchery, they are kept in tanks flushed with ambient Netarts Bay seawater maintained at 18°C and fed with an excess supply of algae (produced in the hatchery) for a minimum of 2 wk to maximize fitness and fecundity while preventing natural spawning by not allowing temperatures to rise above 18°C. Oysters are strip spawned by sacrificing several individual males and females that are visually confirmed to be in peak fitness and fertility. Spawns are performed two to four times each week throughout the growing season, producing cohorts of 2×10^8–2×10^9 larvae per spawn.

Harvested eggs are screened to remove debris and thoroughly rinsed on a 20-μm screen. Clean eggs are resuspended in 0.1-m^3 tanks of filtered seawater, typically at concentrations of 10^{10} m^{-3}, then fertilized with a sperm suspension. Within 30–60 min, eggs are examined microscopically to insure proper fertilization and then placed into 22-m^3 tanks of seawater at concentrations not exceeding 2×10^7 m^{-3}. This represents one cohort of oyster larvae.

After 48 h, D-hinge larvae are collected during tank water change and transferred to 22-m^3 tanks of new seawater pumped from the bay; larvae are stocked initially at a concentration of 5×10^7 m^{-3}. Small larvae are fed a diet of the haptophyte *Isochrysis galbani* at cell densities of ~ 5×10^{10} m^{-3} until 7–10 days after fertilization. For the next few weeks, larvae are fed a mixed diet of diatoms (primarily *Chaetoceros gracilis* and *Thalassiosira* sp. fed at cell densities of ~ 7×10^{10} m^{-3}), and water in the holding tanks is replaced completely every 48 h. Larvae are typically large enough to be retained on 100-μm sieves (nominally 140–150 μm in long-axis shell length) within 1–2 wk after fertilization.

Although we do not discuss later development here, the hatchery produces and rears larvae until roughly 12–24 h before settlement, when they are packaged and shipped to commercial oyster growers across the Pacific Northwest. Rearing of successful cohorts continues for an additional 7–14 d beyond the times described above, at which point

larvae are harvested on 236-μm sieves (310–330-μm shell length) and distributed to growers. Low-production cohorts are not maintained beyond the 150-μm size threshold.

Hatchery operators have found that certain optimal ratios of biomass per volume are ideal for growth and feeding in various settings. In order to balance production rates and expenses associated with heating and maintain water, the hatchery maintains a near-constant tank-specific biomass by condensing or dividing cohorts into or across tanks as required. The hatchery uses a volumetric cone to estimate tank biomass and adjust the number of tanks as needed at each tank change. We can therefore utilize the data collected on the total number of tanks per cohort as a measure of larval biomass production. The change in the number of tanks is thus a proxy for the net production of a cohort of larvae. Relative larval production, P, was calculated from the relative change in the number of tanks between the initial number of tanks at the postfertilization D-hinge stage to the number of tanks when larvae are retained on 100-μm screens, that is, $P = (T_{100} - T_D)/T_D$, where T_{100} and T_D are the numbers of tanks at the 100-μm screen and D-hinge stages of production, respectively. A value of 0 in this case would imply no larval biomass production and could be attained by either limited growth and high survival or high growth and low survival, while a value of 1 would imply a doubling of larval biomass, and a value of -1 would imply complete mortality.

Hatchery personnel estimate larval development and growth by various metrics. Early development is tracked by estimating the number of fertilized eggs that develop to healthy D-hinged larvae as the ratio of healthy D-hinged larvae over successfully fertilized eggs. Growth is tracked by logging the number of days it takes for components of a cohort to reach benchmark sizes based on direct observations and sieves used to capture larvae during regular tank changes. Specifically, the number of days until the first observation of 120-μm larvae and the number of days until all surviving larvae reach 150 μm are cataloged. We utilized these records to estimate early-stage growth and midstage growth related to important transitions in larval feeding and development stages. Early-stage growth was defined as the time required until the first observation of individuals in a cohort reaching a shell length of 120 μm. Up to this size stage, sizes are obtained by microscopic analyses of subsamples from the cohort. Importantly, this metric potentially captures the dynamics of the fittest individuals in the cohort; however, the D-hinge developed integrates the entire early development. We defined midstage growth by calculating the time between first observation of larvae at 120-μm shell length and the time at which all surviving larvae are retained on a 100-μm screen (nominally 150-μm shell length). Larval nutrition in early-stage growth is reported to be mixotrophic with larvae depending on both egg reserves and limited ingestion of microalgae (Rico-Villa et al. 2009; Kheder et al. 2010*a,b*). In midstage growth, larvae become more dependent on exogenous food sources, and changes in diet or food availability have more pronounced effects on growth (Rico-Villa et al. 2009; Kheder et al. 2010*a,b*).

Water samples were collected from the hatchery seawater system weekly in the morning ($\sim$ 08:00 h local time) and afternoon (14:00 h) in addition to a few other times selected by the hatchery personnel. This sampling point was separated from the bay by a flow transit time of a few seconds and coincides with the location in the hatchery where the tanks are filled. Samples for analysis of seawater CO_2 partial pressure (P_{CO2}) and total dissolved CO_2 (T_{CO2}) were collected in 350-mL amber glass bottles with minimal headspace and poisoned with 300 μL of saturated $HgCl_2$ solution before being sealed with urethane-lined crimp-seal metal caps. We have shown in previous research (Hales et al. 2005; Bandstra et al. 2006) that samples collected in this manner are stable for several months or more for CO_2 analyses. Samples were analyzed for T_{CO2} following the method developed in Hales's lab by Bandstra et al. (2006) and for P_{CO2} by recirculating headspace air through the sample and a nondispersive infrared detector using a small air pump and a fritted bubbling stone. Results were corrected for the pressure of analysis headspace and for the temperature difference between analytical and in situ conditions to yield P_{CO2} values in dekapascals rather than the nearly numerically equivalent and more widely used μatm unit at in situ temperature (T) and T_{CO2} values in μmol kg^{-1}. Uncertainty for the T_{CO2} analyses is expected to be less than 0.2% (accuracy and precision), based on the results of Bandstra et al. (2006), and less than 5% for the P_{CO2} analyses, based on comparison between archived samples and measurements made in situ by a variety of methods (membrane and showerhead equilibration of flowing sample streams and moored measurement of P_{CO2} using absorbance-based sensors; Evans et al. 2011).

Inlet water aragonite saturation state (Ω_A) was calculated from the T, salinity (S), and P_{CO2} and T_{CO2} data at in situ conditions using a program written by the authors that accounts for the equilibrium interactions between the carbonic acid species at in situ conditions, using the carbonic acid dissociation constants of Mehrbach et al. (1973) as refit by Lueker et al. (2000), the boric acid constants as defined by Dickson (1990), and the aragonite solubility as defined by Mucci (1983). This choice of constants is appropriate for freshwater and salinities above 20 but is unverified for the range 0–20 and should be used with caution in lower salinity ranges where verification is insufficient. Calcium concentrations were calculated assuming conservative dependence on salinity with a freshwater end member of 1 mmol kg^{-1}, appropriate for local riverine inputs. From these measurements and choice of equilibrium constants, we can calculate pH on the total hydrogen ion scale, hereafter referred to as pH_T. Propagation of analytical uncertainty in the measured P_{CO2} and TCO_2 data (stated above) dominates the uncertainty in calculated Ω_A, which is about 5%, while calculated pH_T is uncertain by about $\pm$ 0.02. We do not consider inaccuracy in the thermodynamic constants used in our calculations. The most significant of these is the inaccuracy of the solubility of aragonite, which, as reported in the literature is uncertain by about $\pm$ 10%,. Absolute values of Ω_A and pH_T will suffer additional accuracy uncertainty if the equilibrium constants are considered, but relative changes in either term are known to within the above-stated propagation of analytical precision. Comparison of these

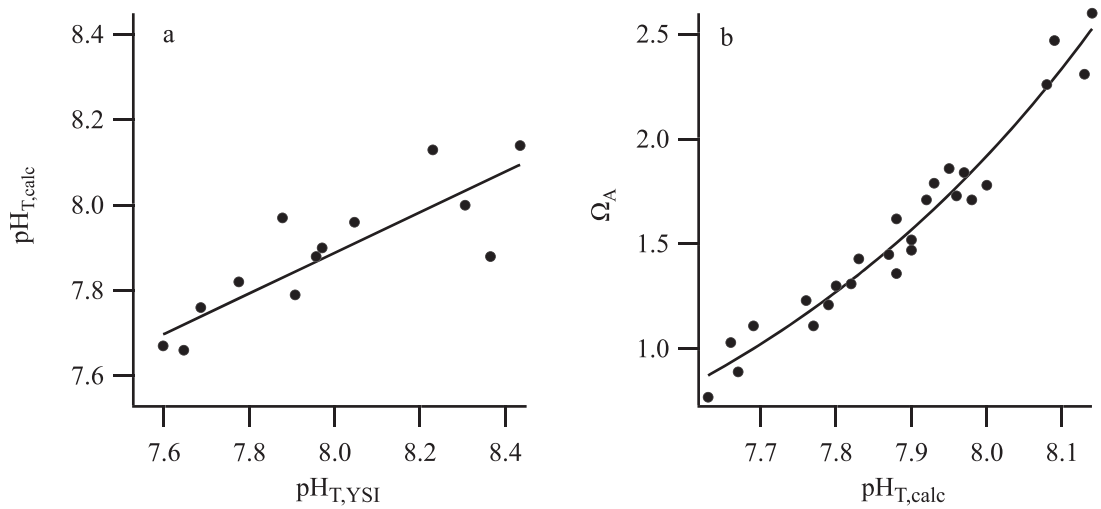


Fig. 2. (a) Correlation between $p\mathrm{H}_{\mathrm{T,calc}}$ calculated from $\mathrm{P}_{\mathrm{CO2}}$ and $\mathrm{T}_{\mathrm{CO2}}$ measured on discrete samples and $\mathrm{pH}_{\mathrm{T,YSI}}$, corrected to the total hydrogen ion scale from the NBS-scale pH measured with the YSI 6000 EDS sonde, for those samples with good T, S agreement. $\mathrm{pH}_{\mathrm{T}} = 0.45\mathrm{pH}_{\mathrm{T,YSI}} + 4.22$, $F_{1,12} = 26.80$, $p = 0.0002$, $R^2 = 0.70$. (b) Relationship between aragonite saturation state (Ω_{A}) and $\mathrm{pH}_{\mathrm{T,calc}}$ for all discrete samples ($\Omega_{\mathrm{A}} = -0.288 + 0.9274e^{(1.7345\Delta\mathrm{pHt})}$, $F_{2,23} = 248.84$, pseudo-$R^2 = 0.96$, $p < 0.0001$, where $\Delta\mathrm{pH}_{\mathrm{T}} = \mathrm{pH}_{\mathrm{T}} - 7.5$ and pseudo-$R^2 = 1 -$ [residual sums of squares divided by the total corrected sums of squares], appropriate for nonlinear regressions).

results with those of other studies will require careful examination of acid dissociation and mineral solubility assumptions.

It is important to note that the data presented here are for the conditions at the hatchery inlet sampling point. We maintain this for two reasons. Part of our objective is quantification of the natural variation in conditions in ambient bay waters, and direct measurements were not made in the water after heating. Treatment of the water approximates isochemical heating, in which alkalinity and $\mathrm{T}_{\mathrm{CO2}}$ do not change because of the quiescent conditions and short time scale of the heating and the slow response of CO_2 to gas exchange. If waters were only warmed, calculated Ω_{A} values would be about 0.2 higher in warmed waters than at the intake at in situ T, and thus the responses we report would take place at more stable mineral solubility conditions. The most likely chemical change would be relaxation of the warmed waters to $\mathrm{P}_{\mathrm{CO2}}$ values in equilibrium with the atmosphere while alkalinity remained constant. This would have two consequences. Warmed waters would mostly lose CO_2 to the atmosphere, resulting in potentially higher mean Ω_{A} values, and moving toward a common $\mathrm{P}_{\mathrm{CO2}}$ would tend to reduce the dynamic range in Ω_{A}. Therefore, our measurements, if anything, underestimate the mean Ω_{A} and overestimate its dynamic range in fertilization and rearing waters. We believe that this choice of Ω_{A} values is the most conservative in implicating ocean acidification effects on larval oysters.

The high variability of the bay-water chemistry and the frequency of the spawning operations made the approximately weekly discrete samples alone insufficient for capturing the conditions experienced during spawning operations, and we were forced to use these discrete samples to ground-truth a continuous record of pH being recorded by an in situ Yellow Springs Instruments (YSI) 6000 EDS sonde, located in the bay at the inlet of the hatchery intake pipe, starting in late May 2009. Along with pH, the sonde also recorded temperature and salinity at 30-s intervals. The sonde was recovered monthly for cleaning and calibrated for pH using Oakton buffers (http://www.4oakton.com) at pH 7 and 10 on the National Bureau of Standards (NBS) scale. One calibration operation occurred in the middle of the presented record on 01 July 2009 and two others preceding and following the sampling interval. No drift was obvious during these calibrations, and no drift correction was applied.

As there can be decoupling between pH and mineral saturation state, pH is an imperfect variable for assessing corrosivity of ocean water to calcium carbonate biominerals. This is especially true in a complicated matrix like seawater where ionic strength effects can lead to large uncertainties in the determination of pH itself. We made two corrections to the in situ pH record. We first corrected the NBS-scale YSI measurements to the total hydrogen ion scale using the nonlinear empirical relationship between the total hydrogen ion activity coefficient and temperature and salinity given by Millero et al. (1988). The corrections made to adjust to the pH_{T} scale were on the order of 0.13 unit, with dependence on the T and S of the sample water. We then synchronized our discrete measurements with the continuous in situ record and used our calculated pH_{T} values to verify the accuracy of the in situ measurements. We limited our analyses only to times when the measured discrete sample T and S agreed with the synchronized in situ observations to within $\pm$ 0.5°C and 0.75, respectively, to ensure comparisons of common water types. The discrete and in situ T and S measurements were quite closely correlated ($R^2 > 0.98$ for both parameters), but periodic deviations occurred, as expected in a dynamic setting such as this. The linear relationship between in situ and discrete-sample calculated pH (Fig. 2a) was used to convert the continuous in situ record into one that was

internally consistent with our CO_2 chemical analyses ($pH_{T,calc} = 0.45pH_{T,YSI} + 4.22$, $F_{1,12} = 26.80$, $p = 0.0002$, $R^2 = 0.70$). This correction is large, with slope < 1 and offset > 0. This is not unexpected, as the YSI electrode is advertised to produce accuracy of only $\pm$ 0.2 unit, although precision is 0.01 unit (http://www.ysi.com/productsdetail.php?6600V2-1). If this inaccuracy exists as nonlinearity in relatively narrow pH ranges ($\sim$ 0.6 in this case) or as function of T and/or S fluctuations, none of which are specified by the manufacturer, it could account for the majority of this disagreement. However, the average residual difference between predicted pH_T from our regression analysis and measured pH_T is 0.06 ($\pm$ 0.06, 1 SD), with a maximum residual value of 0.19; thus, for our purposes, this is adequate.

As stated, pH is not expected to be a perfect predictor for mineral saturation state, but the correlation between pH_T and Ω_A from our discrete samples (Fig. 2b) was strong enough to give good predictive power ($\Omega_A = -0.288 + 0.927e^{(1.735\Delta pHt)}$, $F_{2,23} = 248.84$, pseudo-$R^2 = 0.96$, $p < 0.0001$, where $\Delta pH_T = pH_T - 7.5$ and pseudo-$R^2 = 1 -$ [residual sums of squares divided by the total corrected sums of squares], appropriate for nonlinear regressions). After correcting the in situ pH with the relationship shown in Fig. 2a, we then converted that record to a continuous time series of Ω_A with the relationship shown in Fig. 2b. Conditions for individual spawns were determined by averaging the Ω_A record between 0:800 h and 12:00 h local time on the day of the spawn, corresponding to the interval in which spawn tanks were filled.

A simple linear regression analysis was used to quantify the effect of aragonite saturation state at time of spawn on the proportion of healthy D-hinge, relative production value (both described above), the early-stage growth rate (up to 120-μm shell length), and the midstage growth rate (120–150-μm shell length). The proportional D-hinge data were arcsin square-root transformed because of the problems associated with parametric analysis of proportional data. Assumptions of heteroscedasticity and normality of residuals were checked with visual examination of residuals and by the Shapiro–Wilk statistic, and these assumptions were met. We additionally checked for overly influential data points by examining Studentized residuals and Cook's distance and found one observation that corresponded to an overly influential data point in two of the three regressions. We censored each point and repeated the statistical analyses but have included the point on subsequent graphs. The exclusion of this point did not change the inferences from either analysis. All analyses were run in SAS v9.1, using Proc Reg for linear regressions and Proc Nlin for the nonlinear regression.

Results

Netarts Bay carbonate chemistry—Water sampling and continuous analysis for the period 01 June–03 August 2009 (Figs. 3, 4) show that the bay is subjected to two major forcings that affect its carbonate chemistry. The first is the upwelling state of the adjacent coastal ocean. During upwelling, evidenced by high atmospheric pressure and strong north winds (Fig. 3a), colder, saltier (Fig. 3b), low-pH (Fig. 3c) aragonite-undersaturated (Fig. 3d) deep-origin upwelled water is brought to the surface very near the coast (Hales et al. 2005; Feely et al. 2008), and this water spills over the sill at the bay mouth into the bay. During reduced upwelling or relaxation events, low-CO_2 photosynthetically modified (high pH, high Ω_A) surface waters are forced back onshore and enter the bay. The time scale of this forcing ranges from several days, corresponding to the frequency of relaxation events during the upwelling season, to several months, corresponding to the seasonal shift between winter downwelling- and summer upwelling-favorable winds. During the record of interest in 2009, there were periods of strong upwelling from about year-day 161–186 (10 June–05 July) and again from about year-day 195–212 (14–31 July), punctuated by periods of weak variable to south winds from year-day 153–161 (02–10 June), 187–194 (06–13 July), and 213–217 (01–05 August).

The second forcing factor is diurnal metabolic variability within the bay. This is visible as the high-frequency patterns superimposed on the smoothed records of Fig. 3, and one part of the record is examined in more detail in Fig. 4. The bay contains broad shallows populated by extensive eelgrass beds and benthic microalgae, and on high-insolation days, photosynthetic uptake of CO_2 from bay waters is high. In contrast, nighttime respiration produces abundant CO_2. This results in a clear pattern of high P_{CO2} and T_{CO2} in the morning discrete samples, with greatly reduced levels in the afternoon samples (Fig. 4), that drives the diurnal signal in pH_T and Ω_A (e.g., increasing values during the day and decreasing values through the night). Note that thermal forcing alone would predict higher afternoon P_{CO2}, opposite of what is seen. This effect occurs independent of tidal forcing and is apparent in both upwelling and relaxation (or reversal) oceanic conditions. Unlike the upwelling-modulated changes, the bay's carbonate chemistry is decoupled from its temperature and salinity in these intervals.

Oyster larval performance—Larval performance was assessed by four measures derived from hatchery records: early development to D-hinge, the relative production, early-stage growth when larvae depend on mixotrophic nutrition, and midstage when larvae transition to exogenous food sources. Successful development from fertilized egg to D-hinge is measured by the proportion of fertilized eggs that make it to D-hinge in $\sim$ 24 h. The relative production term provides a proxy for cohort biomass, with positive numbers indicating growth in cohort biomass and negative numbers indicating mortality. Our early- and midstage growth metrics estimate the time required to reach and exceed a benchmark size. Aragonite saturation state at the time of spawning did not have significant effects on D-hinge development ($F_{1,14} = 3.97$, $p = 0.0663$, adjusted $R^2 = 0.17$; Fig. 5a) and early-stage growth ($F_{1,15} = 0.00$, $p = 0.9845$, adjusted $R^2 < 0$; Fig. 5b). However significant effects of carbonate conditions at time of spawn were found on midstage growth ($F_{1,14} = 13.87$, $p = 0.0025$, adjusted $R^2 = 0.47$; Fig. 5c) and relative production ($F_{1,15} = 17.93$, $p = 0.0008$, adjusted $R^2 = 0.53$; Fig. 5d). Differing numbers of

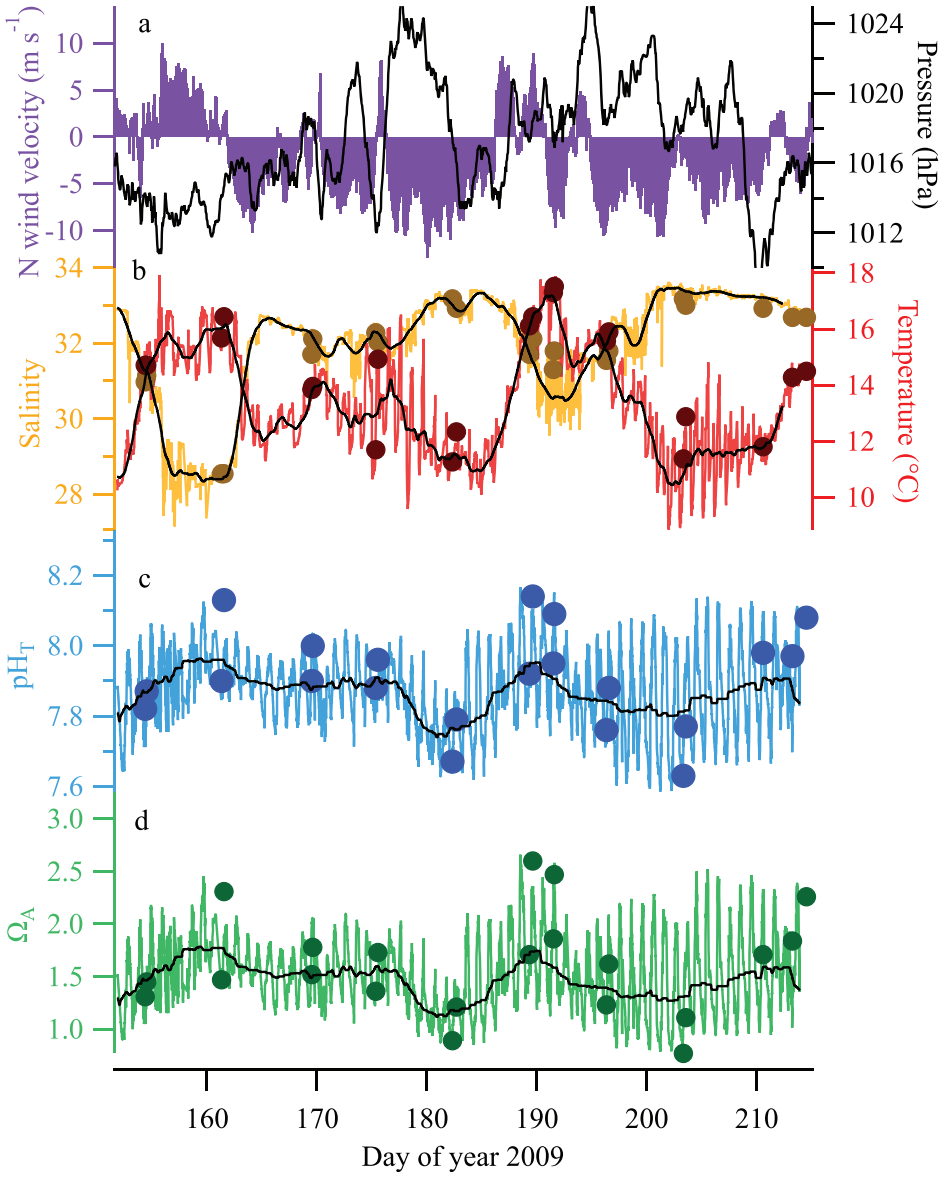


Fig. 3. Variability in environmental conditions in Netarts Bay, 01 June–03 August 2009. (a) Record of north wind velocity (left axis) and atmospheric pressure (right axis), showing the atmospheric conditions driving upwelling variability over the period of interest. (b) Record of salinity (left axis) and temperature (right axis) at the hatchery intake. Black lines are 3-day running-median smoothed representations of each record, distinguishing the upwelling forcing from the diurnal forcing. Similarly colored circles are corrected discrete measurements of each parameter, corresponding to the discrete samples analyzed for CO_2 chemistry. (c) pH_T from the corrected in situ measurements made by the YSI sonde at the hatchery intake (blue line) and calculated from the discrete samples analyzed for P_{CO2} and T_{CO2} (blue circles). Black line is the 3-day filtered pH_T record, as for T and S, above. (d) Aragonite saturation (Ω_A), calculated from the corrected YSI pH_T data and the regression of Fig. 2b (green line) and from the discrete measurements of P_{CO2} and T_{CO2} (green circles).

degrees of freedom result from either missing data points or one data point in each of the midgrowth and relative production regressions having been censored due to the finding that each point was overly influential by Studentized residuals, Cook's distance, and DFFITS diagnostics. These censored data points have, however, been included in the plots of Fig. 5 as filled circles.

Discussion

This work differs from previous studies of calcifying organism responses to changing acid-base chemistry in several key ways. First, we utilized hatchery records of larval performance under naturally fluctuating ambient-water CO_2 chemistry of Oregon's coastal upwelling system

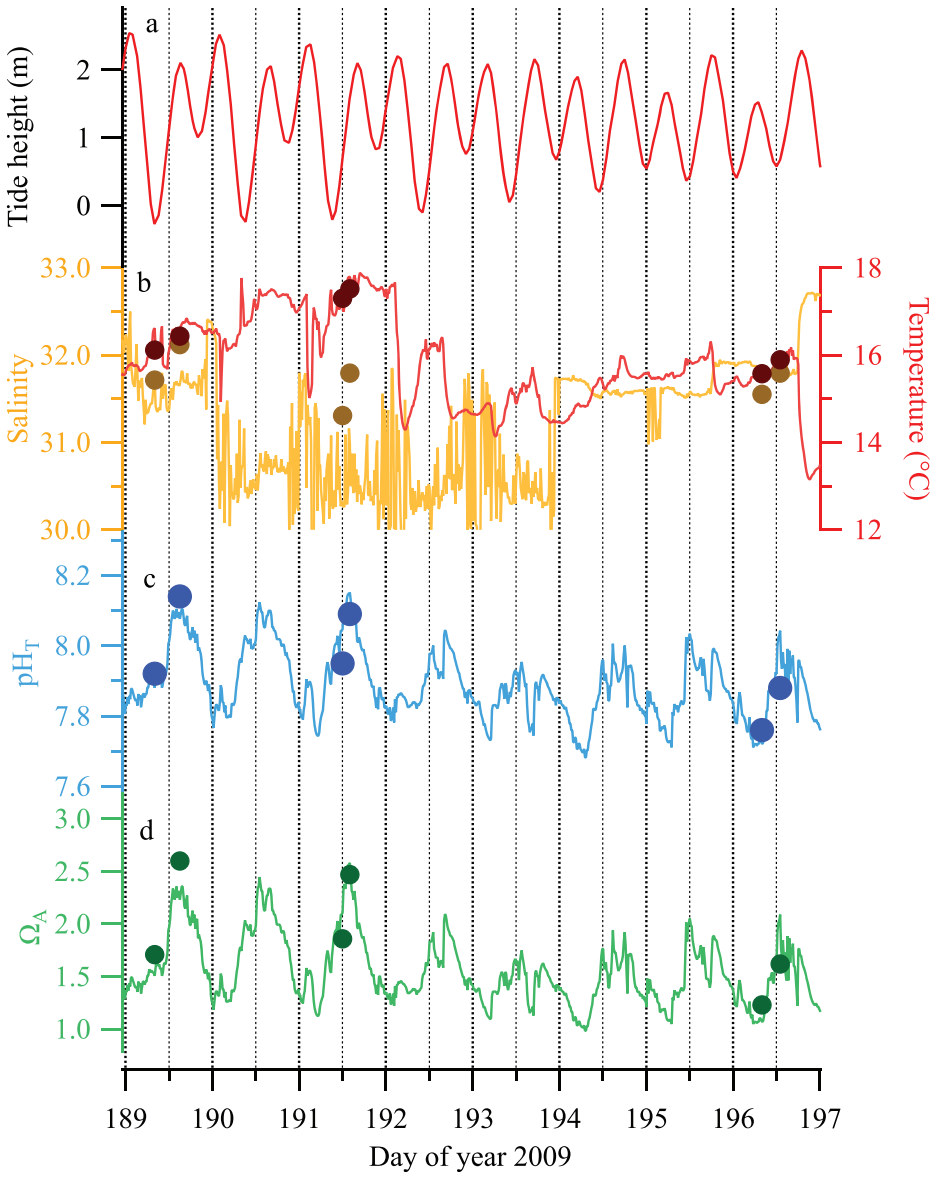


Fig. 4. Demonstration of effect of diurnal variability on Netarts Bay carbonate chemistry for a subset of the data presented in Fig. 3, 08–17 July 2009. (a) Tidal stage is largely uncorrelated with (b) in-bay T and S or (c) pH or (d) aragonite saturation. Bay carbonate chemistry shows regular diurnal variability, with maximum pH_T and Ω_A at or shortly after local noon and minima in early morning. Vertical dashed lines represent local midnight (major ticks) and noon (minor ticks).

with *C. gigas*, a nonnative study organism that has been resident in these waters for nearly a century. No artificial manipulations of water chemistry outside of those occurring naturally were conducted. Second, we have focused on the larval stages of several cohorts grown under commercial hatchery conditions and protocols. Finally, we have resolved the vagaries of in situ pH electrode time-series data with discrete measures of seawater carbonate chemistry analyzed with state-of-the-art methodology. With this novel approach, we have observed significant relationships between aragonite saturation states at the time of spawning and subsequent growth and production of larvae over the size range of ~ 120 to ~ 150-μm shell length (Fig. 5) but not on early growth and development.

Recent work by A. Hettinger (unpubl.) on the native *O. lurida* showed carryover effects of water chemistry experienced at early stage (larvae) to the later postlarval juvenile stage. The carryover effect was evident after 1 mo of growth when juveniles exposed to acidified conditions in their larval stages failed to attain the same size as juveniles derived from larvae exposed to less acidic conditions. Our findings and those of A. Hettinger (unpubl.) illustrate important concepts of how variable acidification may affect coastal and estuarine ecosystems. The timing of spawning

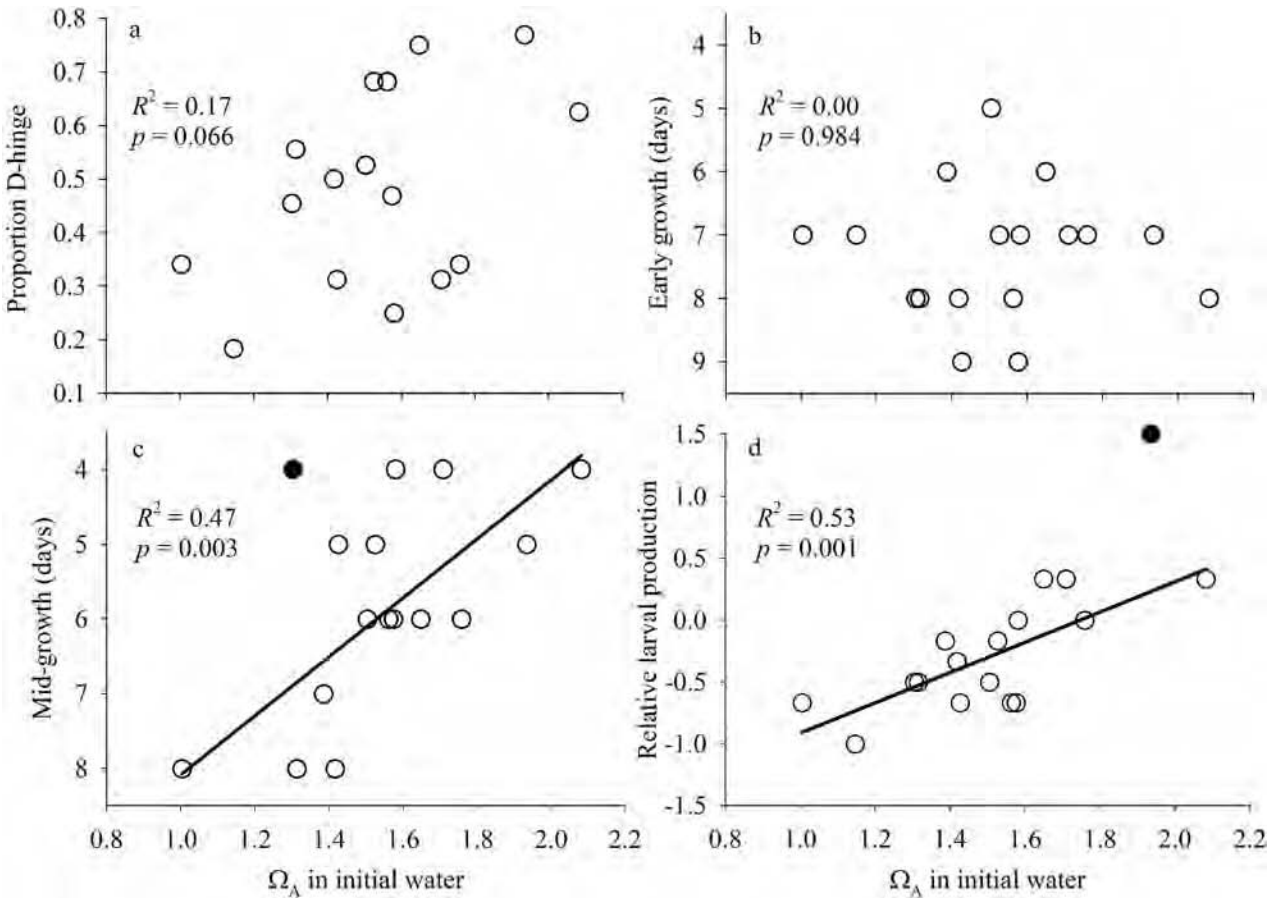


Fig. 5. Relationship between saturation state of aragonite and (a) proportion of larvae developing to D-hinge stage, (b) number of days for larvae to reach a nominal 120-μm size, (c) number of days for larvae to grow from 120- to 150-μm nominal size, and (d) overall relative production of each cohort. As described in the Methods section, relative production does not include the changes in the cohort prior to D-hinge; relative production captures only the changes from the D-hinge stage up to competent to settle. Data points in black on graphs 5c and 5d are statistical outliers and were excluded from regression analysis. Reduced R^2 values and p-values of linear regression analyses are shown in the figure. Other statistics for significant relationships are (c) midstage, growth slope = -3.95 ± 1.05 days, intercept = 12.02 ± 1.67 days and (d) relative production, slope = 1.22 ± 0.29 days, intercept = -2.13 ± 0.44 days.

and release of gametes into the water column are critical in light of variable local conditions occurring on top of a shifting CO_2 chemistry baseline (Waldbusser et al. 2010, 2011). Others have demonstrated the importance of environmental conditions related to climate and weather patterns in controlling interannual oyster recruitment (Kimmel and Newell 2007). Our results further highlight the ecological significance of windows of opportunity for successful recruitment that may be likened to match–mismatch theory (Cushing 1990). However, it is important to note that in the current study, we have evaluated hatchery production and therefore minimized the typically extreme mortality of larval oysters in the wild by growing them under otherwise ideal conditions.

Long-term records of recruitment of Pacific oysters in Willapa Bay, Washington, have previously shown multiple year failures in recruitment (Dumbauld et al. 2011). Recent Pacific Northwest failures in natural recruitment have also corresponded with diminished oyster seed production at WCH (S. Cudd pers. comm.). Within a hatchery setting, oysters are spawned frequently (every few days at WCH over several months) and cohorts distinct, whereas natural populations typically have fewer cohorts per season that are often variable in timing and generally result in an integrated cohort over the spawning season. Within the hatchery, temperature and food concentrations are all held at near-constant and optimal levels, and broodstock are optimized for reproduction, while these conditions vary significantly in natural waters during the spawning season. Additionally, Rumrill (1990) noted that fertilization failure, adverse hydrography, substrate limitation, and predation are the most important determinants of the success of natural populations of meroplankton, such as larval oysters. Recent biochemical studies of oyster larvae indicate the importance of food quality and quantity in conjunction with sensitivity to fluctuating salinity and temperature (Hofmann et al. 2004). Increased food availability and quality generally improved tolerance of *C. gigas* larvae to salinity and temperature variability in these modeling studies. If we view the less favorable aragonite formation energetics associated with high-CO_2 upwelling conditions as sublethal acute stress and recognize

that early development is energetically expensive, as is shell construction (Palmer 1992), then the moderate acidification we measured can be considered a stress that requires additional energy to overcome (Portner 2008).

Many marine bivalve species share similar developmental stages from egg fertilization to metamorphosed juveniles, although the timing and typical sizes at which these stages occur will vary both within and among species. Recent studies suggest that early *C. gigas* larvae up to about 120 microns in shell length are mixotrophic, with egg yolk reserves providing a major source of nutrition (Rico-Villa et al. 2009; Kheder et al. 2010*a,b*). In our analyses of production and growth, there was no significant Ω_A effect (over the natural range of conditions) during early egg development on growth through this early stage (Fig. 5a,b); however, production was ultimately affected (Fig. 5d). This finding suggests that within-cohort variability may allow some individuals to continue to grow without incident while reaching our initial early-growth benchmark (Fig. 5b), while others cannot survive, leading to increased mortality and ultimately decreased cohort production (Fig. 5d). It is important to note that the relative production term uses the change in number of tanks from the D-hinge stage to competent to settle and therefore does not overlap with the proportion of D-hinge value. Growth was, however, significantly affected for midstage larvae that depend primarily on exogenous food sources (Fig. 5c). These findings suggest that the effects of acidification during egg development may have carried over (A. Hettinger unpubl.) to affect the growth and survival of midstage larvae. The nature of this putative carryover effect is unclear but could be due to the cumulative effects of stress on later larval development, metabolism or feeding physiology. Further research on the effects of ocean acidification on larval development and physiology is needed.

The fact that the bay itself experiences large fluctuations in carbonate chemistry driven by primarily natural mechanisms provides an opportunity for adaptive strategies to be implemented by resource managers such as hatchery operators. In the case of Netarts Bay, WCH operators have already implemented a strategy to avoid early morning tank-filling operations during strong upwelling conditions and as a result have restored a significant amount of lost production. Nevertheless, climatic shifts that are likely to move the mean state toward higher-CO_2, more corrosive conditions are a cause for concern (Feely et al. 2009; Rykaczewski and Dunne 2010). Feely et al. (2008) estimated that the most recent exposure of upwelled source waters off the US west coast was a few decades ago, yet the waters were still influenced by addition of anthropogenic CO_2 from a 1960s-vintage atmosphere. If correct, this means that waters already in transit to the upwelling locations have been exposed to more recent, higher-CO_2 atmospheres, and increasingly corrosive upwelled water is inevitable in coming years. Likewise, the intensity and persistence of upwelling that has accompanied hypoxic events (Grantham et al. 2004; Chan et al. 2008) off the Oregon coast has been hypothesized as a recurring feature of a warming climate (Barth et al. 2007; McGregor et al. 2007). Enhanced upwelling will carry increased corrosivity of shelf waters, much as it drives increased hypoxia. In each case, the fluctuations in local CO_2 chemistry will be superimposed on a trend of increasing corrosivity, meaning that the conditions favorable for larval oyster production will have lower frequency of occurrence and shorter persistence. Even if commercial shellfish producers are able to predict favorable conditions, the windows of opportunity for these adaptive strategies to be implemented will be diminished.

Two significant shortcomings exist with regard to understanding acidification effects on natural populations of organisms in variable coastal and estuarine habitats: prediction of how carbonate conditions will vary in coastal and estuarine environments with increasing atmospheric CO_2 and a better understanding of the fundamental biology underlying the responses of multicellular organisms to acidification. The first of these will be addressed by development of better predictive capability (Juranek et al. 2009) such that stakeholders may be able to plan production operations for the most favorable conditions. Our limited experience suggests that the multitude of forcing time scales still requires high-resolution monitoring of water CO_2 chemistry before we are fully capable of developing predictive models.

The second can be addressed only by more focused experimental work on physiological mechanisms of early larval response in conditions with tightly controlled carbonate chemistry. Our findings in the hatchery setting corroborate the laboratory-based experiments of Kurihara et al. (2007) illustrating the sensitivity of the very early developmental stages to ambient chemistry, although the hatchery larvae in this study experienced a much narrower range of carbonate chemistry. The descriptive biology of oyster larvae has been well documented; however, the dynamic responses of initial shell formation to environmental signals is still poorly understood. Initial shell is thought to be formed between the periostracum and the shell gland and thus "protected" from ambient conditions. How protected this initial shell material is, the energetics of this initial shell formation, and physiological plasticity are still poorly constrained. Numerous studies have now illustrated a pattern of negative response to acidification for many marine species, including bivalves (Kurihara et al. 2007; Miller et al. 2009; Watson et al. 2009), and many more will likely follow. In almost all cases, it has been shown that mollusks tend to respond negatively to increasing corrosiveness of water. However, only a handful of studies have addressed the underlying physiological and ecological mechanisms of these responses to develop a clear and well-founded argument for how other related organisms will respond (Wood et al. 2008; Thomsen et al. 2010; Ries 2011). Although shell mineralogy is a possible first-order explanation for the responses of calcifying organisms to acidification (Cooley and Doney 2009), it is clear that many other factors will determine susceptibility of biogenic minerals to dissolution (e.g., Glover and Kidwell 1993), and calcifying organisms may respond in less predictable ways (Ries et al. 2009). Although the history of studies of biomineralization spans several decades, aspects of the mechanisms of shell formation are still poorly understood, in particular during the very early life stages of mollusks. Some

recent work questions our current understanding of biomineralization (Mount et al. 2004), and we are far from understanding the processes underlying the patterns of response.

The fact that multiple parameters in the CO_2 chemistry system (pH, P_{CO2}, Ω_A, and so on) all change in response to perturbations complicates this understanding, as there are very likely differential effects of these variables on different physiological processes. Experimental methods that can elucidate these mechanisms will be important. Finally, the ability to predict the acute and chronic sensitivities of organisms to high-CO_2 waters should be a goal of future research. There has been some suggestion that larval and juvenile organisms need to reach critical development states where they can compensate for the deleterious effects of elevated CO_2 levels (Green et al. 2009; Waldbusser et al. 2010), and it may be that *C. gigas* larvae are especially sensitive to chemical conditions at the time of spawning. This may require development of acute and chronic threshold definitions, assessment of the sensitivity of organisms to the persistence of conditions, and stage-based models of organism responses.

The current study illustrates the value of monitoring efforts, which, if applied with stakeholder interests at hand, can provide valuable economic feedback (as is the case with WCH). Our study, relying on hatchery records and utilizing their production model, does potentially suffer from the lack of strict control as found in other more constrained experimental systems. We therefore acknowledge the correlative and suggestive nature of this study; however, it highlights the significance of current-day variable carbonate chemistry effects on commercially important species (and reliant regional economy) in surface waters and validates previous laboratory-based acidification experiments in which carbonate chemistry was manipulated. The significant effects on hatchery-based oyster production indicates that local and regional acidification effects are already on us, and responses of these coupled natural human systems to increasing CO_2 are likely unfavorable.

Acknowledgments

We thank Sue Cudd and Mark Wiegert, owners and operators of the Whiskey Creek Hatchery, for their assistance with developing a sampling program at the hatchery and for sharing their operational and production data with us. This work was supported by the Pacific Shellfish Institute, through efforts led by Andrew Suhrbier, and the Pacific Coast Shellfish Growers Association, through efforts led by Robin Downey. Conversations with Alan Trimble were greatly instructive in building our understanding of the local shellfish environment and ecology. National Science Foundation grant OCE-1041267 supported G.G.W., and the National Oceanic and Atmospheric Administration Ocean Acidification Program supported R.A.F. in preparation of this manuscript. Pacific Marine Environmental Laboratory contribution number 3720. Two anonymous reviewers provided thoughtful comments that greatly improved the final manuscript.

References

Aydin, K. Y., G. A. McFarlane, J. R. King, B. A. Megreya, and K. W. Myers. 2005. Linking oceanic food webs to coastal production and growth rates of Pacific salmon (*Oncorhynchus* spp.), using models on three scales. Deep-Sea Res. II **52:** 757–780, doi:10.1016/j.dsr2.2004.12.017

Banas, N. S., B. M. Hickey, J. A. Newton, and J. L. Ruesink. 2007. Tidal exchange, bivalve grazing, and patterns of primary production in Willapa Bay, Washington, USA. Mar. Ecol. Prog. Ser. **341:** 123–129, doi:10.3354/meps341123

Bandstra, L., B. Hales, and T. Takahashi. 2006. High-frequency measurement of seawater total carbon dioxide. Mar. Chem. **100:** 24–38, doi:10.1016/j.marchem.2005.10.009

Barth, J. A., and others. 2007. Delayed upwelling alters nearshore coastal ocean ecosystems in the northern California current. Proc. Natl. Acad. Sci. **10:** 3719–3724, doi:10.1073/pnas.0700462104

Chan, F., J. A. Barth, J. Lubchenco, A. Kirincich, H. Weeks, W. T. Peterson, and B. A. Menge. 2008. Emergence of anoxia in the California current large marine ecosystem. Science **319:** 920, doi:10.1126/science.1149016

Cooley, S. R., and S. C. Doney. 2009. Anticipating ocean acidification's economic consequences for commercial fisheries. Environ. Res. Lett. **4:** 024007, doi:10.1088/1748-9326/4/2/024007

Cushing, D. H. 1990. Plankton production and year-class strength in fish populations—an update of the match-mismatch hypothesis. Adv. Mar. Biol. **26:** 249–293, doi:10.1016/S0065-2881(08)60202-3

Dickson, A. G. 1990. Thermodynamics of the dissociation of boric acid in synthetic seawater from 273.15 to 298.15 K. Deep-Sea Res. **37:** 755–766.

Dumbauld, B. R., B. E. Kauffman, A. Trimble, and J. L. Ruesink. 2011. The Willapa Bay oyster reserves in Washington state: Fishery collapse, creating a sustainable replacement, and the potential for habitat conservation and restoration. J. Shellfish Res. **30:** 71–83, doi:10.2983/035.030.0111

Elston, R. A., H. Hasegawa, K. L. Humphrey, I. K. Polyak, and C. C. Hase. 2008. Re-emergence of *Vibrio tubiashii* in bivalve shellfish aquaculture: Severity, environmental drivers, geographic extent and management. Dis. Aquat. Org. **82:** 119–134, doi:10.3354/dao01982

Evans, W., B. Hales, and P. Strutton. 2011. The seasonal cycle of surface ocean pCO_2 on the Oregon shelf, J. Geophys. Res. Oceans **116:** C05012, doi:10.1029/2010JC006625

Fabry, V. J., and others. 2009. Present and future impacts of ocean acidification on marine ecosystems and biogeochemical cycles. Report of the Ocean Carbon and Biogeochemistry Scoping Workshop on Ocean Acidification Research (UCSD, Scripps Institution of Oceanography; 9–11 October 2007).

Feely, R. A., and C.-T. A. Chen. 1982. The effect of excess CO_2 on the calculated calcite and aragonite saturation horizons in the northeast Pacific Ocean. Geophys. Res. Lett. **9:** 1294–1297, doi:10.1029/GL009i011p01294

———, S. C. Doney, and S. R. Cooley. 2009. Ocean acidification: Present conditions and future changes in a high-CO_2 world. Oceanography **22:** 36–47, doi:10.5670/oceanog.2009.95

———, C. L. Sabine, J. M. Hernandez-Ayon, D. Ianson, and B. Hales. 2008. Evidence for upwelling of corrosive "acidified" water onto the continental shelf. Science **320:** 1490–1492, doi:10.1126/science.1155676

Gazeau, F., C. Quiblier, J. M. Jansen, J. P. Gattuso, J. J. Middelburg, and C. H. R. Heip. 2007. Impact of elevated CO_2 on shellfish calcification. Geophys. Res. Lett. **34:** L07603, doi:10.1029/2006GL028554

Glover, C. P., and S. M. Kidwell. 1993. Influence of organic matrix on the postmortem destruction of molluscan shells. J. Geol. **101:** 729–747, doi:10.1086/648271

Grantham, B. A., and others. 2004. Upwelling-driven nearshore hypoxia signals ecosystem and oceanographic changes in the northeast Pacific. Nature **429:** 749–754, doi:10.1038/nature02605

Green, M. A., G. G. Waldbusser, S. L. Reilly, K. Emerson, and S. O'Donnell. 2009. Death by dissolution: Sediment saturation state as a mortality factor for juvenile bivalves. Limnol. Oceanogr. **54:** 1037–1047, doi:10.4319/lo.2009.54.4.1037

Hales, B., T. Takahashi, and L. Bandstra. 2005. Atmospheric CO_2 uptake by a coastal upwelling system Glob. Biogeochem. Cycles **19:** GB1009, doi:10.1029/2004GB002295

Hall-Spencer, J. M., and others. 2008. Volcanic carbon dioxide vents show ecosystem effects of ocean acidification. Nature **454:** 96–99, doi:10.1038/nature07051

Hofmann, E. E., E. N. Powell, E. A. Bochenek, and J. A. Klinck. 2004. A modelling study of the influence of environment and food supply on survival of *Crassostrea gigas* larvae. ICES J. Mar. Sci. **61:** 596–616, doi:10.1016/j.icesjms.2004.03.029

Juranek, L. W., and others. 2009. A novel method for determination of aragonite saturation state on the continental shelf of central Oregon using multi-parameter relationships with hydrographic data. Geophys. Res. Lett. **37:** L01601, doi:10.1029/2009GL040423

Kheder, R. B., C. Quere, J. Moal, and R. Robert. 2010*a*. Effect of nutrition on *Crassostrea gigas* larval development and the evolution of physiological indices. Part A: Quantitative and qualitative diet effects. Aquaculture **305:** 165–173, doi:10.1016/j.aquaculture.2010.04.022

———, ———, ———, and ———. 2010*b*. Effect of nutrition on *Crassostrea gigas* larval development and the evolution of physiological indices. Part B: Effects of temporary food deprivation. Aquaculture **308:** 174–182, doi:10.1016/j.aquaculture.2010.08.030

Kimmel, D. G., and R. I. E. Newell. 2007. The influence of climate variation on eastern oyster (*Crassostrea virginica*) juvenile abundance in Chesapeake Bay. Limnol. Oceanogr. **52:** 959–965, doi:10.4319/lo.2007.52.3.0959

Kudo, M., J. Kameda, K. Saruwatari, N. Ozaki, K. Okano, H. Nagasawa, and T. Kogure. 2010. Microtexture of larval shell of oyster, *Crassostrea nippona*: A FIB-TEM study. J. Struct. Biol. **169:** 1–5, doi:10.1016/j.jsb.2009.07.014

Kurihara, H., S. Kato, and A. Ishimatsu. 2007. Effects of increased seawater pCO_2 on early development of the oyster *Crassostrea gigas*. Aquat. Biol. **1:** 91–98, doi:10.3354/ab00009

Lueker, T. J., A. G. Dickson, and C. D. Keeling. 2000. Ocean pCO_2 calculated from dissolved inorganic carbon, alkalinity, and equations for $K1$ and $K2$: Validation based on laboratory measurements of CO_2 in gas and seawater at equilibrium. Mar. Chem. **70:** 105–119, doi:10.1016/S0304-4203(00)00022-0

McGregor, H. V., M. Dima, H. W. Fischer, and S. Mulitza. 2007. Rapid 20th-century increase in coastal upwelling off northwest Africa. Science **315:** 637–639, doi:10.1126/science.1134839

Mehrbach, C., C. H. Culberson, J. E. Hawley, and R. M. Pytkowicz. 1973. Measurement of the apparent dissociation constants of carbonic acid in seawater at atmospheric pressure. Limnol. Oceanogr. **18:** 897–907, doi:10.4319/lo.1973.18.6.0897

Miller, A. W., A. C. Reynolds, C. Sobrino, and G. F. Riedel. 2009. Shellfish face uncertain future in high CO_2 world: Influence of acidification on oyster larvae calcification and growth in estuaries. PLoS ONE **4:** e5661, doi:10.1371/journal.pone.0005661

Millero, F. J., T. Plese, and M. Fernandez. 1988. The dissociation of hydrogen sulfide in seawater. Limnol. Oceanogr. **33:** 269–274, doi:10.4319/lo.1988.33.2.0269

Morse, J. W., and F. T. Mackenzie. 1990. Geochemistry of sedimentary carbonates. Developments in Sedimentology 48. Elsevier.

Mount, A. S., A. P. Wheeler, R. P. Paradkar, and D. Snider. 2004. Hemocyte-mediated shell mineralization in the eastern oyster. Science **304:** 297–300, doi:10.1126/science.1090506

Mucci, A. 1983. The solubility of calcite and aragonite in seawater at various salinities, temperatures, and 1 atmosphere total pressure. Am. J. Sci. **238:** 780–799, doi:10.2475/ajs.283.7.780

National Research Council. 2010. Ocean acidification: A national strategy to meet the challenges of a changing ocean. National Academies Press.

Pacific Coast Shellfish Growers Association. 2010. Shellfish production on the west coast. Available from http://www.pcsga.org/pub/farming/production_stats.pdf

Palmer, A. R. 1992. Calcification in marine mollusks—how costly is it? Proc. Natl. Acad. Sci. **89:** 1379–1382, doi:10.1073/pnas.89.4.1379

Portner, H. O. 2008. Ecosystem effects of ocean acidification in times of ocean warming: A physiologist's view. Mar. Ecol. Prog. Ser. **373:** 203–217, doi:10.3354/meps07768

Rico-Villa, B., S. Pouvreau, and R. Robert. 2009. Influence of food density and temperature on ingestion, growth and settlement of Pacific oyster larvae, *Crassostrea gigas*. Aquaculture **287:** 395–401, doi:10.1016/j.aquaculture.2008.10.054

Ries, J. B. 2011. A physiochemical framework for interpreting the biological calcification response to CO_2-induced ocean acidification. Geochim. Cosmochim. Acta **14:** 4053–4064, doi:10.1016/j.gca.2011.04.025

———, A. L. Cohen, and D. C. McCorkle. 2009. Marine calcifiers exhibit mixed responses to CO_2-induced ocean acidification. Geology **37:** 1131–1134, doi:10.1130/G30210A.1

Ruesink, J. L., H. S. Lenihan, A. C. Trimble, K. W. Heiman, F. Micheli, J. E. Byers, and M. C. Kay. 2005. Introduction of non-native oysters: Ecosystem effects and restoration implications. Annu. Rev. Ecol. Evol. Syst. **36:** 643–689, doi:10.1146/annurev.ecolsys.36.102003.152638

Rumrill, S. S. 1990. Natural mortality of marine invertebrate larvae. Ophelia **32:** 163–198.

Rykaczewski, R. R., and J. P. Dunne. 2010. A measured look at ocean chlorophyll trends. Nature **472:** E5–E6, doi:10.1038/nature09952

Sabine, C. L., R. A. Feely, R. Wanninkhof, T. Takahashi, S. Khatiwala, and G.-H. Park. 2011. The global ocean carbon cycle. p. S100–S108. *In* State of the Climate in 2010, 3. Glob. Oceans. Bull. Am. Meteorol. Soc. **92:** S100–S108, doi:10.1175/1520-0477-92.6.S1

Steinacher, M., F. Joos, T. L. Frolicher, G. K. Plattner, and S. C. Doney. 2009. Imminent ocean acidification in the Arctic projected with the NCAR global coupled carbon cycle-climate model. Biogeosciences **6:** 515–533, doi:10.5194/bg-6-515-2009

Stenzel, H. B. 1964. Oysters: Composition of the larval shell. Science **145:** 155–156, doi:10.1126/science.145.3628.155

Talmage, S. C., and C. J. Gobler. 2009. The effects of elevated carbon dioxide concentrations on the metamorphosis, size, and survival of larval hard clams (*Mercenaria mercenaria*), bay scallops (*Argopecten irradians*), and eastern oysters (*Crassostrea virginica*). Limnol. Oceanogr. **54:** 2072–2080, doi:10.4319/lo.2009.54.6.2072

Thomsen, J., and others. 2010. Calcifying invertebrates succeed in a naturally CO_2-rich coastal habitat but are threatened by high levels of future acidification. Biogeosciences **7:** 3879–3891, doi:10.5194/bg-7-3879-2010

Waldbusser, G. G., H. Bergschneider, and M. A. Green. 2010. Size-dependent pH effect on calcification in post-larval hard clam *Mercenaria* spp. Mar. Ecol. Prog. Ser. **417:** 171–182, doi:10.3354/meps08809

———, E. P. Voigt, H. Bergschneider, M. A. Green, and R. I. E. Newell. 2011. Biocalcification in the eastern oyster (*Crassostrea virginica*) in relation to long-term trends in Chesapeake Bay pH. Estuar. Coasts **34:** 221–231, doi:10.1007/s12237-010-9307-0

Watson, S-A., P. C. Southgate, P. A. Tyler, and L. S. Peck. 2009. Early larval development of the Sydney rock oyster *Saccostrea glomerata* under near-future predictions of CO_2-driven ocean acidification. J. Shellfish Res. **28:** 431–437, doi:10.2983/035.028.0302

Weiss, I. M., N. Tuross, L. Addadi, and S. Weiner. 2002. Mollusc larval shell formation: Amorphous calcium carbonate is a precursor phase for aragonite. J. Exp. Zool. **293:** 478–491, doi:10.1002/jez.90004

White, J., J. L. Ruesink, and A. C. Trimble. 2009. The nearly forgotten oyster: *Ostrea lurida* Carpenter 1864 (Olympia oyster) history and management in Washington State. J. Shellfish Res. **28:** 43–49, doi:10.2983/035.028.0109

Wood, H. L., J. I. Spicer, and S. Widdicombe. 2008. Ocean acidification may increase calcification rates, but at a cost. Proc. R. Soc. B Biol. Sci. **275:** 1767–1773, doi:10.1098/rspb.2008.0343

Associate Editor: John Albert Raven

Received: 17 May 2011
Accepted: 16 January 2012
Amended: 27 January 2012

nature climate change

LETTERS

PUBLISHED ONLINE: 11 DECEMBER 2011 | DOI: 10.1038/NCLIMATE1291

Reduced early life growth and survival in a fish in direct response to increased carbon dioxide

Hannes Baumann, Stephanie C. Talmage and Christopher J. Gobler*

Absorption of anthropogenic carbon dioxide by the world's oceans is causing mankind's 'other CO_2 problem', ocean acidification[1]. Although this process will challenge marine organisms that synthesize calcareous exoskeletons or shells[2–6], it is unclear how it will affect internally calcifying organisms, such as marine fish[7]. Adult fish tolerate short-term exposures to CO_2 levels that exceed those predicted for the next 300 years (~2,000 ppm; ref. 8), but potential effects of increased CO_2 on growth and survival during the early life stages of fish remain poorly understood[7]. Here we show that the exposure of early life stages of a common estuarine fish (*Menidia beryllina*) to CO_2 concentrations expected in the world's oceans later this century caused severely reduced survival and growth rates. When compared with present-day CO_2 levels (~400 ppm), exposure of *M. beryllina* embryos to ~1,000 ppm until one week post-hatch reduced average survival and length by 74% and 18%, respectively. The egg stage was significantly more vulnerable to high CO_2-induced mortality than the post-hatch larval stage. These findings challenge the belief that ocean acidification will not affect fish populations, because even small changes in early life survival can generate large fluctuations in adult-fish abundance[9,10].

Since the industrial revolution, average atmospheric and oceanic CO_2 concentrations have risen by 40% to 393 ppm (ref. 11; in 2011); levels that now far exceed those of the past one million years (180–280 ppm; ref. 12). Current emission scenarios predict that CO_2 concentrations will increase further and reach ~800 ppm during this century and potentially 2,000 ppm by the year 2300 (ref. 8). Apart from accelerating global climate change, another major concern is the absorption of CO_2 by the world's oceans and the resulting decrease in ocean pH, carbonate ion concentration (CO_3^{-2}) and calcium carbonate ($CaCO_3$) saturation state (Ω; refs 1,2,4). Collectively known as ocean acidification, these shifts in marine chemistry will probably alter the phenotypes and hence the fitness of many marine organisms, particularly those with exoskeletons and shells made from calcium carbonate ($CaCO_3$; ref. 3). Experimentally increased CO_2 conditions have been shown to adversely affect many species of foraminifers[13], coccolithophores[14], corals[3], pteropodes[2], bivalves[5,6], crustaceans[15] and echinoderms[16].

In contrast, direct effects of ocean acidification on fish, the world's most important marine resource, are at present assumed to be negligible[4,7,17]. Fish calcify internal (bones, otoliths) rather than external skeletal elements, and as highly mobile vertebrates have evolved effective acid–base and osmoregulatory mechanisms to overcome high metabolic CO_2 levels[4]. The high CO_2 tolerance of juvenile and adult fish has been extensively documented for decades, suggesting no measureable growth or survival effects even at exposures of up to 16,000 ppm CO_2 (ref. 7; eight times the relevant level for future climate change scenarios). However, the susceptibility of the earliest life stages of fish to increased CO_2 levels has not yet been sufficiently examined[7], even though CO_2 sensitivity is highest during the larval stages in most other marine organisms[5,6,16]. Recent studies have documented that increased CO_2 levels (>700 ppm) can have detrimental behavioural effects on larval reef fish by impairing their ability to detect olfactory cues of predators or nearby reefs[18–20]. White sea bass larvae reared at 1,000–2,500 ppm CO_2 were shown to grow abnormally heavy otoliths[21]. Despite such signs of CO_2 sensitivity in larval fish, however, direct CO_2 effects on larval-fish growth or survival have yet to be demonstrated[7,22].

Here we report on a series of controlled CO_2 exposure experiments with embryos of the inland silverside *Menidia beryllina*. This small, schooling fish occurs naturally in estuaries along the North American Atlantic coast, where it is of ecological importance as both a zooplanktivore and prey for higher trophic animals. In addition, *M. beryllina* is commonly used as a model species in environmental risk assessments. In five separate experiments, we exposed a known number of newly fertilized eggs (<24 h old) to replicated CO_2 concentrations ranging from ~390 to 1,100 ppm (Supplementary Tables S1–S5). CO_2 levels were carefully administered and monitored by adhering to established best practices for ocean acidification research (European Project on Ocean Acidification[23]). Approximately one week after hatching, the surviving larvae were counted, photographed and measured for length.

Our results were surprisingly unambiguous. Despite variable control survival rates, each experiment revealed a consistent decline in larval survival with increasing CO_2 concentration (experiment 1, analysis of variance $F_{1,4} = 5.2$, $p = 0.08$; experiments 2–5, four analyses of variance, $F_{1-3,6-12} = 14.5–38.9$, $p < 0.009$; Fig. 1a). When averaged across replicate means, an exponential decay model best fitted the entire data set ($r^2 = 0.80$) and indicated a 74% reduction in average survival at increased (~1,000 ppm) when compared with control levels (~400 ppm).

An alternative interpretation of the patterns in Fig. 1a is that CO_2 sensitivity in *M. beryllina* remained essentially stable until reaching a threshold (650–800 ppm, consistent with refs 18,20), beyond which survival and growth (Fig. 1a,b) sharply declined. Regardless of the functional relationship, *M. beryllina* seems to show a direct link between early life mortality and the levels of oceanic CO_2 predicted for the twenty-first century. Whether this is common among fish is at present unknown. However, the question has important implications for future fish stock abundance, because larval survival mediates the strength of new year classes in fish populations, including those of commercial importance[9,10].

Compounding these concerns, we also observed a consistent decrease in larval length with increasing CO_2 concentration. Average lengths (±1 s.e.m.) of survivors approximately one week post-hatch declined significantly ($F_{1,6} = 30.6$, $p = 0.001$)

School of Marine & Atmospheric Sciences, Stony Brook University, Stony Brook, NY 11794, USA. *e-mail: christopher.gobler@stonybrook.edu.

© 2011 Macmillan Publishers Limited. All rights reserved.

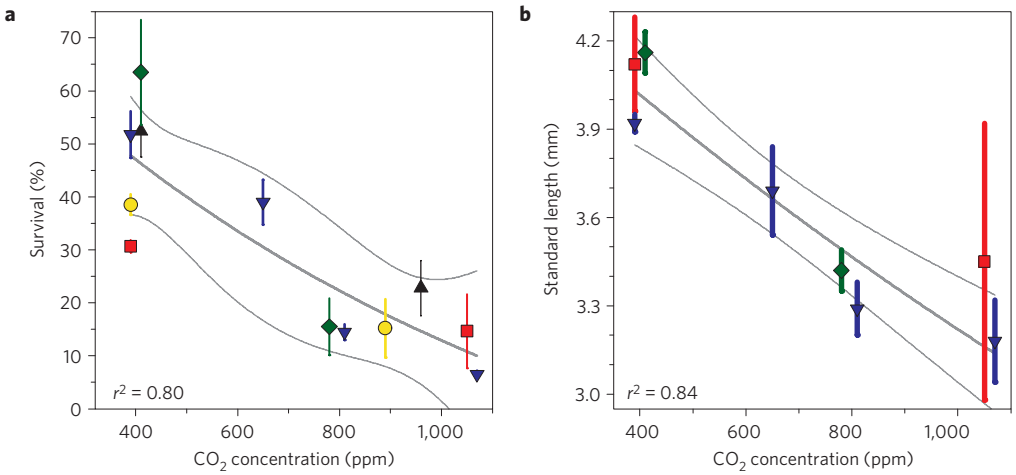


Figure 1 | Effect of increased CO_2 on early life *M. beryllina* survival and length. a, Survival was averaged across replicates (experiment 1, $n = 3$; experiments 2, 3, $n = 4$; experiment 4, $n = 6$; experiment 5, $n = 5$) for each experiment and CO_2 level. **b**, Weighted means (±1 s.e.m.) of standard length averaged across replicates per experiment and CO_2 level. Pooled data in **a** and **b** were fitted with an exponential decay model (thick grey line) with 95% confidence intervals (thin grey lines). Experiment 1, red squares; experiment 2, blue down triangles; experiment 3, green diamonds; experiment 4, yellow circles; experiment 5, black up triangles. Points represent means ± 1 s.d.

by 18% from 4.0 ± 0.07 mm at control levels (400 ppm CO_2) to 3.3 ± 0.3 mm at 1,000 ppm CO_2 (Fig. 1b). In most fish species, larval growth and mortality rates are inversely related, because slower-growing larval cohorts are vulnerable for longer to the suite of ichthyoplankton predators and therefore experience higher cumulative mortalities[24,25]. Reduced early life growth may therefore further reduce the productivity of fish stocks in future acidified oceans.

Precisely how increased CO_2 levels affect survival and growth in fish early life stages is at present unknown. For some fish species, the explanation may involve the high surface-to-volume ratio of eggs and larvae, which may make individuals more vulnerable to diffusive processes across epithelia[26]. The heightened CO_2 sensitivity of the earliest life stages may further reflect poorly developed mechanisms of acid–base regulation and cardiorespiratory control, as both are probably linked to increased gill function and muscle activity due to swimming in later-stage larvae, juveniles and adults[27]. Third, even if fish embryos and early larvae are capable of some level of physiological adaptation to increased CO_2, this would incur further metabolic costs[7] and thus reduce energy available for tissue synthesis (growth reduction) or post-hatch survival on diminished yolk reserves. As some fish eggs, including those of *M. beryllina*, seem to be tolerant of low-pH conditions[28], the high levels of CO_2 or associated changes in carbonate chemistry may be more important to larval-fish survival than hydrogen ion concentrations.

It is possible that the earliest life stages of fish (that is, embryos in eggs) are most susceptible to CO_2. To test this hypothesis, a further experiment was conducted where CO_2 exposure of *M. beryllina* was switched from control (410 ppm) to increased (780 ppm) levels only after eggs had hatched (5 days after fertilization, Fig. 2). Survivorship five days later was then compared with constant-control and constant-increased CO_2 treatments (10 days of 410 or 780 ppm, respectively). As survival in the 'switch' group was only marginally lower than in the constant-control group (t-test, $df = 6, p = 0.5$), but significantly higher than in the constant-increased group (t-test, $df = 6, p < 0.01$), the main CO_2 impact evidently occurred during the egg stage (Fig. 2). This may partly explain why studies so far that have carried out experiments on fish larvae, but not on eggs, have not observed the negative effects of ocean acidification[21,22]. Furthermore, one day post-hatch *M. beryllina* showed a significantly (t-test, $df = 10, p < 0.01$) higher percentage of malformations in increased (37%, 960 ppm) when

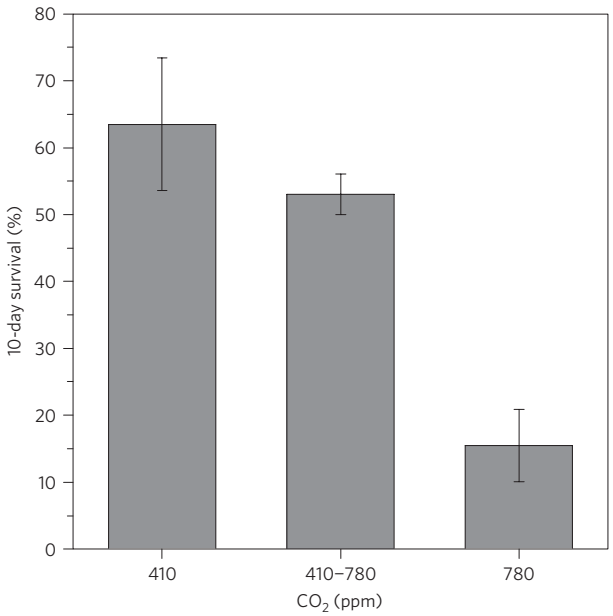


Figure 2 | CO_2 sensitivity of the egg versus early post-hatch stage in *M. beryllina*. Bars depict average survival (±1 s.e.m.) 10 days after fertilization in control (410 ppm), increased (780 ppm) and 'switch', where CO_2 concentration was increased only after eggs hatched (5 days post-fertilization), treatments. Precise CO_2 levels and complete carbonate chemistry from experiments appear in Supplementary Tables S1–S5.

compared with control CO_2 levels (7%, 410 ppm), supporting the particular sensitivity of the egg stage (Fig. 3).

In summary, we present evidence of direct adverse growth and survival effects in the early life stages of fish due to exposure to CO_2 levels that are expected in the world's oceans later this century. Together with other emerging evidence[18–21], such apparently high CO_2 sensitivity contradicts the notion that ocean acidification will have no direct consequences for marine fish populations. Furthermore, the CO_2 levels used in our experiments already occasionally occur in temperate coastal waters, often coinciding with the spawning season of fish[29,30]. Hence, CO_2-induced offspring mortality may already be influencing patterns of adult-fish abundance in the ocean. We

© 2011 Macmillan Publishers Limited. All rights reserved.

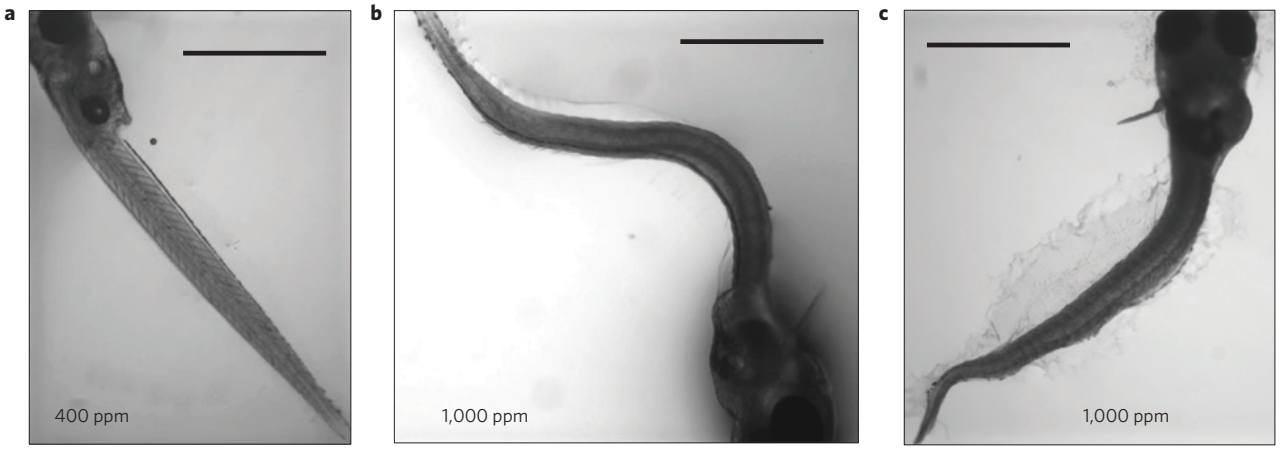


Figure 3 | *M. berylina* larvae exposed to normal and elevated levels of CO_2. a–c, Larvae with curved or curled bodies were significantly more common at increased (**b**,**c**) when compared with control (**a**) CO_2 levels. Scale bar = 1 mm.

expect, however, in a manner similar to what is now emerging from studies on invertebrates[1,3–5], that responses to increased CO_2 levels in fish will be highly species specific. For example, oceanic fish species that spawn pelagic eggs might be more susceptible to CO_2 increases than benthic spawners[22], where eggs may be more adapted to natural CO_2 fluctuations due to elevated rates of microbial respiration.

In light of the broad implications of our findings, we believe that there is now a need to comprehensively investigate not only the incidence, the physiological causes and the form of the functional response of early life CO_2 sensitivity in fish, but also the general potential of marine organisms to adapt to the CO_2 levels projected for future oceans. Our study indicates that future work should focus on the earliest life stages, as the effects of increased CO_2 may be especially acute during this phase of development.

Methods

All five experiments were conducted between July 2010 and January 2011, using <24-h-old *M. beryllina* embryos obtained from a large, commercial brood stock (Aquatic Research Organisms). A known number of eggs (experiments 1–3, $n = 100$; experiments 4, 5, $n = 50$) was randomly placed in each of three to five replicate rearing containers (4 l) per CO_2 level (390–1,060 ppm, Supplementary Tables S1–S5). An air: CO_2 mix, adjusted to desired levels by a gas proportionator system (Cole Parmer Flowmeter), was continuously delivered to each rearing container. Attained CO_2 levels were calculated with the program CO2SYS on the basis of measured total inorganic carbon (EGM-4 Environmental Gas Analyser, PP Systems), pH, temperature and salinity. Fish were reared at constant temperature (24 °C), salinity (30) and photoperiod levels (15L:9D) and were provided with live rotifers and brine shrimp nauplii after hatching. Survival was measured 7 ± 2 days post-hatch as the percentage of live larvae relative to the number of seeded eggs. Our protocols ensured that potential mortality sources unrelated to CO_2, for example due to varying egg quality, transport and handling, minor fluctuations in food and water quality, or the shape of our rearing containers, were random between replicated treatments and experiments. Standard lengths of survivors were measured using calibrated digital pictures and image analysis software (ImagePro 4.5.1, Media Cybernetics). All reported p and F values were derived from analyses of variance to compare survival (arc-sine transformed) and length between CO_2 treatments (PASW Statistics 18). Further details regarding methods are available as Supplementary Methods.

Received 14 January 2011; accepted 19 October 2011; published online 11 December 2011

References

1. Doney, S. C., Fabry, V. J., Feely, R. A. & Kleypas, J. A. Ocean acidification: The other CO_2 problem. *Annu. Rev. Mar. Sci.* **1,** 169–192 (2009).
2. Orr, J. C. *et al.* Anthropogenic ocean acidification over the twenty-first century and its impact on calcifying organisms. *Nature* **437,** 681–686 (2005).
3. Kleypas, J. A. *et al. Impacts of Ocean Acidification on Coral Reefs and Other Marine Calcifiers: A Guide for Future Research* (Report of a workshop held 18–20 April 2005, St. Petersburg, FL, Sponsored by NSF, NOAA, and the US Geological Survey, 2006).
4. Fabry, V. J., Seibel, B. A., Feely, R. A. & Orr, J. C. Impacts of ocean acidification on marine fauna and ecosystem processes. *ICES J. Mar. Res.* **65,** 414–432 (2008).
5. Talmage, S. C. & Gobler, C. J. Effects of past, present, and future ocean carbon dioxide concentrations on the growth and survival of larval shellfish. *Proc. Natl Acad. Sci. USA* **107,** 17246–17251 (2010).
6. Kurihara, H., Kato, S. & Ishimatsu, A. Effects of increased seawater p_{CO_2} on early development of the oyster *Crassostrea gigas*. *Aquat. Biol.* **1,** 91–98 (2007).
7. Ishimatsu, A., Hayashi, M. & Kikkawa, T. Fishes in high-CO_2, acidified oceans. *Mar. Ecol. Prog. Ser.* **373,** 295–302 (2008).
8. Caldeira, K. & Wickett, M. E. Anthropogenic carbon and ocean pH. *Nature* **425,** 365–365 (2003).
9. Sissenwine, M. P. in *Exploitation of Marine Communities* (ed. May, R.) 59–94 (Springer, 1984).
10. Trippel, E. A. & Chambers, R. C. in *Early Life History and Recruitment in Fish Populations* (eds Chambers, R. C. & Trippel, E. A.) xxi–xxxii (Chapman & Hall, 1997).
11. www.esrl.noaa.gov/gmd/ccgg/trends/.
12. Tripati, A. K., Roberts, C. D. & Eagle, R. A. Coupling of CO_2 and ice sheet stability over major climate transitions of the last 20 million years. *Science* **326,** 1394–1397 (2009).
13. Spero, H. J., Bijma, J., Lea, D. W. & Bemis, B. E. Effect of seawater carbonate concentration on foraminiferal carbon and oxygen isotopes. *Nature* **390,** 497–500 (1997).
14. Riebesell, U. *et al.* Reduced calcification of marine plankton in response to increased atmospheric CO_2. *Nature* **407,** 364–367 (2000).
15. Kurihara, H., Matsui, M., Furukawa, H., Hayashi, M. & Ishimatsu, A. Long-term effects of predicted future seawater CO_2 conditions on the survival and growth of the marine shrimp *Palaemon pacificus*. *J. Exp. Mar. Biol. Ecol.* **367,** 41–46 (2008).
16. Dupont, S., Havenhand, J., Thorndyke, W., Peck, L. & Thorndyke, M. C. Near-future level of CO_2-driven ocean acidification radically affects larval survival and development in the brittlestar *Ophiothrix fragilis*. *Mar. Ecol. Prog. Ser.* **373,** 285–294 (2008).
17. Hayashi, M., Kita, J. & Ishimatsu, A. Acid–base responses to lethal aquatic hypercapnia in three marine fishes. *Mar. Biol.* **144,** 153–160 (2004).
18. Munday, P. L. *et al.* Ocean acidification impairs olfactory discrimination and homing ability of a marine fish. *Proc. Natl Acad. Sci. USA* **106,** 1848–1852 (2009).
19. Munday, P. L. *et al.* Replenishment of fish populations is threatened by ocean acidification. *Proc. Natl Acad. Sci. USA* **107,** 12930–12934 (2010).
20. Dixson, D. L., Munday, P. L. & Jones, G. P. Ocean acidification disrupts the innate ability of fish to detect predator olfactory cues. *Ecol. Lett.* **13,** 68–75 (2010).
21. Checkley, D. M. *et al.* Elevated CO_2 enhances otolith growth in young fish. *Science* **324,** 1683–1683 (2009).
22. Munday, P. L., Gagliano, M., Donelson, J. M., Dixson, D. L. & Thorrold, S. R. Ocean acidification does not affect the early life history development of a tropical marine fish. *Mar. Ecol. Prog. Ser.* **423,** 211–221 (2011).
23. Riebesell, U., Fabry, V. J., Hansson, L. & Gattuso, J. P. in *Guide to Best Practices for Ocean Acidification Research and Data Reporting* (Publications Office of the European Union, 2010).
24. Anderson, J. T. A review of size dependent survival during pre-recruit stages of fishes in relation to recruitment. *J. Northw. Atl. Fish. Sci.* **8,** 55–66 (1988).

© 2011 Macmillan Publishers Limited. All rights reserved.

25. Leggett, W. C. & Deblois, E. Recruitment in marine fishes: Is it regulated by starvation and predation in the egg and larval stages? *Neth. J. Sea Res.* **32,** 119–134 (1994).
26. Mangor-Jensen, A. Water balance in developing eggs of the cod *Gadus morhua* L. *Fish Physiol. Biochem.* **3,** 17–24 (1987).
27. Perry, S. F. & Gilmour, K. M. Acid–base balance and CO_2 excretion in fish: Unanswered questions and emerging models. *Respir. Physiol. Neurobiol.* **154,** 199–215 (2006).
28. Hemmer, M. J., Middaugh, D. P. & Moore, J. C. Effects of temperature and salinity on *Menidia beryllina* embryos exposed to terbufos. *Dis. Aquat. Org.* **8,** 127–136 (1990).
29. Feely, R. A., Sabine, C. L., Hernandez-Ayon, J. M., Ianson, D. & Hales, B. Evidence for upwelling of corrosive 'acidified' water onto the continental shelf. *Science* **320,** 1490–1492 (2008).
30. Salisbury, J., Green, M., Hunt, C. & Campbell, J. Coastal acidification by rivers: A threat to shellfish? *EOS Trans. AGU* **89** http://dx.doi.org/10.1029/2008EO500001 (2008).

Acknowledgements

We acknowledge the assistance of L. Merlo, R. Anderson, R. Light and Y. Tang. This work was supported by grants from the New Tamarind Foundation and National Science Foundation Biological Oceanography (no 1129622).

Author contributions

H.B., S.C.T. and C.J.G. designed the experiments, conducted the experiments, generated the data, analysed samples, analysed the data and wrote the manuscript.

Additional information

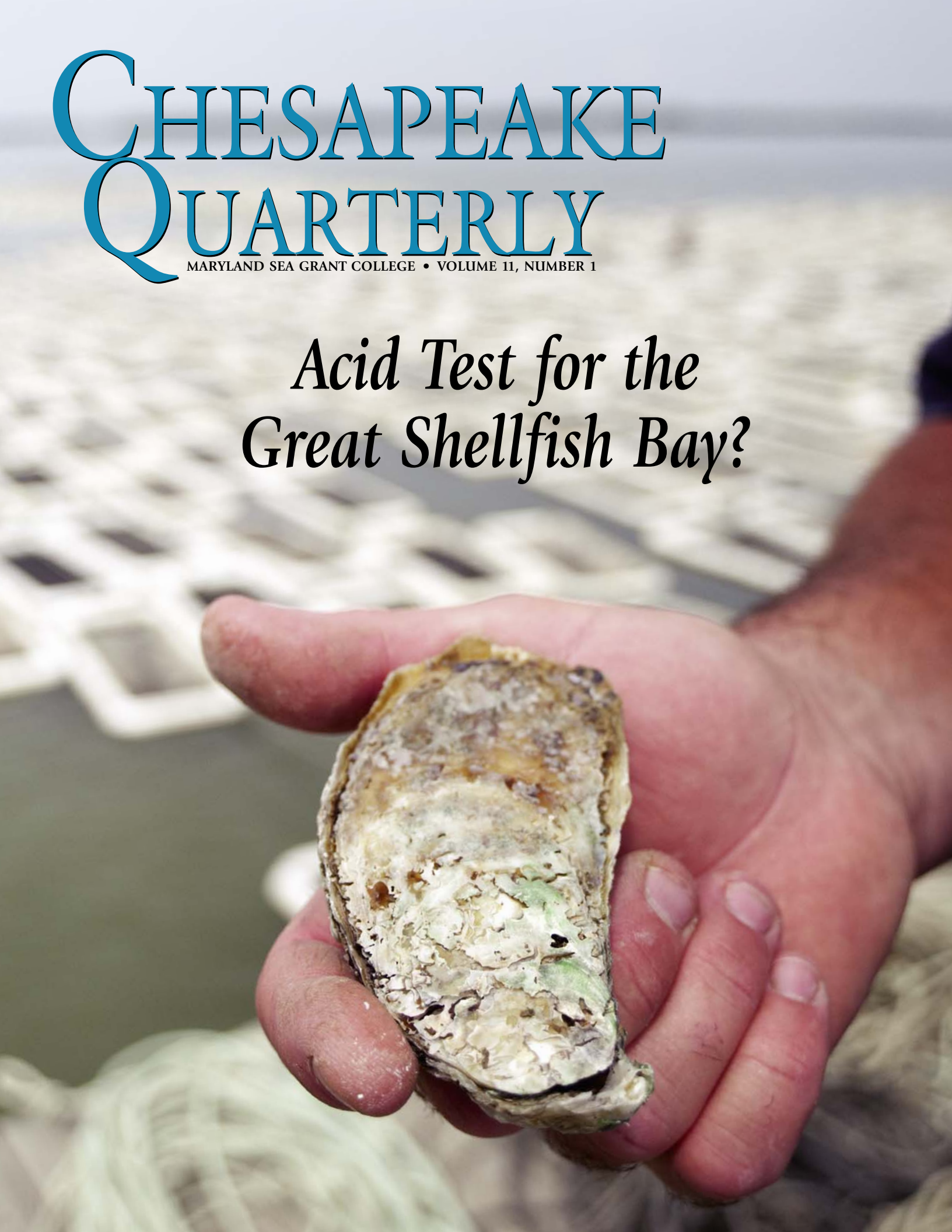
The authors declare no competing financial interests. Supplementary information accompanies this paper on www.nature.com/natureclimatechange. Reprints and permissions information is available online at http://www.nature.com/reprints. Correspondence and requests for materials should be addressed to C.J.G.

© 2011 Macmillan Publishers Limited. All rights reserved.

Chesapeake Quarterly

MARYLAND SEA GRANT COLLEGE • VOLUME 11, NUMBER 1

Acid Test for the Great Shellfish Bay?



contents

Volume 11, Number 1

2 **An Acidifying Estuary?**
As CO_2 changes the ocean, will it also change the Chesapeake?

8 **Shell Game**
Finding answers for acid waters at oyster hatcheries.

13 **Crab vs. Oyster**
It's not the same old story for the Bay's best loved shellfish.

15 **Should We Regulate Acidity in the Bay? If So, How?**
Is the science strong enough to support regulation?

CHESAPEAKE QUARTERLY

March 2012

Chesapeake Quarterly explores scientific, environmental, and cultural issues relevant to the Chesapeake Bay and its watershed.

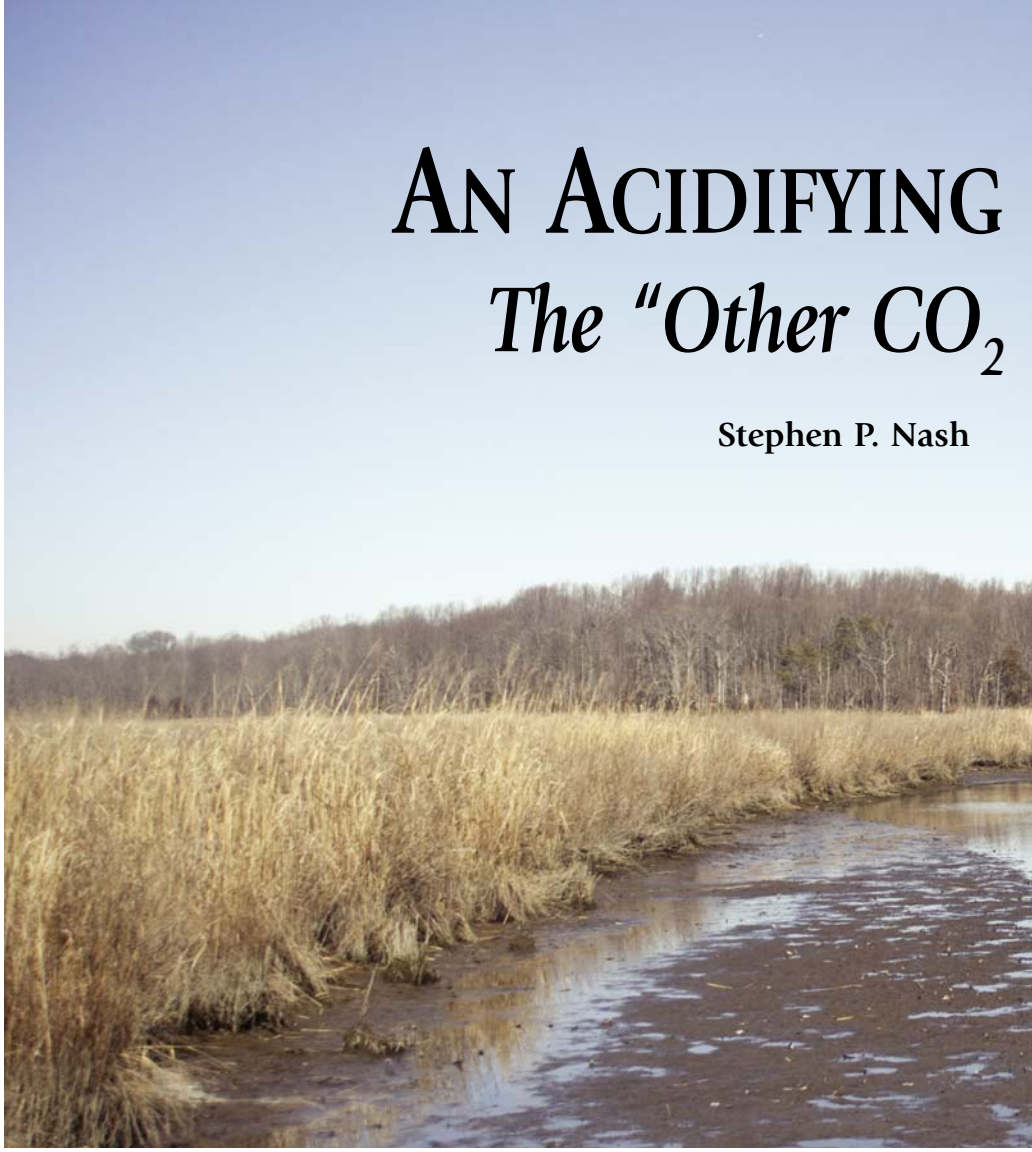
This magazine is produced and funded by the Maryland Sea Grant College Program, which receives support from the National Oceanic and Atmospheric Administration and the state of Maryland. Editors, Michael W. Fincham and Jeffrey Brainard; Production Editor and Art Director, Sandy Rodgers. Send items for the magazine to:

Maryland Sea Grant College
4321 Hartwick Road, Suite 300
University System of Maryland
College Park, Maryland 20740
301.405.7500, fax 301.314.5780
e-mail: mdsg@mdsg.umd.edu
www.mdsg.umd.edu

Cover photo: *Did Algonquin tribes call the Chesapeake a "great shellfish bay?" Scholars disagree on the origins of the Bay's name, but scientists agree that the waters of the Chesapeake were once the greatest oyster grounds in the world. A new generation of oyster farmers could face a new challenge if acid levels rise in the estuary.* ***Pages 2 and 3:*** *Whitman Miller found that oysters did not grow well near Kirkpatrick Marsh on the Rhode River, a study site used by scientists from the Smithsonian Environmental Research Center.*
PHOTOGRAPHS BY MICHAEL W. FINCHAM.

AN ACIDIFYING
The "Other CO_2

Stephen P. Nash



The scene is more than a little askew here in a picnic park along the suburban shore of Maryland's Severn River not far from the Chesapeake Bay. On a drowsy, late-June morning, a high-school kid mows grass in the park, zodiacs and kayaks line the parking lot, and some pleasure craft are bobbing at anchor.

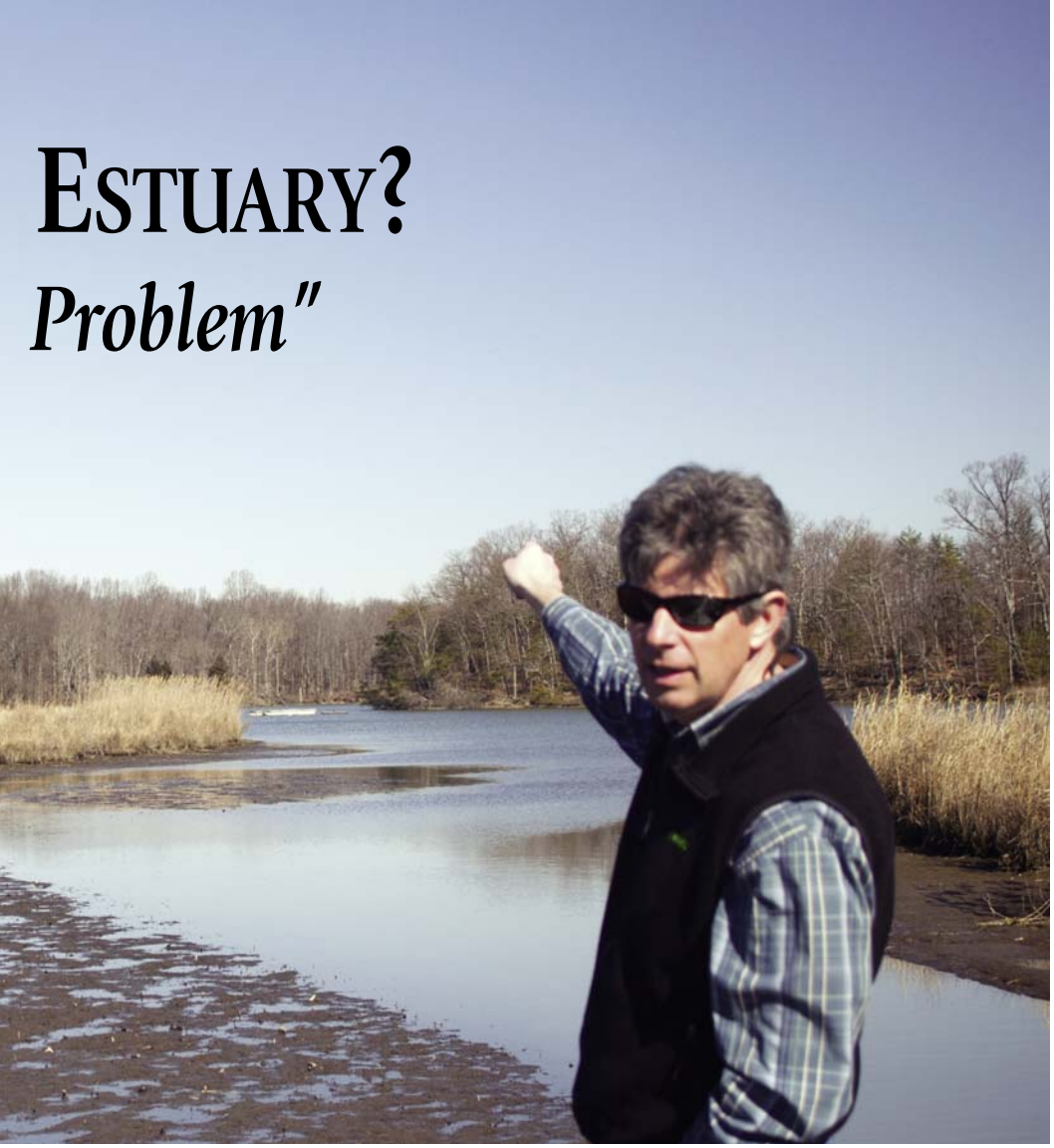
But the picnic tables are laden with an assortment of notebooks and odd tools. And some purposeful folks have unloaded tall green pressure tanks topped with gauges that could be mistaken for a welding kit, now installed in a big plastic chest under the trees. Tubes from their tanks run down into the river and out to a ten-meter square of water. It's marked off by colorful floating foam noodles and attended by two students in snorkel gear.

These are researchers, not welders, and the low-tech look of their apparatus belies the high-stakes questions behind their work. Designed as a kind of time machine, their river setup is meant to simulate Severn River waters as they will be in the future, circa 2050 and 2100. The green tanks are filled with carbon dioxide (CO_2), the same gas that now concentrates in the atmosphere as we burn coal, oil, and forests across the planet.

The Severn River project is part of a suite of new and recent research on the effects of rising CO_2 levels on the Bay. It is a collaboration between Whitman Miller, a marine ecologist with the Smithsonian Environmental Research Center (SERC), and Tom Arnold, a chemical ecologist with Dickinson College.

The two researchers plan to pump CO_2 out of their green tanks and down into that marked-off square of river

ESTUARY?
Problem"



As CO_2 levels are rising in the atmosphere, acidity levels are rising in the ocean, slowing growth rates for coral reefs, oysters, and other shell-building species. What's happening in the Chesapeake Bay?

water. They are doing on a small scale what industrial nations are doing on a global scale by pumping CO_2 into the atmosphere in ever-higher amounts. Nearly a third of that airborne CO_2 ends up absorbed in the world's oceans, unleashing chemical reactions that raise acidity levels and alter life for many marine species, especially shellfish.

What would happen to oysters in an acidifying Chesapeake? Miller plans to answer that question by placing baskets of young oysters out in that marked-off square of river and then raising CO_2 levels. He already knows that oysters don't grow well — at least in a lab — under higher CO_2 levels, but lab studies have their limits. "A lab study by its nature is a controlled environment," Miller notes. "The more controlled it becomes, the less realistic."

It turns out real-world experiments also have their limits, as Miller is discovering. Heavy, early summer rains have filled the Severn River with fresh, low-salinity water. Since oysters need moderate salinities to survive, he may have to delay deploying his samples.

The fate of oysters is a high-stakes question because oyster farming is on the rise in Maryland and Virginia, and both states have ambitious plans to rebuild some of the Bay's historic oyster reefs. Those ancient reefs played a key role in the ecology of an ecosystem once known as the "great shellfish bay."

A second question to be answered: what would happen to seagrasses in an estuary with higher CO_2 levels? Tom Arnold is planning on taking samples from the meadow of widgeon grass that seems to be flourishing in this part of the river. Heavy rains haven't hurt the widgeon grass, so Arnold can simply walk over and turn the handle on the tank regulator to start pumping CO_2 into the Severn River.

Miller and Arnold set up these experiments in the Bay because higher levels of CO_2 in the atmosphere are already changing both the climate of the Earth and the chemistry of the open oceans. We think of sky and water as distinctly separate in their vast reaches, but in fact where they meet, they mingle. As CO_2 gas increases in the atmosphere, it seeks equilibrium, pressing on the surface of open water and steadily diffusing into it, mixed in by wind and waves. As additional CO_2 dissolves into the ocean, it lowers pH and raises acidity. Scientists call this process ocean acidification.

The oceans, as a result, are changing at a faster rate than at any time in the last 300 million years, according to a report published this March in the journal *Science*. And that's worrisome. An earlier episode of rapid ocean acidification not only brought extinction to many one-celled organisms along the ocean bottom, it also caused the collapse of coral reefs and dissolved all the carbonate plankton shells that once littered ancient seafloors.

If the ocean continues to acidify, seawater could once again become corrosive to calcium carbonate structures, dissolving coral reefs and the shells of many marine organisms. The oceans are already 30 percent more acidic than they were 250

years ago, in the pre-industrial era. According to the *Science* paper, the current rate of acidification raises the possibility "that we are entering an unknown territory of marine ecosystem change."

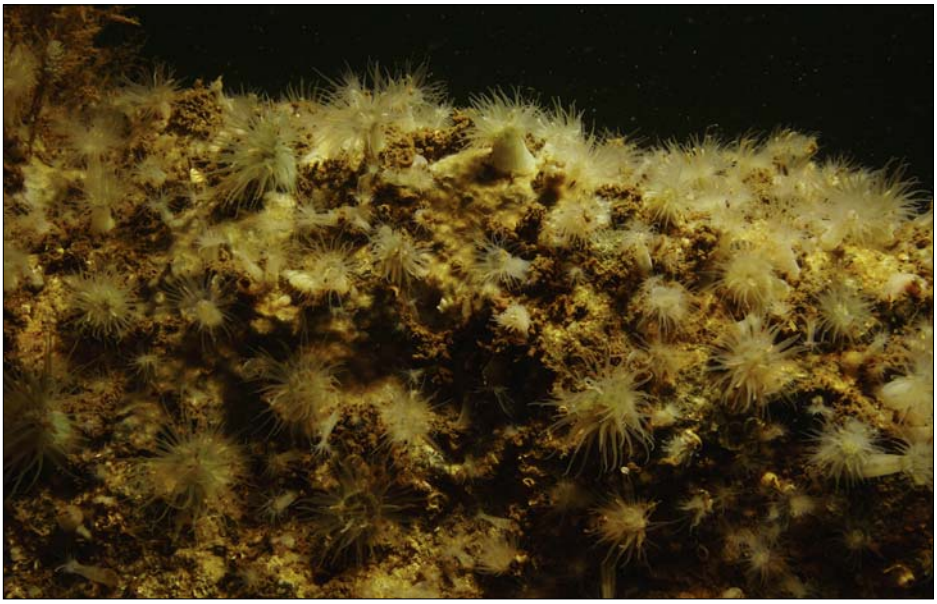
What's happening in the ocean could affect the Chesapeake, according to scientists who launched several of the early studies on acidity in the estuary. It's difficult to draw sweeping conclusions, however, since only a handful of studies have been completed so far. If ocean acidification research is in its infancy, as the National Research Council suggests, then the research on estuaries like the Chesapeake is embryonic.

Basic questions, such as what are acidification levels and trends in the Chesapeake, are difficult to answer. To determine acidity, scientists test for pH, the classic scale used to measure the balance between acidity and alkalinity. In an estuary with changing salinities, most pH readings come with an inevitable — and large — margin of error. Checking on acidification, it turns out, is not as easy as dunking some litmus paper in the water.

As a result, "we don't really know how the Bay is going to respond," says ecologist George Waldbusser, whose expertise is in bottom-dwelling organisms. While at the University of Maryland Center for Environmental Science, he was lead author of a recent study that reviewed historical water quality data and tried to reconstruct how acidity has changed in the Bay over the past two decades. The records, he found, were imprecise and tricky to evaluate.

One trend, nevertheless, seems clear: acidity has increased sharply in the Bay's saltier waters — more than can be explained by CO_2 in the atmosphere. In the Bay, acidity levels are also driven by other factors, especially by the runoff of nitrogen and phosphorus pollution from farms and sewage. These nutrients generated by human activity lead to explosive growth of phytoplankton in the Bay's waters as photosynthesis converts nutrients and sunlight and CO_2 into plant material and oxygen. When all those excess plankton die, however, the decay process sucks oxygen out of the water, creating the Bay's famous dead zones every summer. It also releases a lot of little-noticed CO_2. The net effect can be a rise in the Bay's acidity.

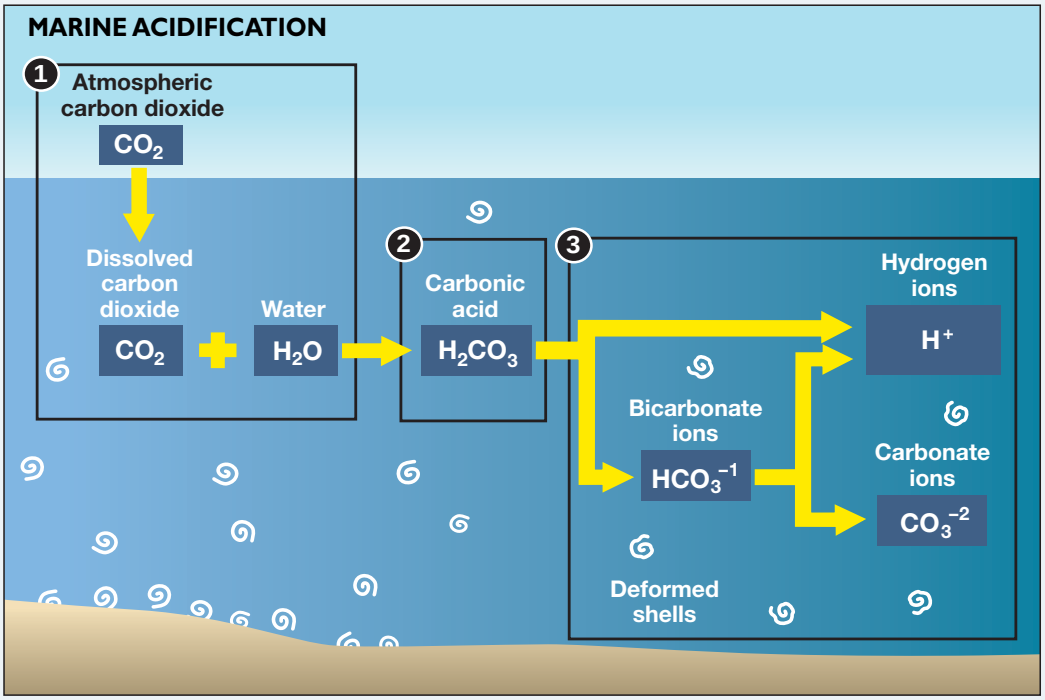
"If you say there is no CO_2 problem in the Bay, then you have to say there is no oxygen problem in the Bay," says Waldbusser, now a researcher at Oregon State University. "Hypoxia is a byproduct of that oxygen uptake and CO_2 release. Those things are linked through biology." For now, that biology and those land-borne nutrients create more acidity in the water than airborne CO_2 does.



***Coral reefs are falling victim to** disease, warming waters, and acidifying oceans. The growth of coral reefs, especially in colder waters, is slowing in the open ocean as levels of CO_2 are rising in the global atmosphere. The oceans absorb nearly a third of the airborne CO_2, creating chemical changes that raise acidity and even threaten warm-water coral reefs, like this one (top) in St. Croix, U.S. Virgin Islands. Oyster reefs in the Chesapeake Bay (bottom) and other coastal waters could also suffer from growing acidification. Laboratory research indicates that higher levels of CO_2 would slow shell growth of young oysters.* PHOTOGRAPHS: NOAA (TOP) AND MICHAEL EVERSMIER (BOTTOM).

CHEMISTRY 101

How Rising Carbon Dioxide Threatens Shell-Builders



Higher CO_2 reduces a key ingredient in shells.

1 CO_2 absorbed by seawater (H_2O).

2 CO_2 reacts to form carbonic acid; makes water more acidic (more hydrogen atoms).

3 Carbonic acid breaks down into bicarbonate and hydrogen ions (H^+). Bicarbonate breaks down into more H^+ and carbonate, key to organisms like oysters, clams, corals, and other marine organisms that make shells and skeletons. But as acidity increases, less bicarbonate changes into carbonate.

Higher CO_2 causes shells to dissolve.

Calcium carbonate is the main building block in the shells of marine animals. As seawater becomes more acidic, calcium carbonate — and the shells — can dissolve.

ACIDIFICATION AND OYSTER SHELLS IN THE CHESAPEAKE BAY

In an estuary like the Chesapeake Bay, sources other than the atmosphere — like runoff of excess nutrients — may add additional CO_2 to the water, contributing to acidification. Evidence suggests that higher acidity in the Bay could slow the rate of growth in the shells of young oysters, making them thinner and more vulnerable to predators.

David Littschwager, National Geographic Images

The pteropod, *or "sea butterfly," is a tiny sea snail about the size of a small pea. The photos above show what happens to a pteropod's shell when placed in seawater with pH and carbonate levels projected for the year 2100. The shell slowly dissolved over 45 days.*

Other trends he found: some historical records show decreases in acidity in the less salty mid-Chesapeake, but surprisingly they also show sharp increases in acidity in the southern Bay where saltier waters have greater buffering power. Waldbusser's explanation: while more plankton dieoffs occur in the mid-Bay, much of the resulting CO_2 is transported downstream to the southern Bay.

When Waldbusser applied his findings to laboratory research, he was able to measure some of the impacts of acidification on young oysters and on shell reefs that are critical for the establishment and survival of oyster colonies. His three conclusions: At current average pH levels in some parts of the Bay, the rates of shell growth in young oysters are slowed, creating shells that are likely to be abnormally thin and more vulnerable to predators. In addition, the saltier waters in the southern Bay are likely to become even more acidic and increasingly corrosive to oyster shell. And, finally, some Chesapeake waters, he claims, may already be unsuitable for shell preservation in areas that once supported oyster populations. His analysis, says Waldbusser in his recent paper,

CHEMISTRY 102

The pH Scale: Yardstick for Acidity

The pH scale — that staple of introductory chemistry courses — is the measuring stick that scientists use to gauge acidity in the Chesapeake Bay.

The scale normally runs from 0 to 14; the lower the number, the greater the acidity. A pH of 7, the middle point, is regarded as neutral pH. That is the reading for distilled water, or pure H_2O with nothing dissolved in it.

The pH scale indicates the concentration of hydrogen ions in a solution. Strong acids like sulfuric acid contain lots of unattached hydrogen ions floating in them; strong alkalines, like bleach, contain few.

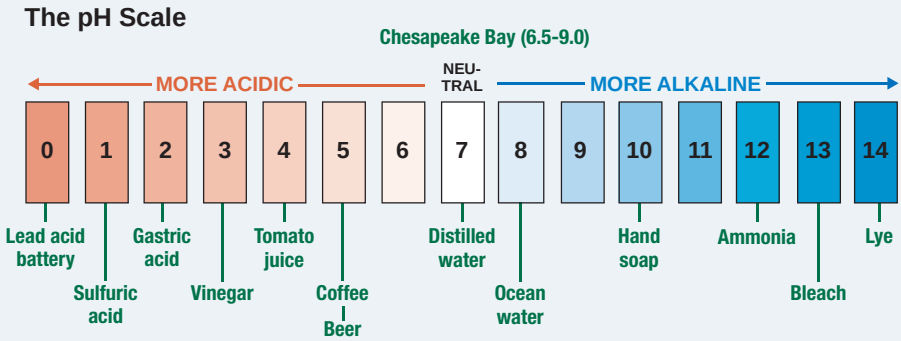
The scale is logarithmic, which means it measures a very big range. A difference of one pH unit is equivalent to a 10-fold difference in hydrogen ions. A reading of "4" is 10 times as acidic as a 5 and 100 times as acidic as a 6.

The "H" in pH refers to hydrogen, but the "p" has several meanings (for example, "power"), depending on which science historian you ask.

The pH in the Bay varies widely over time and along its length, influenced by salinity and temperature. The open ocean's different chemistry makes its pH range tighter and, on average, less acidic than the Bay's.

Neither the Bay's waters nor surface waters of the ocean typically register an average pH in the acidic range, below 7. Their waters are not acidic. Scientists, however, speak of ocean water as "acidifying" or "acidified" because its pH has dropped over time, making it more acidic than a few decades ago. On average, the ocean's pH has dropped from 8.2 to 8.1 since the industrial age began. That seemingly small change translates to a 30-percent increase in relative acidity. Scientists forecast a further drop of about 0.3 pH units by the year 2100, lowering pH to 7.8. That level would translate to a further increase of 150 percent in relative acidity.

Parts of the Bay are already at least as acidic as pH 7.8. As for the trend of pH in the Chesapeake, that is less extensively studied.



SOURCE: FIGURE ADAPTED FROM *CURRENT: THE JOURNAL OF MARINE EDUCATION*, VOLUME 25, NUMBER 1, 2009.

should be viewed "cautiously" and followed up with additional research.

If acidification is affecting oysters in the laboratory, could it also be stressing animals and plants out in the waters of the Chesapeake Bay? That's the question that brought scientists Whitman Miller and Tom Arnold to the Severn River last summer. According to Miller, "Nobody's really been looking."

That may be changing. Arnold designed a project to examine the impact of rising CO_2 levels on the Bay's underwater grasses by pumping CO_2 gas into an underwater research plot that held widgeon grass. His focus was photosynthesis and the protective compounds that grasses use to ward off predators and disease.

Perhaps more CO_2 in the water could improve prospects for the Bay's underwater seagrasses. Under ideal conditions, CO_2, sunlight, and water all combine to drive photosynthesis for these submerged grasses, a key support system for the health of the Bay. Since the 1970s, unfortunately, conditions have been less than ideal, and nearly all seagrass species have declined dramatically. The Bay's waters remain so clouded with silt pollution that the grasses no longer get much light.

Arnold's findings from last summer's fieldwork were less than hopeful. Starved for light, seagrasses, he found, are unlikely to take advantage of added CO_2. There may not be enough light to help CO_2 stimulate photosynthesis, but there is enough acidification, unfortunately, to erode the grasses' protective compounds. By the end of the season, his research established that a rise in CO_2 levels was followed by a sharp reduction of those protective compounds.

"I've been doing this for about 20 years now, and it's the largest change I've ever seen," Arnold said recently, summing up last year's experiment. "Everything we see so far tells us that it's not going to be so great for the seagrasses after all."

There were no findings at all, however, about oysters in the Severn River. Miller had planned to chart the physical development of juvenile native oysters as they matured over the summer, sitting in a basket amid plumes of elevated CO_2 and rising acidification. When heavy rains kept salinities too low for oyster survival, Miller decided he would have to postpone his river experiment.

Based on his earlier lab findings, Miller suspects that rising acidification will make it more difficult for the Bay's oysters to form calcium carbonate structures — shells and skeletons. When he charted shell growth under a variety of CO_2 regimes, he found that higher CO_2 brought slower shell growth. At levels predicted for the year 2100, he found that the shell area of maturing native oysters decreased by 16 percent, and their calcium content by 42 percent.

Similar failures have already shown up among ocean calcifiers such as sea butterflies, some planktons, and corals. In oyster hatcheries along the northwest Pacific Coast, large dieoffs of oyster larvae have been linked to upwellings of acidic ocean water (see "Shell Game," p. 8). In the Chesapeake, acidification could also affect plankton as well as some or all of the many mollusk species that grow shells (see "Crab vs. Oyster," p. 13).

Not all scientists agree, however, that

How would small oysters *like these (above) grow in more acidic waters? Researchers Whitman Miller (far right) and Tom Arnold (right) wanted to pump CO_2 from tanks into a patch of the Severn River to see whether small oysters and widgeon grass would grow well in an acidifying river.* PHOTOGRAPHS BY STEPHEN P. NASH.

rising acidity levels will be a threat to the Bay's ecology. This brand of optimism, for example, is sometimes heard: because plants and animals in the Chesapeake ecosystem are adapted to variability, they will not be sensitive to added increments of acidification. Native species may in a sense be pre-adapted, from an evolutionary standpoint, to at least some degree of future change in acidity.

But shellfish calcification in particular is a long-term process of integration, says Richard Feely, a senior scientist who studies ocean acidification with the National Oceanic and Atmospheric Administration. "When they calcify, they calcify all day long, so what matters is that average value, over the course of time," he says. "That's what affects their rates. So yeah, you see a lot of variability, but you want to know what the overall trend is."

The overall trend is clear, at least according to scientists like Waldbusser, Miller, and Feely. The change in atmospheric CO_2 is permanent — it will be with us for hundreds or thousands of years — and it is all in one direction.

And it's that long-term, one-way direction that may pose a future threat to the Chesapeake Bay. "What we're most concerned about is a baseline shift of the whole system," Miller says. "You can imagine if we increase the CO_2 just a bit more, what happens is you will still have variability, but you'll shift that variability to a different place on the pH scale. And it may not take a tremendous amount before many parts of the cycle of variation are outside the tolerance level of the organisms. If we have a shift in the baseline, that could mean bad news for lots and lots of organisms, no matter how adaptable they are."

The idea is worth dwelling on. You can think of the Chesapeake's normal cycles as rising and falling within certain limits, as if they were inside a picture frame. Aquatic life has adapted to live within those boundaries, but survival rates fall off near the edges. If you move the whole picture frame incrementally — as atmospheric CO_2 rises, for example — survival chances diminish. The Miller-Arnold experiments are moving that frame, simulating acidification that is an evolving threat to the already sorely challenged, unstable health of the Bay.

Because their projects simulated CO_2 in the years 2050 and 2100, they may give the illusion that we have plenty of time to figure things out and ease the threat. The changes, though, are incremental — they won't arrive all at once, a few decades out. "Fifty to 100 years is not a very relaxed schedule, actually, given the magnitude of the problem," Miller says. "We're fighting against a time clock here."

The fight, however, has to focus first on the immediate, and crucial, job of throttling back on land-based pollution threats, especially farm runoff and sewage flows (see "Should We Regulate Acidity in the Bay?" p. 15). For now, these nutrients generate more CO_2 in estuaries like the Chesapeake than atmospheric CO_2 does. According to Waldbusser and Miller and other scientists who study acidification in the Chesapeake, these nutrients are the strongest factors that may move the baseline, tipping acidification levels beyond the tolerances of life in the Bay.

"We've altered coastal ecosystems in ways that have affected that carbonate chemistry and already pushed the system to levels that are predicted for [the open ocean] a hundred years from now, or even further into the future," adds Waldbusser. "I think we're worse off than the open ocean."

Stephen P. Nash teaches journalism at the University of Richmond and has written for Bioscience, The New York Times, The New Republic, The Scientist *and* The Washington Post. *His most recent book is* Millipedes and Moon Tigers: Science and Policy in an Age of Extinction.

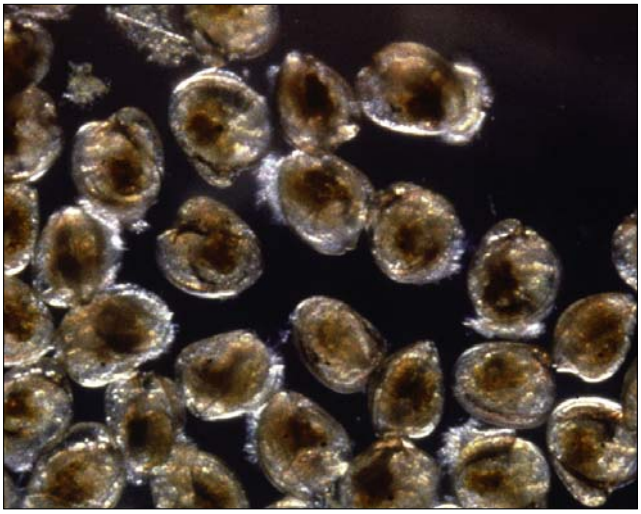
LEARN
PLAY
OBSERVE
EXPLORE
CONNECT

Shell Game
Finding Answers for Acid Waters

Michael W. Fincham

Mike Congrove was cruising through a pretty good season for spawning oysters when the water in the Piankatank River began to change. He runs a commercial hatchery down on Gwynn Island where this Virginia river meets the Chesapeake Bay. There he takes in water from the river and the Bay, coaxes males and females to spawn, and sells their offspring to a growing number of oyster farmers and gardeners in Virginia.

About the middle of June 2011, his good season hit a big slowdown. At his hatchery, Oyster Seed Holdings LLC, new oyster larvae began dying in large numbers. Larval production dropped from 100 million larvae a week down to 10 million. "We were spawning the same amount of oysters, fertilizing the same number of eggs," he said, "but we were getting extremely low survival rates."



Mike Congrove checks oysters *(opposite page) spawned and raised by Oyster Seed Holdings, LLC at its Gwynn Island hatchery down on Virginia's Piankatank River. Hatcheries like this are supplying larvae (above) and disease-resistant oyster seed to a growing number of oyster farmers in Virginia. After episodes of acidic water led to dieoffs of early-stage larvae, hatchery owners began adapting new monitoring techniques and workarounds to keep turning out new oysters for a growing industry.* PHOTOGRAPHS: TOM CHILLEMI (OPPOSITE PAGE) AND DONALD MERITT (ABOVE).

A slim, bearded, and blonde-haired young man, Congrove has worked in the seafood business long enough to know this kind of sudden slowdown could have multiple causes. In this dieoff, however, one major change drew his attention: he was seeing river water with lower levels of pH, a traditional measure of the balance between acidity and alkalinity. The Piankatank and the Chesapeake — at least for a while — had become more acidic.

The Piankatank incident raised a red flag with Congrove — and with operators at half a dozen other Virginia hatcheries who also were seeing a lot of dying and deformed larvae. Their problems here in the Chesapeake sounded a lot like the dieoffs of oyster larvae that struck two Pacific Coast hatcheries back in 2008 and 2009. Researchers and hatchery experts eventually concluded that upwellings from deep in the Pacific had brought acidic water up into the intake pipes for these hatcheries. One result of the acidic influx was a series of oyster dieoffs at Taylor Shellfish Farms in Washington state and at Whiskey Creek Hatchery in Oregon. A second result was a crisis for the entire West Coast oyster industry.

Those two large hatcheries produced much of the oyster seed used by growers from Canada down to Mexico. Oysters seemed to be dying because acidity levels in the ocean were rising.

The West Coast crisis, according to a number of scientists, was another dramatic sign of a disturbing global change. The world's oceans are becoming more acidic as levels of carbon dioxide (CO_2) are rising in the earth's atmosphere. The oceans are a great carbon sink absorbing much of the CO_2 that industries and automobiles send into the atmosphere when they burn fossil fuels like coal and oil. All that extra CO_2 alters the chemistry of seawater, eventually lowering the levels of calcium carbonate, a key ingredient for many ocean dwellers that use calcium to build skeletons or shells. Around the globe, the poster child for ocean acidification is the coral reef. In the Chesapeake Bay and other coastal waters, it may be the oyster.

For hatchery operators in the Chesapeake, the West Coast crisis comes at a time when oyster farming in the Bay seems to be entering a growth phase after decades of decline. In Virginia, the sale of farmed oysters has increased nearly tenfold since 2004, and growers in 2010 planted three times more seed oysters than ever before. And in Maryland, the state is hoping to imitate the Virginia boom by opening up more areas of Bay bottom for leasing by would-be oyster farmers.

Is rising acidity also a risk for oyster

hatcheries and farms here in the Chesapeake? It's a question with no easy answer. An estuary, of course, is not an ocean. The Chesapeake is shallower and less salty than coastal waters along the Pacific, so hatchery operators and farmers here are not exposed to upwellings of acidic waters from the bottom of the ocean. That combination of shallows and lower salinities, however, actually makes the Bay more vulnerable, at least in theory, to rising acidity and declining pH. "At low salinity, the water is less buffered," says Whitman Miller, a researcher at the Smithsonian Environmental Research Center. "So that means any molecule of CO_2 has a much bigger effect on a lower-buffered system."

Miller is one of several researchers who've run laboratory studies looking at how rising acidity could affect oysters in the Chesapeake. When he spawned groups of oysters under differing acidities, he did, in fact, find that larvae fared poorly under high acidities. "The larvae grew slower, and they calcified less at high CO_2," he says. It was not, he cautions, a real-world test. In the lab he subjected larvae to CO_2 levels well above those currently found in the Bay, reaching heights projected for 50 and 100 years from now. And his oyster larvae, he points out, were living under steady-state exposures while larvae in Bay waters would experience fluctuating CO_2 levels. Despite these caveats, Miller thinks the risk from rising acidity is real. "The answer is yes," he says. "There is a potential effect."

Acidic water, he found, seems to have its greatest effect on early-stage larvae, a finding that matches the experience of hatchery operators on both the East and West Coasts. After males and females spawn, fertilization of new oysters occurs in the water, creating larvae that begin to float and feed. At his hatchery down on the Piankatank River, Mike Congrove has seen new larvae begin to struggle almost immediately as they try to form their first shell and digestive tracts. "It's probably in the first 48 hours or so that these animals are being compromised," he says, "so they don't make it past the first week or so of life."

Those early larval failures in Chesapeake hatcheries are similar to what had happened on the West Coast, according to Alan Barton, the man who was hired to find out what was killing oysters at the Whiskey Creek hatchery in Oregon. "The larvae are dead on day one," he says, "but they don't die until 10 days later."

What Barton discovered has led hatchery operators to change their thinking about the water they let into their tanks. When he went to work at Whiskey Creek, he instinctively focused first on bacteria, specifically a bacterium called *Vibrio tubiashi* that was known to kill larvae. After engineering a system to filter the bacterium out of the water, he discovered that the larvae kept dying. "We always looked at diseases and bacteria as the culprits killing larvae, and they are," says Barton. "But it turns out those problems get a leg up because of carbon chemistry problems."

The key to the mystery, he decided, was the correlation between dieoffs of larvae and surges of acidic water. When winds blowing from the north push surface waters out to sea, cold water wells up from the deep carrying little or no oxygen, a lot of nutrients, and a lot of dissolved CO_2 gases. Not only does this acidic water kill off larvae, it helps support bacteria that also kill larvae. Before these episodes, says Barton, most hatchery operators had not been paying much attention to carbonate chemistry.

Not only are they paying attention now, they are busily looking for workarounds that could keep hatcheries profitable. The first step — monitoring the water coming into the hatchery — seems obvious, but it proved neither easy nor inexpensive. West Coast hatcheries joined together to seek federal grants to install instruments and establish protocols for measuring pH, temperature, salinity, and dissolved oxygen.

Their early monitoring efforts led to their first workaround. CO_2 levels, they

The oyster crash that hit Pacific Coast hatcheries *in 2008 drove home the need for close monitoring of coastal and estuarine waters. Federal agencies began adding carbon and pH sensors to open ocean and coastal moorings already in place. International Ocean Observing System (IOOS) partners in the Northwest Association of Networked Ocean Observing Systems (NANOOS) deployed this buoy in 2010 as part of a three-piece observing array to assess issues in the Pacific Northwest, including ocean acidification, hypoxia, harmful algal blooms, and climate change. The coastal buoy aids computer models that predict ocean and atmospheric conditions. Known as "Chá bă," the buoy is named for the Native American word (pronounced "chay buh") for "whale tail." Data from these buoys can now alert hatchery operators to oncoming acidic upwellings in time to adjust their water intake schedules. Workarounds like this have helped hatcheries increase their production again.* PHOTOGRAPH COURTESY OF JOHN PAYNE, PACIFIC OCEAN SHELF TRACKING PROJECT.

found, were lowest late in the afternoon, after photosynthesis had spent the day turning CO_2 and sunlight and water into plant material and oxygen. Photosynthesis, of course, shuts down during nighttime, but phytoplankton and seagrasses don't turn off their natural respiration. The metabolism that keeps them alive continues around the clock, creating CO_2 as waste and releasing it into the water column during all the dark hours. As a result, levels of CO_2 are always highest at the start of the day. Hatchery operators now time their water intakes for later in the day.

A second workaround came from weather buoys moored in coastal waters by the U.S. Integrated Ocean Observing System (IOOS), a program led by the National Oceanic and Atmospheric Administration (NOAA). By tracking wind shifts that unleash upwellings, the buoys give early warning alerts to shut down intake pipes. At Taylor's Shellfish Farms, located along inlets off Puget Sound, operators found relief by raising their intake pipes out of deep waters and positioning them near the surface. In 2009, NOAA also began adding carbon and pH sensors to many of its monitoring buoys in coastal waters.

Another solution that helped: adding sodium carbonate and bicarbonate when hatchery waters got too acidic or too alkaline. It's the same ploy the rest of us try when we get an acid stomach: we pop Alka Seltzer to lower the acidity, but its greatest effect is to raise the alkalinity. The chemistry in hatcheries may get a little more complicated than in our stomachs, but the principle seems to be working. "We're probably still in business because of that," says Barton.

Thanks to a combination of research and practical workarounds, West Coast hatcheries have been able to increase their annual production of oyster larvae in recent years.

Those solutions may not be as helpful along the East Coast, where hatcheries in the Chesapeake face a different kind of acid test. The Chesapeake is not the Pacific. Upwellings from the ocean and deposition from the atmosphere are not the major sources of CO_2 in the Bay. According to Whitman Miller, "The issue is really how much stuff is coming off the land."

Researcher Whitman Miller on how biology drives CO_2 cycles: *"In the open ocean, if something dies, it sinks down to the abyss, and that carbon is lost from the surface waters for hundreds of thousands of years. If something dies in the water column in the Chesapeake, it falls a few meters to the bottom, rots, CO_2 comes off, and it's back in the water column."* PHOTOGRAPH BY MICHAEL W. FINCHAM.

The ongoing overenrichment of the Chesapeake is a well-publicized problem — but it has a little-noted effect. Nutrients running off the land include decaying detritus, animal waste, fertilizer, and sewage, all of which help create blooms of phytoplankton and zooplankton. When these floating plants and animals die, the process of their decomposition lowers oxygen levels, creating well-documented dead zones in the Bay every summer. The surprise kicker: all that decay also recycles CO_2 back into the water. And, in the shallows of rivers and creeks, all that CO_2 could slow shell growth for young oysters.

Estuarine acidification, it turns out, is quite different from ocean acidification, according to Miller. With acidification in the ocean, the chemistry is driving the biology, but it's the exact opposite with acidification in estuaries. "If you come into the coastal systems," he says, "it is the biology that is driving the chemistry."

Miller saw dramatic evidence of how biology drives CO_2 chemistry when he grew test oysters in the Rhode River. He placed one batch next to a dock and another batch next to a nearby salt marsh. Most salt marshes are traps that become biological decay zones for plant material washing off the land, and Miller discovered that CO_2 levels coming out of the salt marsh biology were often 10 times higher than levels at the dock. His test oysters grew well near the dock — but not near the marsh. "The ones near the marsh are sort of devastated," says Miller. "Lots of them die. They barely grow at all, even after months and months and months."

There's obviously a lesson there for Chesapeake oyster farming and restoration. "If I were going to site an oyster restoration," says Miller, "it would not be right at a [marsh] location." Oyster growers are already advised not to plan in river areas with high fecal counts. Now they need to avoid low pH levels before planting seed oysters, paying close attention to fringe habitats like sewage outfalls and tidal marshes. Some places in the Bay are better to grow oysters than others.

Some lessons from the West Coast crisis are helpful, some are not. On the Piankatank River, Congrove is already shutting down his intakes when CO_2 levels are high and adding sodium carbonate to his hatchery water when pH levels are low. A West Coast solution like early warning buoys is less relevant in the enclosed waters of the Bay. What's needed here, according to Miller, are close readings of water chemistry on smaller, regional scales like the rivers, creeks, and coves where hatcheries or farms might be located.

The search for that kind of Bay-area solution is already underway. This year a coalition of six hatcheries in Virginia is beginning an ambitious effort to monitor a suite of water quality conditions that could be at play in their larvae dieoffs. With funding from the Virginia Secretary for Natural Resources and the Coastal Zone Management Program, hatchery operators will be able to work with more sophisticated gear, train their staff, send samples out for laboratory analysis, and follow a consistent testing protocol at each hatchery.

A skeptic at work. *Like most hatchery operators, Kevin McClarren has seen mysterious dieoffs of newly spawned oyster larvae, but he's unconvinced acidity is at fault. As manager of the Choptank Oyster Company, he runs one of the most successful oyster farms in Maryland and the only privately funded oyster hatchery in the state. "I don't know that anybody who's had a bad season could say it's because of acidity," he says. "I don't know." He's also skeptical about predictions of rising acidity. "People are guessing what the pH is going to be in 30 years."* PHOTOGRAPH BY MICHAEL W. FINCHAM.

Costs could run up to $30,000 per hatchery, according to Congrove. That's a hefty outlay, he says, for hatcheries that are "capital limited," but the payoff could be survival and expansion of the growing oyster industry in the state.

The chemistry of acidic water will not be the only focus for the monitoring. "We're looking at a number of things we've never looked at in the past," says Dave Kuhn, a researcher at Virginia Tech who will be analyzing data collected from all the hatcheries. In addition to carbonate chemistry like pH and alkalinity, the tests will cover parameters that include algae toxins, pesticides, minerals such as silicates, and nutrients such as ammonia, nitrates, nitrites, phosphorus, and total organic carbon. They will also look for vibrio and other disease-causing microorganisms.

Not every operator, at the moment, is convinced that acidic water is the killer at fault in the Chesapeake. "It's something that's on people's radar," says Kevin McClarren, who manages the Choptank Oyster Company in Cambridge, Maryland. "But pH is not something we really look at in oyster culture." His skepticism is rooted in a familiar experience: oyster spawning can be notoriously variable and the causes for larval dieoffs can be maddeningly mysterious. "There are so many things that are going to do in your larvae. But whether it's one problem or another, we can never figure out," says McClarren. "Once the larvae are dead, the larvae are dead. It's a guessing game after that."

A game changer may yet come out of the new monitoring, a finding that could cut down most of the guessing about dieoffs of oyster larvae. Perhaps some clear correlations will leap out of all that data. Which factors correlate with growth? Which with dieoffs? Mike Congrove has also noticed high ammonia levels in his hatchery water during larvae dieoffs at his Piankatank River hatchery. If larvae are dying at different hatcheries with similar readings — whether it's high levels of acidity or ammonia or some other parameter — then scientists and hatchery operators can start to zero in on the oyster killers.

And they can try more precise solutions, whether it's better filtration to keep out disease vibrios or some new kind of oyster Alka Seltzer to rebalance the acidity of their hatchery waters. "The real purpose of all this monitoring is the practical application," says Congrove. "How can we change the parameters that seem to be problematic?"

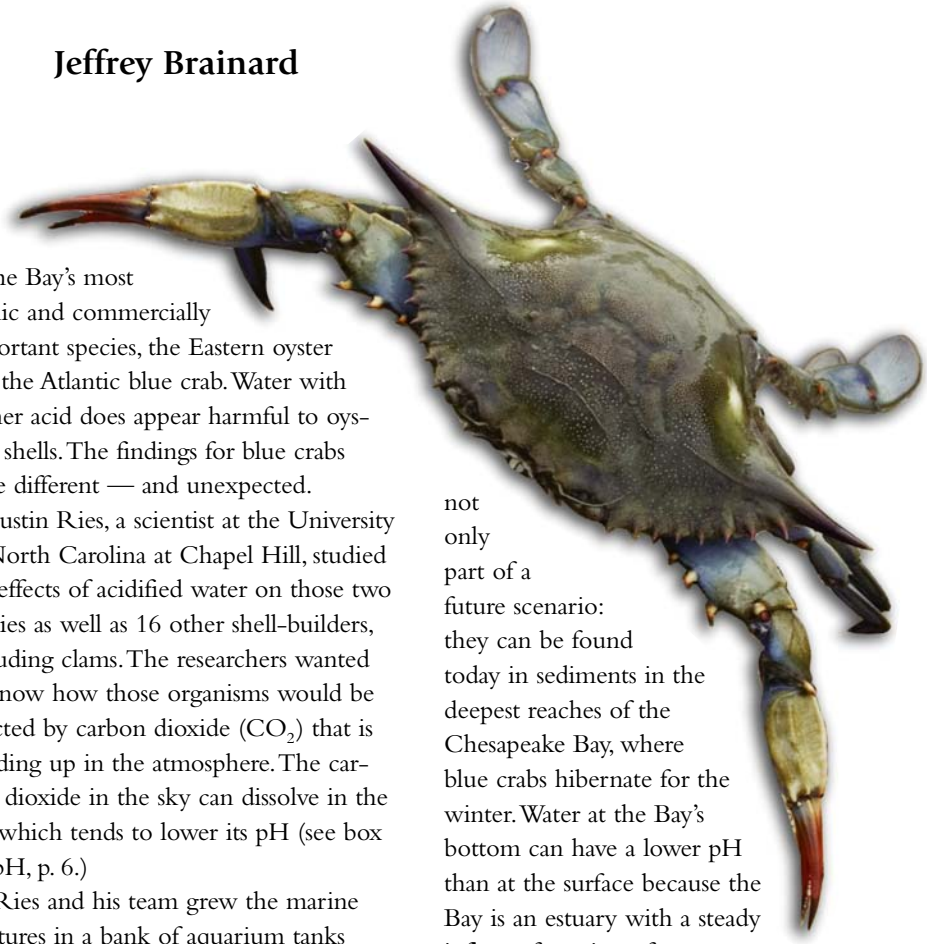
Perhaps that's the silver lining in the West Coast crisis. Now that hatchery operators on both coasts have started paying close attention to acidification, carbonate chemistry, and dozens of other factors, they may start finding answers to some old mysteries about what was killing off their baby oysters. And they may find some workarounds to keep their oysters alive and their industry growing.

— *fincham@mdsg.umd.edu*

Crab vs. Oyster

As Acidity Increases, Some Species May Win and Others Lose

Jeffrey Brainard

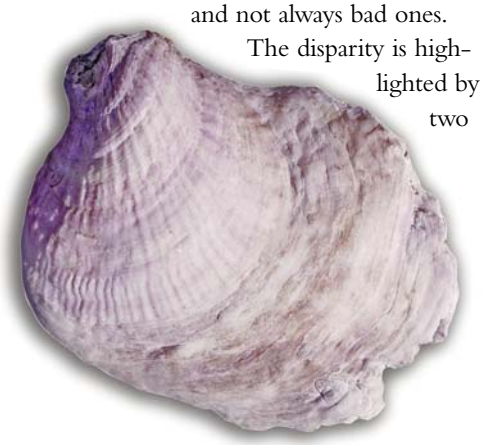


In the Chesapeake Bay and the open ocean, waters with rising acidity are poison for some species and tonic for others.

Those discoveries came from recent laboratory studies about how Bay species are affected by water with different levels of pH, the laboratory scale that describes acidity. (Lower pH readings correspond to higher acidity.) That's important because of predictions that the Bay and the open ocean will slowly become more acidic in coming decades. Water in parts of the Bay is already naturally more acidic than in the open ocean.

This water threatens to degrade the shells and skeletons of marine organisms. As pH in seawater falls, so does the level of a form of carbon (called carbonate) that the creatures need for building those structures. Their shells could grow smaller or even dissolve, making them more vulnerable to predators and threatening their survival.

The laboratory findings suggest, however, that decreases in pH may have quite different effects on different species — and not always bad ones.



The disparity is highlighted by two of the Bay's most iconic and commercially important species, the Eastern oyster and the Atlantic blue crab. Water with higher acid does appear harmful to oysters' shells. The findings for blue crabs were different — and unexpected.

Justin Ries, a scientist at the University of North Carolina at Chapel Hill, studied the effects of acidified water on those two species as well as 16 other shell-builders, including clams. The researchers wanted to know how those organisms would be affected by carbon dioxide (CO_2) that is building up in the atmosphere. The carbon dioxide in the sky can dissolve in the sea, which tends to lower its pH (see box on pH, p. 6.)

Ries and his team grew the marine creatures in a bank of aquarium tanks similar to those in pet stores but outfitted to bubble carbon dioxide into the water. The pH in some tanks corresponded to today's level of carbon dioxide. The pH in other tanks reflected levels of CO_2 two and three times higher than amounts before the Industrial Revolution; those levels are projected to occur by the year 2100 if carbon dioxide in the atmosphere continues to increase at the rate seen in recent decades. Another set of tanks held a still bigger dose of CO_2, representing 10 times the pre-industrial level, predicted to occur within the next millennium.

Acidity at the Bay's Bottom

Ries says it was relevant to study water with CO_2 levels that high because they're not only part of a future scenario: they can be found today in sediments in the deepest reaches of the Chesapeake Bay, where blue crabs hibernate for the winter. Water at the Bay's bottom can have a lower pH than at the surface because the Bay is an estuary with a steady inflow of nutrients from streams and rivers. The nutrients feed algae that bloom in the summer and eventually sink to the Bay's bottom, where they decompose. The process of decomposition creates more carbon dioxide, a waste product of metabolism, and raises acidity.

Ries's interest in what is going on in the Chesapeake sediments is more than purely academic. He grew up in Baltimore and spent summers at his grandfather's marina on Gunpowder Cove and at a summer home on Harris Creek in Maryland. In fact, he obtained the blue crabs he used in the study from the breeders at the Institute of Marine and Environmental Technology (part of the University of Maryland Center for Environmental Science) in Baltimore Harbor.

His laboratory experiments included a seemingly surprising result: the lower the pH in the tanks, the heavier and larger were the crabs' shells. Higher acidity seemed to help blue crabs grow bigger. "Honestly I did not expect it," he says.

Ries chalks this up to the blue crab's body type. Its outer shell or exoskeleton is covered by a substance, called chitin, that protects it from the corrosive effects of surrounding water. What's more, crabs appear able to regulate the pH of fluid inside that covering, keeping the level higher (or less acidic) than the surrounding water. That's important because organisms like crabs build their shells from calcium and a form of carbon called carbonate. The pH level affects the amount of carbonate available for the crab to incorporate into its shell: a higher pH leads to more carbonate, and a lower pH results in less. So when crabs control their internal pH, they can generate more raw material for shell building.

The blue crab probably evolved that capacity because it molts, Ries explains. Its survival depends on building and solidifying a new shell within days after it sheds its old one. So blue crabs needed a physiology that could maximize the amount of carbonate available to build their shells. That same mechanism can probably help blue crabs grow larger shells when carbon dioxide levels are higher than today's, Ries says. The effect is similar to what we see in modern humans, who grow taller on average than people who lived during the Middle Ages because today we enjoy diets richer in protein and calcium.

"We think that the crabs have evolved a sophisticated mechanism, not necessarily to prevent the effects of acidification, but just to go about their normal molting process," Ries says. "The carryover effect is that it makes them more resilient to acidification."

Elevated levels of carbon dioxide and acidity *affected the shells of marine animals in a study by Justin Ries of the University of North Carolina (above, with tanks used in his study). High levels of acidity hurt growth in Eastern oysters but helped it in Atlantic blue crabs. Surprisingly, crabs grown experimentally (bottom) at the highest levels of carbon dioxide (10 times pre-industrial levels, right) developed larger shells than those grown at today's levels (left.) The scale is in centimeters after 60 days of growth.* PHOTOGRAPHS COURTESY OF JUSTIN RIES.

Different Oysters, Different Effects

Other Bay species tested by Ries and his colleagues included hard- and soft-shell clams, and he found that their shells actually dissolved at the highest levels of carbon dioxide. That's an effect of acidification that doesn't offer good news for the blue crab, because clams are among its prey. It's an example of why scientists say that the effects of acidification have to be studied holistically within ecosystems, not just species by species.

When Ries tested Eastern oysters (*Crassostrea virginica*) from Cape Cod, he found effects that were less severe than in clams but still significant: the rate of growth in its shell steadily declined as the water's pH fell. Other researchers studying the Eastern oyster have found similar effects (see "Shell Game," p. 8.)

At least one species of oyster, it turns out, can survive in higher acidity waters. When Whitman Miller ran laboratory experiments at the Smithsonian Environmental Research Center, he found that the Asian oyster (*Crassostrea*

ariakensis) showed no loss of shell when exposed to higher acidity levels. Scientists think that the Asian oyster, native to the rivers of China, is better adapted to low pH waters because it evolved under more acidic conditions.

Oysters' bodies and shells are different from crabs' in several important respects. Oysters have a protective covering (called the periostracum), but it doesn't completely cover their shells. And unlike crabs, they don't molt. An oyster builds its shell continuously.

Other researchers have looked beyond shellfish to study other species that dwell in coastal waters. A study of moon jellyfish by researchers at Western Washington University showed they reproduced just fine in highly acidified water. And although underwater grasses might be expected to fare well in water rich with carbon dioxide, which they photosynthesize, they instead appear to sustain damage under some circumstancess (see "An Acidifying Estuary?" p. 2.)

A big caveat to these laboratory studies, acknowledged by Ries and other researchers, is that they may not accurately predict what would happen to those same creatures in the natural environment of the Bay. Ries gave the oysters a constant dose of low pH for two months, something they would not experience in nature, where pH levels fluctuate. In the Bay, the creatures may be able to compensate and build their shells despite these unfavorable conditions — although at a cost in energy that could reduce their survival, scientists say.

Still, these studies are significant because some earlier reports about acidifying water have implied that lower pH puts all shell-building organisms at risk. Ries and other scientists have offered a more nuanced picture, one that highlights the importance of differences in adaptations among different species. The impact on marine life of higher atmospheric carbon dioxide, Ries wrote, "is more varied than previously thought."

— *brainard@mdsg.umd.edu*

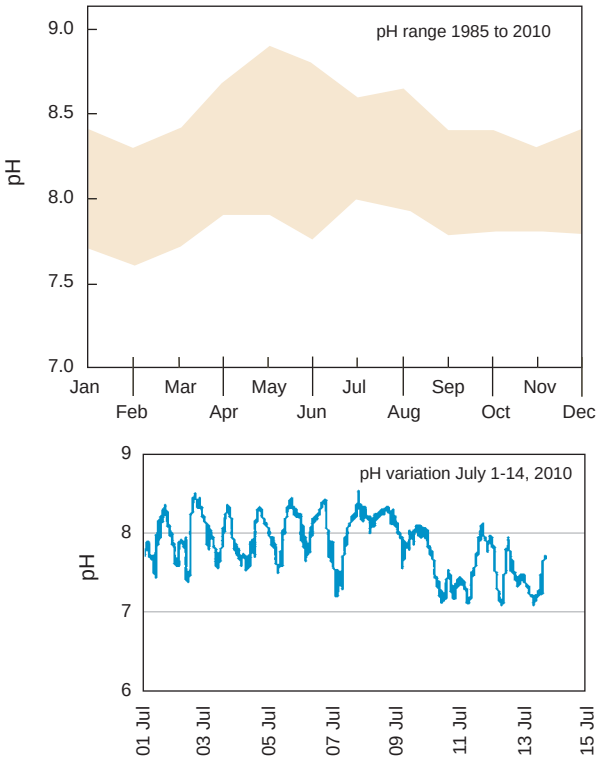
Should We Regulate Acidity in the Bay? If So, How?

Jeffrey Brainard

Levels of acidity may be rising in parts of the Chesapeake Bay, and as a result, oyster larvae may be dying and oyster shells may be thinning. According to state and federal regulators, there isn't yet enough proof to say the Bay is suffering from acid indigestion or to prescribe an antacid. One environmental advocacy group, however, has been working the regulatory umpires to persuade them otherwise.

A byproduct of carbon dioxide inputs from the land and the air, acid levels can fluctuate wildly in an estuary like the Bay, presenting a challenge for anyone seeking to measure or control them. Levels of pH, the numerical scale for acidity, can vary hugely over months and even hours, much more than in the open ocean. In the Bay, measurements in a single year at a single location can vary from 6.5 (more acidic) to 8.5 (less acidic) on the pH scale (see box on pH, p. 6.) That's a difference of more than one hundred times. Ebbs and flows of fresh water from rivers contribute to this unevenness; so do tides that stir the water.

The federal government acknowledged the natural variability of pH in estuaries when it wrote an allowable range for acidity in salt water under the Clean Water Act back in the 1980s: the range is between 6.5 and 8.5 in pH. Maryland adopted the same range.



Measuring pH in the Bay *is a challenge. Levels can vary widely over time and at different locations, and officials say that the available data don't indicate that acidity is rising in Maryland. The top graph shows the ranges of monthly pH at one station in the Bay, Cedar Point, from 1985 to 2010. The bottom graph shows variation of pH over 14 days in July 2010 at Fort Howard.* SOURCE: MARYLAND DEPARTMENT OF NATURAL RESOURCES, EYES ON THE BAY.

So far, the Maryland Department of the Environment, which has lead responsibility for enforcing the act in Maryland, has not seen evidence that the Bay's acidity has transgressed those boundaries, says the agency's Matt Stover. The data don't indicate that the water is growing more acidic. "At this point, it doesn't seem like the localized data are conclusive enough

Continued on p. 16

Maryland Sea Grant College
4321 Hartwick Road, Suite 300
University System of Maryland
College Park, Maryland 20740

Address Service Requested

Non-Profit Org.
U.S.Postage
PAID
Permit No. 04386
College Park, MD

Chesapeake Quarterly is printed on recycled paper, processed chlorine free, using soy-based inks

Regulating Acid, from p. 15

to show that there's an immediate effect on shellfish" from acidity levels, he says.

That conclusion doesn't satisfy Miyoko Sakashita, ocean director at the Center for Biological Diversity, an environmental advocacy group. The organization, based in Tucson, Arizona, has been pushing Maryland, Virginia, and other states to declare their coastal waters to be in violation of the law, so far without success. Sakashita argues that Maryland has grounds to declare portions of the Bay as "impaired" because new research, based on laboratory tests, indicates that current pH levels threaten to damage oysters (see "Shell Game," p. 8.) The oysters are "the elephant in the corner," Sakashita says.

The U.S. Environmental Protection Agency, which oversees how the states enforce the Clean Water Act, weighed in on the issue in 2010, encouraging the states to pay attention to acidification in their coastal waters. (The EPA made the statement to settle a lawsuit filed against it by Sakashita's organization.) States should declare waters as impaired by acidity "where data and assessment methods are available," said the agency, while recognizing "that information is absent or limited for [acidification] parameters and impacts at this point in time in many states." The EPA encouraged states to focus on protecting vulnerable ecosystems, including those where shellfish live.

If acidified water indeed threatens the Bay's ecology, communities in the Chesapeake watershed are already planning measures that could ease the risk, by controlling the excess nutrients that contribute to acidified water. According to Sakashita, it's not clear that Maryland would need to do more to control acidity beyond the steps it has already promised to control nutrient threats to the Bay's water quality. Those steps include a reduction of about 25 percent each in nitrogen and phosphorus by 2025. Excess levels of these nutrients feed algal blooms that kill fish and contribute to rising acidity. The EPA has directed Maryland and other states in the Chesapeake watershed to improve stormwater systems and expand vegetative buffer areas that can filter out those nutrients before they reach the Bay.

While those efforts might help control acidity in the Bay, Sakashita and many scientists believe the controls on nutrients must be complemented by new national and global measures to curtail carbon dioxide emissions into the atmosphere. Those gases can dissolve in waters of both the Bay and the open ocean. The EPA won the legal authority to regulate CO_2 emissions under the federal Clean Air Act and began doing so in 2011. Legal challenges to that authority, however, are still underway.

— *brainard@mdsg.umd.edu*

For Further Reading

NOAA Pacific Marine Environmental Lab. Carbon Program website. http://www.pmel.noaa.gov/co2/story/Ocean+Acidification

The Geological record of ocean acidification. Bärbel Hönisch et al. Science Magazine. March 2, 2012. http://www.sciencemag.org/content/335/6072/1058.full?sid=0dc92a9a-fe7e-48a3-888d-e5f0397e659e

Shellfish face uncertain future in high CO_2 world: influence of acidification on oyster larvae calcification and growth in estuaries. A.W. Miller, A.C. Reynolds, C. Sobrino, and G.F. Riedel. PLoS ONE 4(5):e5661, 2009.

Biocalcification in the Eastern Oyster (*Crassostrea virginica*) in relation to long-term trends in Chesapeake Bay pH. George G. Waldbusser, Erin P. Voigt, Heather Bergschneider, Mark A. Green, and Roger I. E. Newell. Estuaries and Coasts 34:221-231, 2011.

Marine calcifiers exhibit mixed responses to CO_2-induced ocean acidification. Justin B. Ries, Anne L. Cohen, and Daniel C. McCorkle. Geology 37(12): 1131-1134, December 2009.

Anticipating ocean acidification's economic consequences for commercial fisheries. Sarah R. Cooley and Scott C. Doney. Environmental Research Letters 4:024007, 2009. 8 pp.

Special Issue on the Future of Ocean Biogeochemistry in a High-CO_2 World. Oceanography 22(4), December 2009. http://tos.org/oceanography/issues/issue_archive/22_4.html

Biogeosciences, 8, 3697–3707, 2011
www.biogeosciences.net/8/3697/2011/
doi:10.5194/bg-8-3697-2011
© Author(s) 2011. CC Attribution 3.0 License.

Biogeosciences

Effect of ocean acidification on early life stages of Atlantic herring (*Clupea harengus* L.)

A. Franke and C. Clemmesen

Department of Marine Ecology, Leibniz-Institute of Marine Sciences (IFM-GEOMAR), Düsternbrooker Weg 20, 24105 Kiel, Germany

Received: 1 July 2011 – Published in Biogeosciences Discuss.: 18 July 2011
Revised: 30 November 2011 – Accepted: 2 December 2011 – Published: 15 December 2011

Abstract. Due to atmospheric accumulation of anthropogenic CO_2 the partial pressure of carbon dioxide (pCO_2) in surface seawater increases and the pH decreases. This process known as ocean acidification might have severe effects on marine organisms and ecosystems. The present study addresses the effect of ocean acidification on early developmental stages, the most sensitive stages in life history, of the Atlantic herring (*Clupea harengus* L.). Eggs of the Atlantic herring were fertilized and incubated in artificially acidified seawater (pCO_2 1260, 1859, 2626, 2903, 4635 µatm) and a control treatment (pCO_2 480 µatm) until the main hatch of herring larvae occurred. The development of the embryos was monitored daily and newly hatched larvae were sampled to analyze their morphometrics, and their condition by measuring the RNA/DNA ratios. Elevated pCO_2 neither affected the embryogenesis nor the hatch rate. Furthermore the results showed no linear relationship between pCO_2 and total length, dry weight, yolk sac area and otolith area of the newly hatched larvae. For pCO_2 and RNA/DNA ratio, however, a significant negative linear relationship was found. The RNA concentration at hatching was reduced at higher pCO_2 levels, which could lead to a decreased protein biosynthesis. The results indicate that an increased pCO_2 can affect the metabolism of herring embryos negatively. Accordingly, further somatic growth of the larvae could be reduced. This can have consequences for the larval fish, since smaller and slow growing individuals have a lower survival potential due to lower feeding success and increased predation mortality. The regulatory mechanisms necessary to compensate for effects of hypercapnia could therefore lead to lower larval survival. Since the recruitment of fish seems to be determined during the early life stages, future research on the factors influencing these stages are of great importance in fisheries science.

Correspondence to: C. Clemmesen
(cclemmesen@ifm-geomar.de)

1 Introduction

The atmospheric CO_2 concentration is constantly increasing primarily due to human activities causing an acidification of the ocean (Feely et al., 2004). While the CO_2 concentration ranged between 180 and 300 ppm over the last 650 000 years (Siegenthaler et al., 2005), the recent global mean is ∼390 ppm (Conway and Tans, 2011). A further rise up to ∼790 ppm until the end of the century is predicted; as a result the seawater carbonate chemistry is changing and the present mean surface pH_T of ∼8.1 is expected to decrease by ∼0.3 units (Gattuso and Hansson, 2011; Orr, 2011). However, there are naturally CO_2 enriched habitats such as upwelling regions (Feely et al., 2008). In the Baltic Sea acidification of coastal surface waters occurs as a result of its strong seasonal stratification, which is causing hypoxia in deeper water layers and subsequent upwelling of CO_2-enriched waters (Thomsen et al., 2010). In our study area, the Kiel Fjord, the pCO_2 is elevated for large parts of the year with peak values of >2300 µatm during late summer, which could increase to >4000 µatm in the future according to simple model calculations (Thomsen et al., 2010).

Studies reporting the potential impact of ocean acidification on marine organism have focussed on calcifying organisms (e.g., Langdon, 2002; Fabry, 2008). Furthermore, a variety of physiological traits of non-calcifiers such as acid-base regulation, metabolic rate and growth under elevated CO_2 concentrations have been analysed (Larsen et al., 1997;

Michaelidis et al., 2007; Gutowska et al., 2010; Hu et al., 2011) and reviewed (Pörtner et al., 2004; Ishimatsu et al., 2008; Pörtner and Peck, 2010). By using meta-analytic techniques it has been shown that the biological effects of ocean acidification are negative yet variable amongst organisms (e.g., Kroeker et al., 2010). It is hypothesised that the response of marine organisms to acidified seawater does not only vary between different groups of organisms, but also at the species level generating ecological winners and losers (Doney et al., 2009; Ries et al., 2009). Generally, organisms with efficient acid-base regulatory mechanisms e.g. fish and cephalopods are found to be less adversely affected. However, early life history stages even of the more tolerant taxa are assumed to be most susceptible to ocean acidification (Raven et al., 2005; Melzner et al., 2009). Considering that they are generally known to be most affected by abiotic conditions such as oxygen availability, temperature and salinity (Blaxter, 1956; Rosenthal and Alderdice, 1976), particular importance should be given to the potential effect of acidified seawater on their development. Most studies on the influence of ocean acidification on early life stages have been conducted on invertebrates such as molluscs, crustaceans and echinoderms (e.g., Dupont et al., 2010) indicating that its impact is highly variable amongst different species, even within closely related taxa (Dupont and Thorndyke, 2009).

So far only very few studies on the effect of hypercapnia on early developmental stages of marine teleosts, using $p\mathrm{CO_2}$ concentrations in the range of future predictions, have been published (Checkley et al., 2009; Munday et al., 2009, 2011a, b). Higher $p\mathrm{CO_2}$ levels (up to 150 000 µatm) were used by Kikkawa et al. (2003) to investigate the acute lethal effect of $p\mathrm{CO_2}$ on early life stages of marine fishes.

In this study we examined to what extent elevated $p\mathrm{CO_2}$ concentrations affect the embryonic development and the condition of newly hatched larvae of the Atlantic herring, a teleost fish of major commercial importance in the Baltic Sea. We tested whether acidified conditions influence the embryogenesis and hatch rate as well as morphometrics, otolith area and RNA/DNA ratio of newly hatched herring larvae.

2 Materials and methods

2.1 Experimental setup and water chemistry

Adult Atlantic herring from a local spring-spawning stock were caught in Kiel Fjord, one of the most important spawning grounds in the Western Baltic Sea, in April 2007. The gametes of 3 females and 3 males with a total length of 28 cm each were used to perform a laboratory experiment in filtered (0.5 µm) and UV sterilized seawater from Kiel Fjord (salinity 14.0) in a temperature constant room set at 12 °C with a day/night cycle of 12/12 h.

We used 10 l gas-proof high-density polyethylene (HDPE) containers firmly closed with lids as experimental units for egg incubation. A centrifugal pump (1005 21-5 Eheim) was attached to every container with one tube each plugged in the aspiration port and the discharge port going through the container lid ensuring a constant water circulation within the sealed systems to avoid fungal infestation on the eggs. Temperature, oxygen content and $\mathrm{pH_F}$ (free scale) were measured daily in each experimental unit (WTW Multi 350i with SenTix 21 electrode). The pH meter was calibrated by a three-point calibration procedure with NBS buffer solutions of pH 4.01, 7.00 and 10.00 (WTW TEP Trace). The incubation temperature (mean ± standard deviation: 13.6 ± 0.4 °C) was above the set room temperature due to the heat production of the centrifugal pumps.

Before starting the experiment total dissolved inorganic carbon (C_T), total alkalinity (A_T), temperature and salinity of the stock seawater were determined. C_T was measured photometrically in duplicate after Stoll et al. (2001) using a Bran & Lübbe Quattro Analyzer equipped with a XY-2 autosampler. A_T was measured in duplicate through potentiometric titration after Dickson (1981) with a Metrohm Titrando 808. To quantify the measurement accuracy of C_T and A_T certified reference material (provided by A. G. Dickson, Scripps Institution of Oceanography) was used.

We set up 6 different treatment levels (in 4 replicates) composed of a control treatment (non-manipulated Baltic Sea water) and 5 treatment levels with elevated $p\mathrm{CO_2}$ concentrations. The different concentrations were adjusted by decreasing A_T at constant C_T through the addition of a strong acid (1M HCl), according to the Guide to best practices for ocean acidification research and data reporting (Gattuso et al., 2010) one of the most useful techniques to manipulate the seawater chemistry, despite the fact that it does not fully mimic future changes in the carbonate system.

At the beginning, intermediate phase and end of the experiment water samples for C_T and A_T measurements were taken in each experimental unit and processed as described above. Unfortunately, the C_T water samples could not be used due to problems during storage. Thus, the carbonate system was calculated with the CO2SYS macro for low salinities (modified by Körtzinger after Pierrot et al., 2006) using the measured A_T and $\mathrm{pH_F}$ values. The dissociation constants $\mathrm{K_1}$ and $\mathrm{K_2}$ according to Roy et al. (1993) were used.

To reduce the chance of low quality gametes and to simulate natural variability, we incubated eggs from all 3 females in each experimental unit. 50 eggs of each female were strip-spawned on a plastic plate (48 plates in total, each 9 cm × 2.5 cm). The eggs of every female were arranged in a single row to ensure equal gas exchange and comparable environmental conditions. Fertilization was performed in water of the respective treatment level adding a sperm mixture of 3 males. Subsequently, 2 plates each (plate 1 and plate 2) were put in a holder at the bottom of every HDPE container. Fertilization rates were determined 2 h later for every plate under a

stereomicroscope (Leica MZ8). From the second day on eggs of plate 1 were photographed daily with a Canon Digital Ixus camera connected via C-mount to a stereomicroscope (Leica MZ8) to monitor the embryonic development, to determine the proportion of malformed eggs and the overall egg mortality. Plate 2 was not taken out of the containers at any time during the course of the experiment.

To reduce the drift from the originally set pCO_2 levels due to respiration of the eggs, 40 % of the water was exchanged at day 6 in every experimental unit by using stock seawater (non-manipulated and adjusted to the different CO_2 concentrations, respectively) which was stored in completely filled and sealed plastic containers at 12 °C since the beginning of the experiment.

2.2 Analysis of eggs and larvae

After the main hatch occurred, yolk sac larvae were transferred into 1.5 ml Eppendorf safe-lock tubes with seawater and frozen at −70 °C. Hatch rate was determined by counting empty eggshells under a stereomicroscope (Leica MZ8). For the following analysis, larvae were thawed and photographed with a QImaging MicroPublisher 3.3 RTV camera connected via C-mount to a stereomicroscope (Leica MZ95) in order to measure the total length and the yolk sac area using the program UTHSCSA Image Tool 3.0.

To determine the dry weight, larvae were rinsed in distilled water to avoid salt residues, put individually in 1.5 ml Eppendorf safe-lock tubes and freeze-dried (Christ Alpha 1-4 freeze-drier) for 16 h at −55 °C. They were subsequently weighed to the nearest 0.1 µg (Sartorius microbalance SC2) and either used for removal of otoliths or biochemical analysis.

For otolith removal, larvae were put in a drop of distilled water on a microscope slide. Right and left sagittae and lapilli were dissected under a stereomicroscope (Leica MS5) equipped with a polarizing filter using 2 fine dissecting needles and fixed with clear nail polish. Digital pictures of the otoliths were taken at 1250x magnification using a microscope (Leitz Laborlux S) equipped with a QImaging MicroPublisher 3.3 RTV camera. Sagitta and lapillus areas were measured with the image analysis software Image-Pro Plus 5.0.

Larvae were analysed for RNA and DNA concentrations using a modification of the method of Clemmesen (1993) and Belchier et al. (2004) as described in Malzahn et al. (2007). For the determination of RNA/DNA ratios, nucleic acids were quantified fluorometrically in a microtitre fluorescence reader (Labsystems, Fluoroskan Ascent) using ethidium bromide as a fluorophore. At first total nucleic acids were measured and subsequently RNase (Serva, Ribonuclease A) was applied. For RNA and DNA calibrations 16S and 23S rRNA (Boehringer 206938) and Lambda DNA (Boehringer 745782), respectively, were used. RNA amounts were calculated using the RNA standard calibration curves. DNA amounts were calculated using the relationship between RNA and DNA fluorescence described by Le Pecq and Paoletti (1966) resulting in a slope ratio of 2.2 (Caldarone et al., 2006).

2.3 Statistical analysis

Since the experiment was set up to evaluate a broad range of pCO_2 levels, linear regression analysis was the statistical method of choice. Statistical analyses were performed using the software Statistica 6.1 (StatSoft, Inc.). All data were tested for normality using the Shapiro-Wilk test. Non-normally distributed data were log-transformed and percentage data were arcsine transformed prior to linear regression analysis. Data were tested for homogeneity of variances using Levene's test if a significant linear relationship was found. The difference between right and left otolith areas (sagitta and lapillus, respectively) was analyzed using a paired t-test after data were tested for normality and homogeneity of variances. Since no difference between right and left otolith areas was observed, the data were combined and the resulting mean values were used for linear regression analysis. We also calculated effect sizes and 95 % confidence intervals around effect sizes using the results from control (pCO_2 480 µatm) and highest treatment (pCO_2 4635 µatm) applying the methodology of Hedges and Olkin (1985). Results are reported as mean ± standard deviation (SD).

3 Results

We incubated herring eggs at 6 mean pCO_2 values of 480 ± 81, 1260 ± 218, 1859 ± 240, 2626 ± 197, 2903 ± 204 and 4635 ± 340 µatm (corresponding to pH_F values between 8.08 ± 0.07 and 7.05 ± 0.03) until the main hatch occurred (Table 1). The mean oxygen saturation was above 80 % in all cases until the end of the experiment.

Fertilization was successful, resulting in rates between 86 and 90 % at all treatment levels. Neither the daily observation of the herring eggs nor the evaluation of the daily taken digital photographs showed any difference or time delay in the embryonic development between the 6 treatment levels. The herring embryos showed the same stage of development regarding blastoderm formation, epiboly, appearance of eyes and myomeres, beginning of embryonic movement, heart pulsation, eye pigmentation, appearance of otoliths and main hatch at the respective time of monitoring.

There was neither a significant linear relationship between the pCO_2 level and the incidence of embryonic malformations such as deformation and irregular cleavage of blastomeres (Fig. 1a; $r^2 = 0.02$, $P = 0.52$), nor the mortality rate during the embryonic development (Fig. 1b; $r^2 = 0.02$, $P = 0.53$).

There was no effect on the embryonic duration, since the main hatch occurred during the night of day 8 at all pCO_2

Table 1. Seawater carbonate system speciation for the different treatment levels during the course of the experiment. Variables were calculated using measured pH_F, A_T, salinity (14.0) and temperature (13.6 ± 0.4 °C) of the respective replicates at the beginning, in the middle and at the end of the experiment. Values are means ± SD.

Seawater measurements			Calculations					
Treatment	pH_F (free scale)	A_T [µmol kg^{-1}]	C_T [µmol kg^{-1}]	pCO_2 [µatm]	CO_2 [µmol kg^{-1}]	HCO_3^- [µmol kg^{-1}]	CO_3^{2-} [µmol kg^{-1}]	Ω_{arag}
1 (control)	8.08 ± 0.07	2070.2 ± 4.1	1989.9 ± 20.5	480 ± 81	21.5 ± 3.4	1887.8 ± 28.2	80.6 ± 11.2	1.27 ± 0.17
2	7.67 ± 0.07	1965.8 ± 4.7	1981.2 ± 15.4	1260 ± 218	55.7 ± 8.8	1894.1 ± 11.2	31.5 ± 4.3	0.49 ± 0.07
3	7.49 ± 0.05	1922.6 ± 5.1	1977.4 ± 14.9	1859 ± 240	81.6 ± 9.0	1874.9 ± 8.2	20.9 ± 1.9	0.33 ± 0.03
4	7.33 ± 0.03	1870.2 ± 4.1	1967.4 ± 7.6	2626 ± 197	115.4 ± 6.6	1837.9 ± 3.8	14.1 ± 0.7	0.22 ± 0.01
5	7.28 ± 0.03	1854.8 ± 3.1	1967.1 ± 8.0	2903 ± 204	128.5 ± 7.3	1826.2 ± 3.0	12.5 ± 0.6	0.20 ± 0.01
6	7.05 ± 0.03	1737.5 ± 4.9	1934.4 ± 16.6	4635 ± 340	206.0 ± 18.5	1721.5 ± 4.0	6.9 ± 0.7	0.11 ± 0.01

conditions. The hatch rate varied between 66 and 96 %, except for one replicate of the highest treatment level having a hatch rate of only 48 %. However, no significant linear relationship between pCO_2 level and hatch rate was found (Fig. 2; r^2 = 0.09, P = 0.17).

The elevated pCO_2 conditions neither affected the total length ranging from 5.91 to 6.96 mm (Fig. 3a; r^2 = 0.001, P = 0.87), the dry weight ranging from 38.8 to 54.3 µg (Fig. 3b; r^2 = 0.07, P = 0.23), nor the yolk sac area ranging from 0.38 to 0.60 mm^2 (Fig. 3c; r^2 = 0.03, P = 0.40) of the newly hatched larvae.

The left and right otolith areas did not differ significantly from each other (paired t-test for sagitta and lapillus, respectively: $P > 0.05$). The mean sagitta area varied from 371 to 470 µm^2 and the mean lapillus area from 314 to 419 µm^2. No significant linear relationship between the pCO_2 level and the otolith area was found (sagitta: Fig. 4a; r^2 = 0.02, P = 0.47; lapillus: Fig. 4b; r^2 = 0.10, P = 0.13), but a slight trend for greater lapillus area at higher pCO_2 was observed.

In contrast to all the other examined parameters, the RNA/DNA ratio, ranging from 2.5 to 3.8, was negatively affected by acidification (Fig. 5a; r^2 = 0.47, $P < 0.01$, y = 3.42–0.00019 × x). The relative RNA content (RNA/dry weight) was lowered significantly (r^2 = 0.31, $P < 0.05$, y = 37.72–0.0019 × x), while the relative DNA content (DNA/dry weight) was not affected (r^2 = 0.02, $P = 0.62$). However, a significant correlation could no longer be detected when excluding the highest treatment level from the statistical analysis (Fig. 5b; r^2 = 0.25, P = 0.10).

Calculated effect sizes and 95 % confidence intervals around effect sizes for embryonic malformations, mortality rate during embryogenesis, hatch rate, total length, dry weight, yolk sac area, sagitta area, lapillus area and RNA/DNA ratio based on differences of control (pCO_2 480 µatm) versus highest treatment (pCO_2 4635 µatm) are presented in Fig. 6. These showed clear overlap with zero for all variables tested, except for the lapillus area and the RNA/DNA ratio.

4 Discussion

4.1 Effects on early development

In this study we examined the effect of ocean acidification on the embryogenesis and the condition of newly hatched larvae of the Atlantic herring, *Clupea harengus*. We found no significant effect of elevated pCO_2 on the occurence of embryonic malformations, the mortality rate of eggs, the embryonic duration, the hatch rate as well as the total length, dry weight, yolk sac area and otolith area at hatching based on linear regression analysis. The only parameters exhibiting a significant linear relationship were the RNA content and the RNA/DNA ratio, which decreased with increasing pCO_2. Since non-significant results can be inconclusive (Fisher, 1935; Nakagawa and Foster, 2004), additional statistical support can be provided by 95 % confidence intervals around statistical effect sizes (Nakagawa and Foster, 2004). When calculating effect sizes and 95 % confidence intervals around effect sizes for embryonic malformation, egg mortality rate, hatch rate, total length, dry weight, yolk sac area and sagitta area at hatching a clear overlap with zero was found. Therefore, we concluded that the egg stage of *C. harengus* is tolerant to pCO_2 levels up to 4635 µatm, exceeding future predictions of ∼4300 µatm for Kiel Fjord (Thomsen et al., 2010). However, when using the effect size statistics a positive effect of ocean acidification on the lapillus area and a negative effect on the RNA/DNA ratio were shown.

Our results agree with those of Munday et al. (2009) who found no detectable effect on the embryonic duration, egg survival, hatch rate and size at hatching of the coral reef fish *Amphiprion percula* at pCO_2 levels up to 1030 µatm. *A. percula* is a benthic spawner that lays clutches of eggs on hard surfaces in coral reefs where water pH varies during the day and sometimes reaches values below 8.0. Consequently, the eggs might be adapted to variations in ambient pCO_2 levels (Munday et al., 2009). Herring spawns its benthic eggs on plant substrate or hard substrate during spring, when pCO_2 reaches its minimum value (385 µatm) in Kiel

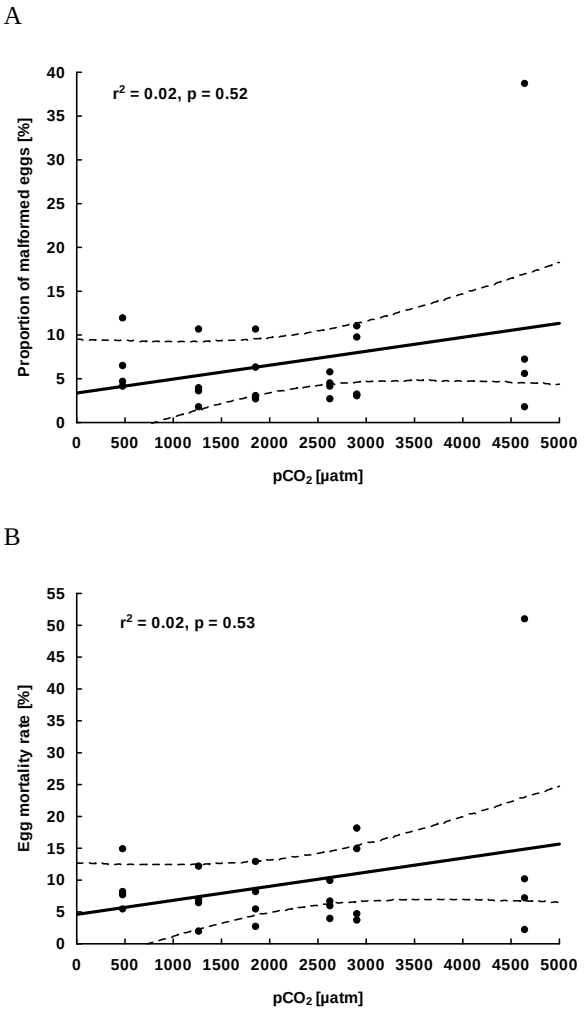


Fig. 1. (A) Proportion of malformed eggs and **(B)** mortality rate during the embryonic development as a function of $p\mathrm{CO_2}$ (4 replicates per treatment level). Data points are percentages of malformed eggs and mortality rates, respectively, of incubation plate 1 of the respective replicate. The solid line shows the regression line, whereas the dashed lines represent the 95 % confidence intervals. The r^2 and P-value were derived from log-transformed data.

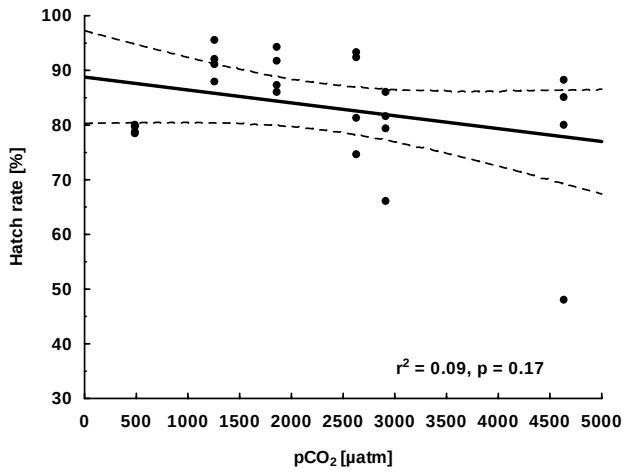


Fig. 2. Hatch rate (%) of Atlantic herring eggs as a function of $p\mathrm{CO_2}$ (4 replicates per treatment level). Data points are percentages of hatched larvae of incubation plate 1 of the respective replicate. The solid line shows the regression line, whereas the dashed lines represent the 95 % confidence intervals. The r^2 and P-value were derived from arcsine transformed data.

Fjord, however, the $p\mathrm{CO_2}$ of the surface water rises from spring to late summer up to a value of 2300 µatm (Thomsen et al., 2010). Thus, herring eggs and larvae develop under rising $p\mathrm{CO_2}$ conditions in Kiel fjord.

Gutowska and Melzner (2009) showed that $p\mathrm{O_2}$ in the perivitelline fluid of cephalopod (*Sepia officinalis*) eggs decrease during their embryonic development, while $p\mathrm{CO_2}$ increases reaching values tenfold higher than in ambient seawater. Accordingly, pH of the perivitelline fluid decreased to 7.2. A decrease of $p\mathrm{O_2}$ during the embryogenesis of shark (*Scyliorhinus canicula*) eggs was observed by Diez and Davenport (1987). Since the egg case serves as a diffusion barrier, high $p\mathrm{CO_2}$ values in developing fish eggs are expected and powerful net proton excretion mechanisms should be present already in early developmental stages to cope with high perivitelline fluid $p\mathrm{CO_2}$ (Melzner et al., 2009). These mechanisms could possibly explain the observed capability of Atlantic herring eggs to cope with elevated $p\mathrm{CO_2}$ conditions.

Kikkawa et al. (2003) found the cleavage and juvenile stages of four teleost species to be the most susceptible to acute CO_2 stress, the most tolerant stages being the embryo, preflexion and flexion stages. The reason for the ontogenetic changes in CO_2 tolerance might be the development of ion-regulatory chloride cells during the course of embryogenesis (Ishimatsu et al., 2004). While cleavage stages have no ion-regulatory chloride cells, they have been found in the yolk sac membrane and body skin of embryos and larvae in various teleost species (Shiraishi et al., 1997; Hiroi et al., 1998; Katoh et al., 2000). Preliminary results from experiments in our laboratory indicate that these chloride cells are also found in herring embryos (Bodenstein and Clemmesen, 2011). The gradual fall in CO_2 tolerance from larval to juvenile stage observed by Kikkawa et al. (2003) was also shown in Atlantic cod (*Gadus morhua*) by Frommel et al. (2011) and may result from the energy demanding transition from one acid-base regulatory site (yolk sac) to the other (gill) (Melzner et al., 2009).

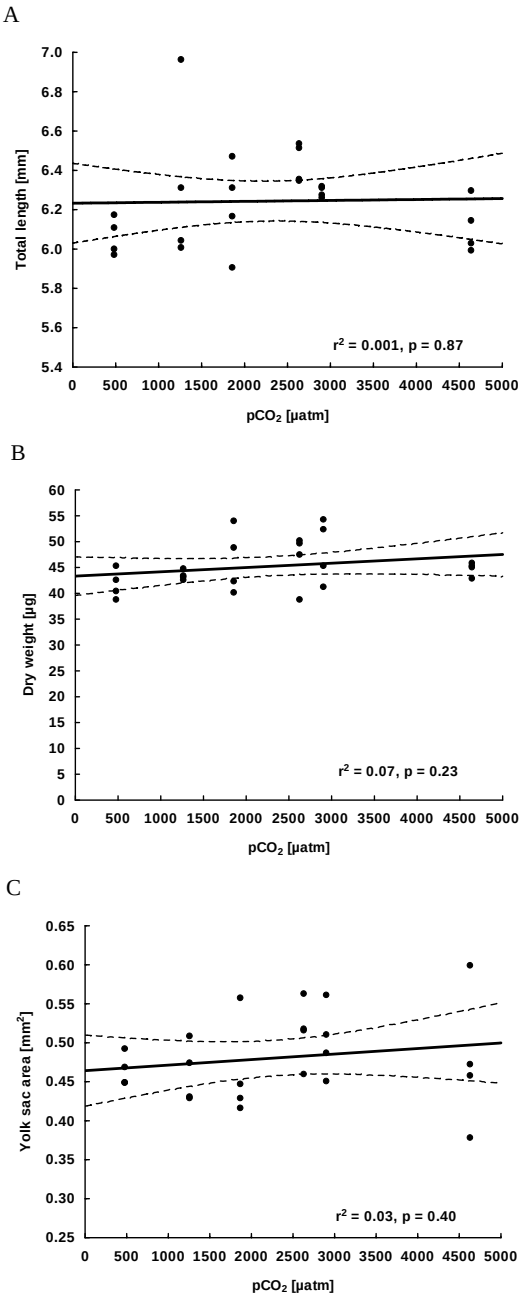


Fig. 3. (A) Total length, **(B)** dry weight and **(C)** yolk sac area of newly hatched Atlantic herring larvae as a function of pCO_2 (4 replicates per treatment level). Data points are mean values of 6 individual larvae. The solid line shows the regression line, whereas the dashed lines represent the 95 % confidence intervals. **(A)** The r^2 and P-value were derived from log-transformed data.

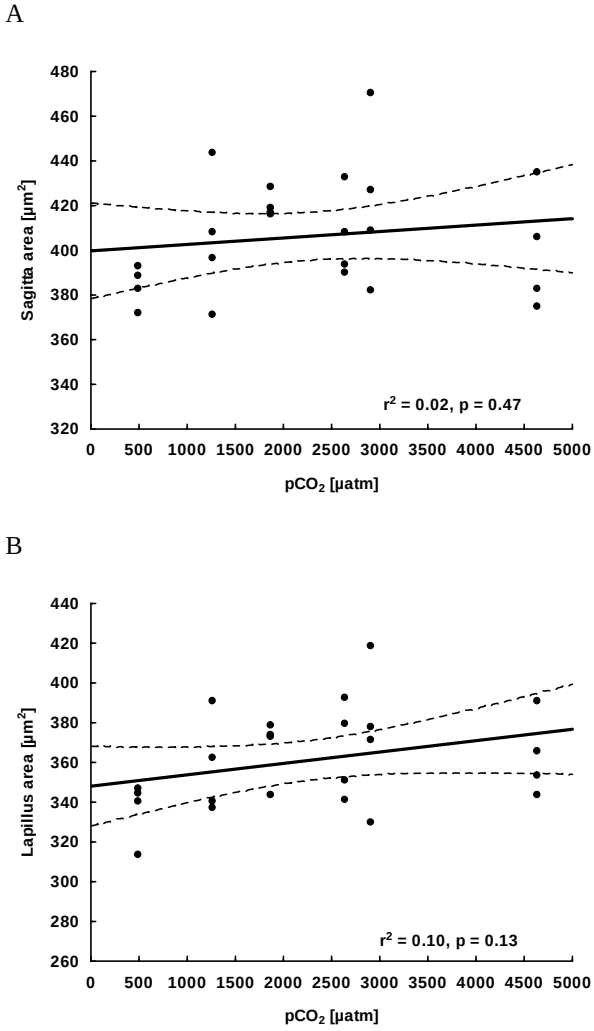


Fig. 4. (A) Sagitta area and **(B)** lapillus area of Atlantic herring larvae at hatch as a function of pCO_2 (4 replicates per treatment level). Data points are mean values of 3 individual larvae (6 otoliths). The solid line shows the regression line, whereas the dashed lines represent the 95 % confidence intervals.

4.2 Biochemical indicator – RNA/DNA ratio

Analyses of larval fish nucleic acid ratios provide a powerful tool to analyze and assess larval growth and condition (Clemmesen, 1994; Pepin et al., 1999; Buckley et al., 2008). The applicability of the nucleic acid ratio is based on the fact that DNA concentrations within individual cells remain fairly constant while RNA concentrations increase as protein synthesis increases (Buckley at al., 1999). The RNA/DNA ratio is therefore used as an indicator of protein biosynthesis and thus metabolic changes (e.g., Bulow, 1970; Buckley, 1984; Bergeron, 1997). It has been shown to be dependent on the nutritional condition and correlated to growth rate (Voss et al., 2006; Huwer et al., 2011). Hence, the RNA/DNA ratio allows to detect a change in the condition of fish larvae at the biochemical level before it can be observed at higher levels of biological organization (Sprague, 1971).

The RNA/DNA ratios of the newly hatched herring larvae were negatively affected by the increasing pCO_2 level. Since

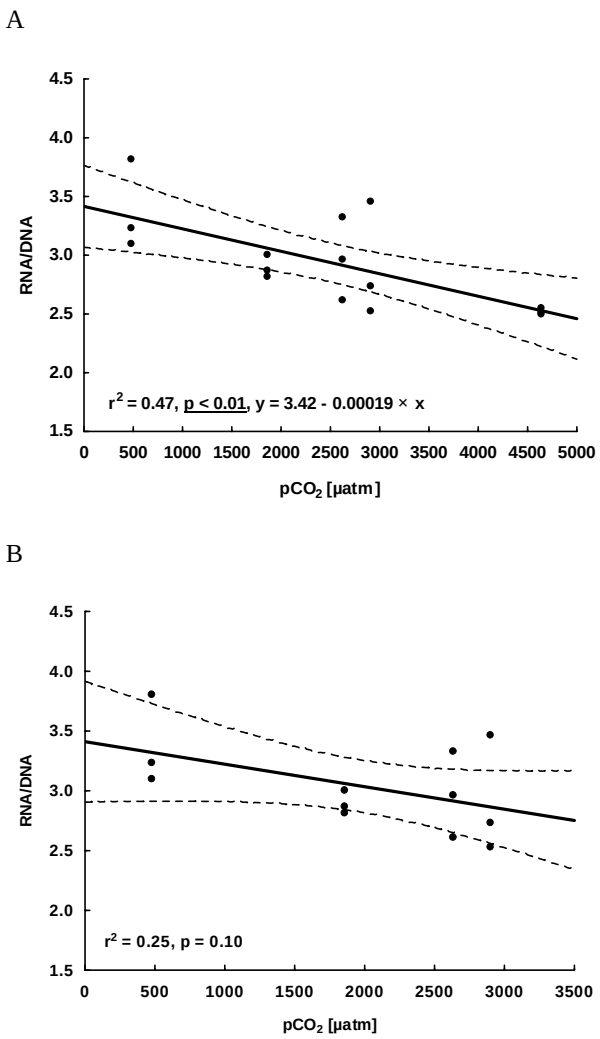


Fig. 5. (A) RNA/DNA ratio of newly hatched Atlantic herring larvae across the entire pCO_2 gradient and **(B)** without the highest treatment level. Due to accidental loss of samples larvae of only 3 replicates per treatment level could be used for nucleic acid determination. Furthermore, nucleic acids could not be examined for treatment level 2. Data points are mean values of 6 individual larvae. The solid line shows the regression line, whereas the dashed lines represent the 95 % confidence intervals.

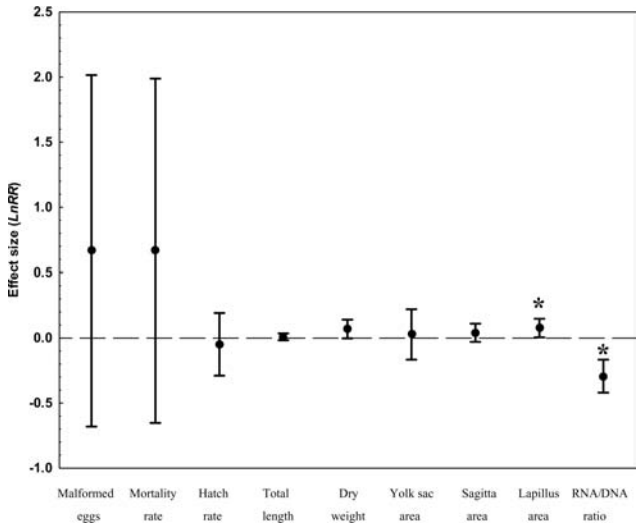


Fig. 6. Effect sizes and 95 % confidence intervals for all examined variables (embryonic malformations, mortality rate of eggs, hatch rate and total length, dry weight, yolk sac area, sagitta area, lapillus area and RNA/DNA ratio of newly hatched Atlantic herring larvae) based on the differences between control (pCO_2 480 µatm) and highest treatment (pCO_2 4635 µatm). The effect size is significant when the 95 % confidence interval does not overlap with zero (*).

the DNA content per larval dry weight did not change in relation to the treatment levels, the number of cells per unit body weight was not affected. The change in the ratio was achieved by a reduction in the amount of RNA, indicating a decrease in protein biosynthesis. So far a reduction in growth and changes in the metabolic profile under hypercapnia have been shown in fish by Foss et al. (2003) in juvenile spotted wolffish (*Anarhichas minor*) and by Michaelidis et al. (2007) in adult gilthead seabream (*Sparus aurata*). Since the negative linear correlation could no longer be detected when the highest treatment level was deleted, a potential tipping point could be located between the two highest pCO_2 levels (2903 and 4635 µatm).

Even though no effects on size and dry weight of newly hatched herring larvae were observed in this study, the question remains whether effects could appear later during the larval phase. Results on the impact of ocean acidification on Atlantic cod larvae from mesocosm experiments indicate that the stressor gradually shows an effect on the developing larvae and causes organ damage during transition phases (Frommel et al., 2011).

A reduction in growth as a result of a decreased protein biosynthesis could have large consequences for larval fish, since the smaller and slower growing individuals have a lower survival potential due to lower feeding success and increased mortality through predation (Houde, 1987, 2008; Anderson, 1988; Leggett and DeBlois, 1994). Pörtner et al. (2004, 2005) and Denman et al. (2011) concluded that reduced growth as a reaction to compensation for energy demanding regulatory mechanisms could lead to lower survival, lower reproductive potential and reduction in population size.

4.3 Effects on otoliths

The otoliths (ear bones) are made of an aragonite structure within a protein matrix and are located in the labyrinth organ of fish. They are involved in sound detection, body orientation and acceleration based on their movement over sensory hairs. They are already formed during the embryonic development (Panella, 1971; Campana and Neilson, 1985) and any change in their size or shape could have implications for the ecological performance and individual fitness of the fish (Gagliano et al., 2008). Contrary to shells and exoskeletons of calcifying organisms, which are directly affected by chemical changes in the ambient seawater, the otoliths are protected in the inner ear of the fish. Therefore, the calcification process is dependent on the chemical composition of the endolymph (Borelli et al., 2003; Payan et al., 2004). In order to deposit aragonite in the protein matrix of the otoliths, the endolymph must be supersaturated with respect to aragonite (Romanek and Gauldie, 1996). Since the aragonite saturation state is correlated with the carbonate ion concentration, which is largely determined by pH, endolymph pH regulation is needed for the aragonite crystallization (Takagi, 2002). Otolith growth may therefore be affected by mechanisms used to compensate extracellular pH decrease. It is noted that in our study the seawater carbonate chemistry was manipulated by adding acid. Compared to CO_2-treated water this results in a decrease (instead of an increase) in the bicarbonate ion concentration and in a larger decrease in the carbonate ion concentration towards higher pCO_2 levels. The bicarbonate ion concentration, however, remains high and should consequently not affect the ion-regulatory ability of the eggs and larvae.

There was a trend for a greater lapillus area at elevated pCO_2 levels, but no significant relation could be found. However, when calculating the effect size a positive effect of ocean acidification on the lapillus area was shown, consistent with previous studies by Checkley et al. (2009) and Munday et al. (2011a). They concluded that pH regulation possibly caused the carbonate ion concentration to increase within the otolith endolymph. However, Munday et al. (2011b) found no effect on spiny damselfish (*Acanthochromis polyacanthus*) otoliths. Juvenile *Sepia officinalis* maintain calcification of the cuttlebone, a structure in the mantle of cuttlefish used for buoyancy control and functioning as an internal skeleton, under pCO_2 levels as high as $\sim$6000 µatm (Gutowska et al., 2008). During long-term exposure to elevated pCO_2 concentrations calcification rates in *Sepia officinalis* even increased, but the spacing of the cuttlebone lamellae decreased which could possibly cause a negative influence on the animal's buoyancy (Gutowska et al., 2010).

The reason for the different responses of the sagitta and the lapillus to an increased pCO_2 shown in our study is unknown. A possible explanation could be that the chemical composition of the endolymph is not spatially uniform. Payan et al. (1999) suggest that increasing bicarbonate and pH gradients occur from the proximal to the distal zone in the saccular endolymph of trout (*Oncorhynchus mykiss*) and turbot (*Psetta maxima*). Thus, ionic gradients within the labyrinth organ might be the reason for the different responses of sagittal and lapillar otoliths.

5 Conclusions and outlook

Even though active taxa with high metabolic rates, such as teleosts and cephalopods, have the ability to compensate acid-base disturbances actively due to their efficient ion-regulatory machinery, their early embryonic stages lack specialized ion-regulatory epithelia. Thus, they may be the true bottleneck for ecological success (Melzner et al., 2009).

The present study has shown that herring eggs can cope at current temperature conditions with an increase in pCO_2, exceeding future predictions of CO_2-driven ocean acidification, but that the yolk sac larvae show a reduced protein biosynthesis capacity and therefore a potential growth reduction. Since the recruitment of fish seems to be determined during the early life stages (Koester et al., 2003; Houde, 2008), knowledge of the factors influencing growth and survival rates of these stages are of great importance in fisheries science. Future studies should analyse carry-over effects that may be passed from adult to offspring and the synergistic effect of changes in pCO_2 and temperature to be able to make predictions, how early life stages of fishes will react to climate-induced changes.

Supplementary material related to this article is available online at: http://www.biogeosciences.net/8/3697/2011/ bg-8-3697-2011-supplement.zip.

Acknowledgements. The authors wish to thank Sebastian Krug, Armin Form and Michael Meyerhöfer for their help and suggestions, Helgi Mempel and Jan Büdenbender for the laboratory work introduction and Uwe Waller for providing a part of the experimental setup. The study was partially supported through the European Community's Seventh Framework Programme (FP7/2007–2013) "European Project on Ocean Acidification" (EPOCA, grant agreement N211384) and the project "Biological Impacts of Ocean ACIDification" (BIOACID), funded by the German Ministry for Education and Research (BMBF).

Edited by: J.-P. Gattuso

References

Anderson, J. T.: A review of size dependent survival during pre-recruit stages of fishes in relation to recruitment, J. Northw. Atl. Fish. Sci., 8, 55–66, 1988.

Belchier, M., Clemmesen, C., Cortes, D., Doan, T., Folkvord, A., Garcia, A., Geffen, A., Høie, H., Johannessen, A., Moksness, E., de Pontual, H., Ramirez, T., Schnack, D., and Sveinsbo, B.: Recruitment studies: Manual on precision and accuracy of tools, ICES Tech. Mar. Environ. Sci., 33, 35 pp., 2004.

Bergeron, J. P.: Nucleic acids in ichthyoplankton ecology: a review, with emphasis of recent advances for new perspectives, J. Fish Biol., 51, 284–302, 1997.

Blaxter, J. H. S.: Herring rearing II. The effect of temperature and other factors on development, Mar. Res. Scot., 5, 19 pp., 1956.

Bodenstein, S. and Clemmesen, C.: Chloride cell distribution in early life stages in Atlantic herring (*Clupea harengus* L.), in prep., 2011.

Borelli, G., Guibbolini, M. E., Mayer-Gostan, N., Priouzeau, F., De Pontual, H., Allemand, D., Puverel, S., Tambutte, E., and Payan, P.: Daily variations of endolymph composition: relationship with the otolith calcification process in trout, J. Exp. Biol., 206, 2685–2692, 2003.

Buckley, L. J.: RNA-DNA ratio: an index of larval fish growth in the sea, Mar. Biol., 80, 291–298, 1984.

Buckley, L. J., Caldarone, E. M., and Ong, T. L.: RNA-DNA ratio and other nucleic-acid based indicators for growth and condition of marine fishes, Hydrobiol., 401, 265–277, 1999.

Buckley, L. J., Caldarone, E. M., and Clemmesen, C.: Multi-species larval fish growth model based on temperature and fluorometrically derived RNA/DNA ratios: results from a meta-analysis, Mar. Ecol. Prog. Ser., 371, 221–232, 2008.

Bulow, F. J.: RNA-DNA ratios as indicators of recent growth rates of a fish, J. Fish. Res. Bd. Can., 27, 2343–2349, 1970.

Caldarone, E. M., Clemmesen, C., Berdalet, E., Miller, T. J., Folkvord, A., Holt, G. J., Olivar, M. P., and Suthers, I. M.: Intercalibration of four spectrofluorometric protocols for measuring RNA/DNA ratios in larval and juvenile fish, Limnol. Oceanogr.-Meth., 4, 153–163, 2006.

Campana, S. E. and Neilson, J. D.: Microstructure of fish otoliths, Can. J. Fish. Aquat. Sci., 42, 1014–1032, 1985.

Checkley, D. M., Dickson, A. G., Takahashi, M., Radich, J. A., Eisenkolb, N., and Asch, R.: Elevated CO_2 enhances otolith growth in young fish, Science, 324, 1683, 2009.

Clemmesen, C.: Improvements in the fluorometric determination of the RNA and DNA content of individual marine fish larvae, Mar. Ecol. Prog. Ser., 100, 177–183, 1993.

Clemmesen, C.: The effect of food availability, age or size on the RNA/DNA ratio of individually measured herring larvae: laboratory calibration. Mar. Biol., 118, 377–382, 1994.

Conway, T. and Tans P., NOAA/ESRL, http://www.esrl.noaa.gov/gmd/ccgg/trends/global.html, last access: November 2011.

Denman, K., Christian, J. R., Steiner, N., Pörtner H. O., and Nojiri, Y.: Potential impacts of future ocean acidification on marine ecosystems and fisheries: current knowledge and recommendations for future research, ICES J. Mar. Sci., 68, 1019–1029, 2011.

Dickson, A. G.: An exact definition of total alkalinity, and a procedure for the estimation of alkalinity and total inorganic carbon from titration data, Deep-Sea Res., 28, 609–623, 1981.

Diez, J. M. and Davenport, J.: Embryonic respiration in the spiny dogfish (*Scyliorhinus canicula* L.), J. Mar. Biol. Assoc. UK, 67, 249–261, 1987.

Doney, S. C., Fabry, V. J., Feely, R. A., and Kleypas, J. A.: Ocean acidification: The other CO_2 problem, Annu. Rev. Mar. Sci., 1, 169–192, 2009.

Dupont, S. and Thorndyke, M. C.: Impact of CO_2-driven ocean acidification on invertebrates early life-history - What we know, what we need to know and what we can do, Biogeosciences Discuss., 6, 3109–3131, doi:10.5194/bgd-6-3109-2009, 2009.

Dupont, S., Lundve, B., and Thorndyke, M.: Near future ocean acidification increases growth rate of the lecithotrophic larvae and juveniles of the sea star *Crossaster papposus*, J. Exp. Zool. (Mol. Dev. Evol.), 314B, 382–389, 2010.

Fabry, V. J.: Ocean science – marine calcifiers in a high-CO_2 ocean, Science, 320, 1020–1022, 2008.

Feely, R. A., Sabine, C. L., Lee, K., Berelson, W., Kleypas, J., Fabry, V. J., and Millero, F. J.: Impact of anthropogenic CO_2 on the $CaCO_3$ system in the oceans, Science, 305, 362–366, 2004.

Feely, R. A., Sabine, C. L., Hernandez-Ayon, J. M., Ianson, D., and Hales, B.: Evidence for upwelling of corrosive "acidified" water onto the continental shelf, Science, 320, 1490–1492, 2008.

Fisher, R. A.: The design of experiments, Oliver and Boyd, Edinburgh, 1935.

Foss, A., Røsnes, B. A., and Øiestad, V.: Graded environmental hypercapnia in juvenile spotted wolffish (*Anarhichas minor* Olafsen): effects on growth, food conversion efficiency and nephrocalcinosis, Aquaculture, 220, 607–617, 2003.

Frommel, A. Y., Maneja, R., Lowe, D., Malzahn, A. M., Geffen, A. J., Folkvord, A., Piatkowski, U., Reusch, T. B. H., and Clemmesen, C.: Severe tissue damage in Atlantic cod larvae under increasing ocean acidification, Nature Clim. Change, doi:http://dx.doi.org/10.1038/NCLIMATE1324, 2011.

Gagliano, M., Depczynski, M., Simpson, S. D., and Moore, J. A. Y.: Dispersal without errors: Symmetrical ears tune into the right frequency for survival, Proc. R. Soc. B., 275, 527–534, 2008.

Gattuso, J.-P. and Hansson, L.: Ocean acidification: background and history, 1–20, in: Ocean acidification, edited by: Gattuso, J.-P. and Hansson, L., Oxford: Oxford University Press, 326 pp., 2011.

Gattuso, J.-P., Lee, K., Rost, B., Schulz, K., and Gao, K.: Approaches and tools to manipulate the carbonate chemistry, 41–52, in: Guide to best practices for ocean acidification research and data reporting, edited by: Riebesell U., Fabry V. J., Hansson L., and Gattuso J.-P., Luxembourg: Publications Office of the European Union, 260 pp., 2010.

Gutowska, M. A. and Melzner, F.: Abiotic conditions in cephalopod (*Sepia officinalis*) eggs: Embryonic development at low pH and high pCO_2, Mar. Biol., 156, 515–519, 2009.

Gutowska, M. A., Pörtner, H. O., and Melzner, F.: Growth and calcification in the cephalopod *Sepia officinalis* under elevated seawater pCO_2, Mar. Ecol. Prog. Ser., 373, 303–309, 2008.

Gutowska, M. A., Melzner, F., Langenbuch, M., Bock, C., Claireaux, G., and Pörtner, H. O.: Acid-base regulatory ability of the cephalopod (*Sepia officinalis*) in response to environmental hypercapnia, J. Comp. Physiol. B, 180, 323–335, 2010.

Hedges, L. V. and Olkin, I.: Statistical methods for meta-analysis, Academic Press, Orlando, FL, 369 pp., 1985.

Hiroi, J., Kaneko, T., Seikai, T., and Tanaka, M.: Developmental sequence of chloride cells in the body skin and gills of Japanese

flounder (*Paralichthys olivaceus*) larvae, Zool. Sci., 15, 455–460, 1998.

Houde, E. D.: Fish early life dynamics and recruitment variability, Am. Fish. Soc. Symp., 2, 17–29, 1987.

Houde, E. D.: Emerging from Hjort's Shadow, J. Northw. Atl. Fish. Sci., 41, 53–70, 2008.

Hu, M. Y., Tseng, Y. C., Stumpp, M., Gutowska, M. A., Kiko, R., Lucassen, M., and Melzner, F.: Elevated seawater pCO_2 differentially affects branchial acid-base transporters over the course of development in the cephalopod *Sepia officinalis*, Ame. J. Physiol.-Reg. I., 300, R1100–R1114, 2011.

Huwer, B., Clemmesen, C., Gronkjaer, P., and Koester, F. W.: Vertical distribution and growth performance of Baltic cod larvae – Field evidence for starvation-induced recruitment regulation during the larval stage?, Prog. Oceanogr., 91, 382–396, 2011.

Ishimatsu, A., Kikkawa, T., Hayashi, M., Lee, K. S., and Kita, J.: Effects of CO_2 on marine fish: Larvae and adults, J. Oceanogr., 60, 731–741, 2004.

Ishimatsu, A., Hayashi, M., and Kikkawa, T.: Fishes in high-CO_2, acidified oceans, Mar. Ecol. Prog. Ser., 373, 295–302, 2008.

Katoh, F., Shimizu, A., Uchida, K., and Kaneko, T.: Shift of chloride cell distribution during early life stages in seawater-adapted killifish, *Fundulus heteroclitus*, Zool. Sci., 17, 11–18, 2000.

Kikkawa, T., Ishimatsu, A., and Kita, J.: Acute CO_2 tolerance during the early developmental stages of four marine teleosts, Environ. Toxicol., 18, 375–382, 2003.

Koester, F. W., Hinrichsen, H. H., Schnack, D., St John, M. A., Mackenzie, B. R., Tomkiewicz, J., Möllmann, C., Kraus, G., Plikshs, M., Makarchouk, A., and Aro, E.: Recruitment of Baltic cod and sprat stocks: identification of critical life stages and incorporation of environmental variability into stock-recruitment relationships, Sci. Mar., 67, 129–154, 2003.

Kroeker, K. J., Kordas, R. L., Crim, R. N., and Singh, G. G.: Meta-analysis reveals negative yet variable effects of ocean acidification on marine organisms, Ecol. Lett., 13, 1419–1434, 2010.

Langdon, C.: Review of experimental evidence for effects of CO_2 on calcification of reef-builders, Proc. 9th Int. Coral Reef Sym., 2, 1091–1098, 2002.

Larsen, B. K., Pörtner, H. O., and Jensen, F. B.: Extra- and intracellular acid-base balance and ionic regulation in cod (*Gadus morhua*) during combined and isolated exposures to hypercapnia and copper, Mar. Biol., 128, 337–346, 1997.

Leggett, W. C. and DeBlois, E.: Recruitment in marine fishes: is it regulated by starvation and predation in the egg and larval stages?, Neth. J. Sea Res., 32, 119–134, 1994.

Le Pecq, J. B. and Paoletti, C.: A new fluorometric method for RNA and DNA determination, Anal. Biochem., 17, 100–107, 1966.

Malzahn, A. M., Clemmesen, C., Wiltshire, K. H., Laakmann, S., and Boersma, M.: Comparative nutritional condition of larval dab *Limanda limanda* and lesser sandeel *Ammodytes marinus* in a highly variable environment, Mar. Ecol. Prog. Ser., 334, 205–212, 2007.

Melzner, F., Gutowska, M. A., Langenbuch, M., Dupont, S., Lucassen, M., Thorndyke, M. C., Bleich, M., and Pörtner, H. O.: Physiological basis for high CO_2 tolerance in marine ectothermic animals: pre-adaptation through lifestyle and ontogeny?, Biogeosciences, 6, 2313–2331, doi:10.5194/bg-6-2313-2009, 2009.

Michaelidis, B., Spring, A., and Pörtner, H. O.: Effects of long-term acclimation to environmental hypercapnia on extracellular acid-base status and metabolic capacity in mediterranean fish *Sparus aurata*, Mar. Biol., 150, 1417–1429, 2007.

Munday, P. L., Donelson, J. M., Dixson, D. L., and Endo, G. G. K.: Effects of ocean acidification on the early life history of a tropical marine fish, P. Roy. Soc. B-Biol. Sci., 276, 3275–3283, 2009.

Munday, P. L., Hernaman, V., Dixson, D. L., and Thorrold, S. R.: Effect of ocean acidification on otolith development in larvae of a tropical marine fish, Biogeosciences, 8, 1631–1641, doi:10.5194/bg-8-1631-2011, 2011a.

Munday, P. L., Gagliano, M., Donelson, J. M., Dixson, D. L., and Thorrold, S. R.: Ocean acidification does not affect the early life history development of a tropical marine fish, Mar. Ecol. Prog. Ser., 423, 211–221, 2011b.

Nakagawa, S. and Foster, T. M.: The case against retrospective statistical power analyses with an introduction to power analysis, Acta. Ethol., 7, 103–108, 2004.

Orr, J. C.: Recent and future changes in ocean carbonate chemistry, 41–66, in: Ocean acidification, edited by: Gattuso, J.-P. and Hansson, L., Oxford, Oxford University Press, 326 pp., 2011.

Panella, G.: Fish otolith: Daily layers and periodical patterns, Science, 173, 1124–1127, 1971.

Payan, P., Edeyer, A., De Pontual, H., Borelli, G., Boeuf, G., and Mayer-Gostan, N.: Chemical composition of saccular endolymph and otolith in fish inner ear: Lack of spatial uniformity, Am. J. Physiol.-Reg. I., 277, R123–R131, 1999.

Payan, P., De Pontual, H., Boeuf, G., and Mayer-Gostan, N.: Endolymph chemistry and otolith growth in fish, C. R. Palevol., 3, 535–547, 2004.

Pepin, P., Evans, G. T., and Shears, T. H.: Patterns of RNA/DNA ratios in larval fish and their relationship to survival in the field, ICES J. Mar. Sci., 56, 697–706, 1999.

Pierrot, D., Lewis, E., and Wallace, D. W. R.: MS Excel program developed for CO_2 system calculations. Macro for low salinities, Carbon Dioxide Information Analysis Center, Oak Ridge National Laboratory, US Department of Energy, Oak Ridge, Tennessee, 2006.

Pörtner, H. O. and Peck, M. A.: Climate change effects on fishes and fisheries: Towards a cause-and-effect understanding, J. Fish Biol., 77, 1745–1779, 2010.

Pörtner, H. O., Langenbuch, M., and Reipschlaeger, A.: Biological impact of elevated ocean CO_2 concentrations: Lessons from animal physiology and earth history, J. Oceanogr., 60, 705–718, 2004.

Pörtner, H. O., Langenbuch, M., and Michaelidis, B.: Synergistic effects of temperature extremes, hypoxia, and increases in CO_2 on marine animals: From earth history to global change, J. Geophys. Res.-Oceans, 110, C09S10, doi:10.1029/2004jc002561, 2005.

Raven, J., Caldeira, K., Elderfield, H., Hoegh-Guldberg, O., Liss, P. S., Riebesell, U., Shepherd, J., Turley, C., and Watson, A. J.: Ocean acidification due to increasing atmospheric carbon dioxide, Policy Document 12/05, London, The Royal Society, 60 pp., 2005.

Ries, J. B., Cohen, A. L., and McCorkle, D. C.: Marine calcifiers exhibit mixed responses to CO_2-induced ocean acidification, Geology, 37, 1131–1134, 2009.

Romanek, C. S. and Gauldie, R. W.: A predictive model of otolith growth in fish based on the chemistry of the endolymph, Comp.

Biochem. Physiol. A, 114, 71–79, 1996.

Rosenthal, H. and Alderdice, D. F.: Sublethal effects of environmental stressors, natural and pollutional, on marine fish eggs and larvae, J. Fish. Res. Board Can., 33, 2047–2065, 1976.

Roy, R. N., Roy, L. N., Vogel, K. M., Portermoore, C., Pearson, T., Good, C. E., Millero, F. J., and Campbell, D. M.: The dissociation constants of carbonic acid in seawater at salinities 5 to 45 and temperatures 0 to 45 °C, Mar. Chem., 44, 249–267, 1993.

Shiraishi, K., Kaneko, T., Hasegawa, S., and Hirano, T.: Development of multicellular complexes of chloride cells in the yolk-sac membrane of tilapia (*Oreochromis mossambicus*) embryos and larvae in seawater, Cell Tissue Res., 288, 583–590, 1997.

Siegenthaler, U., Stocker, T. F., Monnin, E., Lüthi, D., Schwander, J., Stauffer, B., Raynaud, D., Barnola, J.-M., Fischer, H., Masson-Delmotte, V., and Jouzel, J.: Stable carbon cycle-climate relationship during the late Pleistocene, Science, 310, 1313–1317, 2005.

Sprague, J. B.: Measurement of pollutant toxicity to fish III. Sublethal effects and "safe" concentrations, Water Res., 5, 245–266, 1971.

Stoll, M. H. C., Bakker, K., Nobbe, G. H., and Haese, R. R.: Continuous-flow analysis of dissolved inorganic carbon content in seawater, Anal. Chem., 73, 4111–4116, 2001.

Takagi, Y.: Otolith formation and endolymph chemistry: a strong correlation between the aragonite saturation state and pH in the endolymph of the trout otolith organ, Mar. Ecol. Prog. Ser., 231, 237–245, 2002.

Thomsen, J., Gutowska, M. A., Saphörster, J., Heinemann, A., Trübenbach, K., Fietzke, J., Hiebenthal, C., Eisenhauer, A., Körtzinger, A., Wahl, M., and Melzner, F.: Calcifying invertebrates succeed in a naturally CO2-rich coastal habitat but are threatened by high levels of future acidification, Biogeosciences, 7, 3879–3891, doi:10.5194/bg-7-3879-2010, 2010.

Voss, R., Clemmesen, C., Baumann, H., and Hinrichsen, H.-H.: Baltic sprat larvae: Coupling food availability, larval condition and survival, Mar. Ecol. Prog. Ser., 308, 243–254, 2006.

nature climate change

LETTERS

PUBLISHED ONLINE: 22 JANUARY 2012 | DOI: 10.1038/NCLIMATE1372

Detecting regional anthropogenic trends in ocean acidification against natural variability

T. Friedrich[1]*, A. Timmermann[1]*, A. Abe-Ouchi[2], N. R. Bates[3], M. O. Chikamoto[2], M. J. Church[4], J. E. Dore[5], D. K. Gledhill[6], M. González-Dávila[7], M. Heinemann[1], T. Ilyina[8], J. H. Jungclaus[8], E. McLeod[9], A. Mouchet[10] and J. M. Santana-Casiano[7]

Since the beginning of the Industrial Revolution humans have released ∼500 billion metric tons of carbon to the atmosphere through fossil-fuel burning, cement production and land-use changes[1,2]. About 30% has been taken up by the oceans[3]. The oceanic uptake of carbon dioxide leads to changes in marine carbonate chemistry resulting in a decrease of seawater pH and carbonate ion concentration, commonly referred to as ocean acidification. Ocean acidification is considered a major threat to calcifying organisms[4–6]. Detecting its magnitude and impacts on regional scales requires accurate knowledge of the level of natural variability of surface ocean carbonate ion concentrations on seasonal to annual timescales and beyond. Ocean observations are severely limited with respect to providing reliable estimates of the signal-to-noise ratio of human-induced trends in carbonate chemistry against natural factors. Using three Earth system models we show that the current anthropogenic trend in ocean acidification already exceeds the level of natural variability by up to 30 times on regional scales. Furthermore, it is demonstrated that the current rates of ocean acidification at monitoring sites in the Atlantic and Pacific oceans exceed those experienced during the last glacial termination by two orders of magnitude.

Skeletons and shells of marine calcifiers are made of different crystalline forms of calcium carbonate, such as calcite or aragonite. A decrease in the saturation state of calcium carbonate can result in decreased calcification and increased dissolution of calcium carbonate[5,7,8]. As aragonite is the more soluble form, its saturation state (Ω_{Ar}, see Supplementary Information for details) can be regarded as a measure for ocean acidification.

Here we present results from a model simulation over 1,300 years (800–2099 AD) that was conducted with a state-of-the-art coupled carbon cycle–climate model (MPI-ESM, see Supplementary Information for details) forced by the most recent reconstructions of solar and volcanic radiative perturbations, land-use changes, aerosols and orbital variations. The model is also subject to historical CO_2 emissions and the A1B greenhouse-gas emission scenario (Fig. 1a,b; ref. 9). The knowledge of the pre-industrial, natural variability (defined here by the years 800 AD to 1750 AD) of the surface aragonite saturation state (Ω_{Ar}^{surf}) will permit a robust determination of regional signal-to-noise ratios using recent observed and future projected anthropogenic negative trends in Ω_{Ar}^{surf}. Furthermore, we present simulations of the Last Glacial Maximum (LGM) conducted with the models LOVECLIM and MIROC (see Supplementary Information for details) to quantify the impact of the reconstructed ∼90 ppmv increase in atmospheric CO_2 between the LGM and pre-industrial times on Ω_{Ar}^{surf}. This will allow us to put recent anthropogenic trends in ocean acidification into the context of the most recent natural event of carbon cycle–climate reorganization.

According to the MPI-ESM simulation of the pre-industrial surface waters, local marine ecosystems have been exposed to a diverse range of natural variability in both the amplitude of the annual cycle and the interannual variability of Ω_{Ar}^{surf} (Supplementary Fig. S1). For example, the Galápagos Islands are located in the centre of upwelling-driven variability, whereas reefs in the Caribbean are only exposed to small interannual changes in carbonate ion concentration. This spatial heterogeneity in natural variability, together with the local equilibration timescale of surface waters to increasing atmospheric pCO_2 and the net air–sea flux of CO_2 are likely to affect the regional impacts of ocean acidification on calcifying marine ecosystems.

Figure 1c,d shows the simulated spatially averaged Ω_{Ar}^{surf} for the main coral reef locations in the Pacific, the Southern Indian Ocean and the Caribbean (see Fig. 1e, dashed blue lines for the averaging regions). Our results reveal that current levels of Ω_{Ar}^{surf} are already considerably lower than the long-term pre-industrial mean. In a recent study[8] a linear relationship was proposed between Ω_{Ar}^{surf} and coral calcification rates. Using this estimate, our model results suggest that calcification rates at coral reef locations in the western tropical Pacific and the Caribbean may have already dropped by ∼15% with respect to their pre-industrial values. This result extends the findings of a previous study[10] that used present-day Ω_{Ar}^{surf} as a reference. Using historical CO_2 emissions and the A1B greenhouse-gas emission scenario (Fig. 1a,b), a drop to about 60% in coral reef calcification is projected for the end of the twenty-first century. It is important to note that carbonate chemistry is only one factor controlling coral calcification rates. Other factors include the effects of light, nutrients and temperature. The synergistic or combined effects are as yet poorly understood.

[1]International Pacific Research Center (IPRC), SOEST, University of Hawai'i, Honolulu, Hawaii 96822, USA, [2]Research Institute for Global Change, Japan Agency for Marine Science and Technology, Yokohama 236-0001, Japan, [3]Bermuda Institute of Ocean Sciences, St George's, GE 01, Bermuda, [4]Department of Oceanography, University of Hawai'i, Honolulu, Hawaii 96822, USA, [5]Department of Land Resources and Environmental Sciences, Montana State University, Bozeman, Montana 59717, USA, [6]NOAA AOML, Cooperative Institute of Marine and Atmospheric Studies, Rosenstiel School of Marine and Atmospheric Science, University of Miami, Miami, Florida 33149, USA, [7]Departamento de Química, Facultad de Ciencias del Mar, Universidad de Las Palmas de Gran Canaria, Las Palmas de Gran Canaria 35.017, Spain, [8]Max Planck Institute for Meteorology, Hamburg 20146, Germany, [9]The Nature Conservancy, Hawai'i Field Office, Honolulu, Hawaii 96817, USA, [10]Département Astrophysique, Géophysique et Océanographie, Université de Liège, Liège B-4000, Belgium. *e-mail: tobiasf@hawaii.edu; axel@hawaii.edu.

© 2012 Macmillan Publishers Limited. All rights reserved.

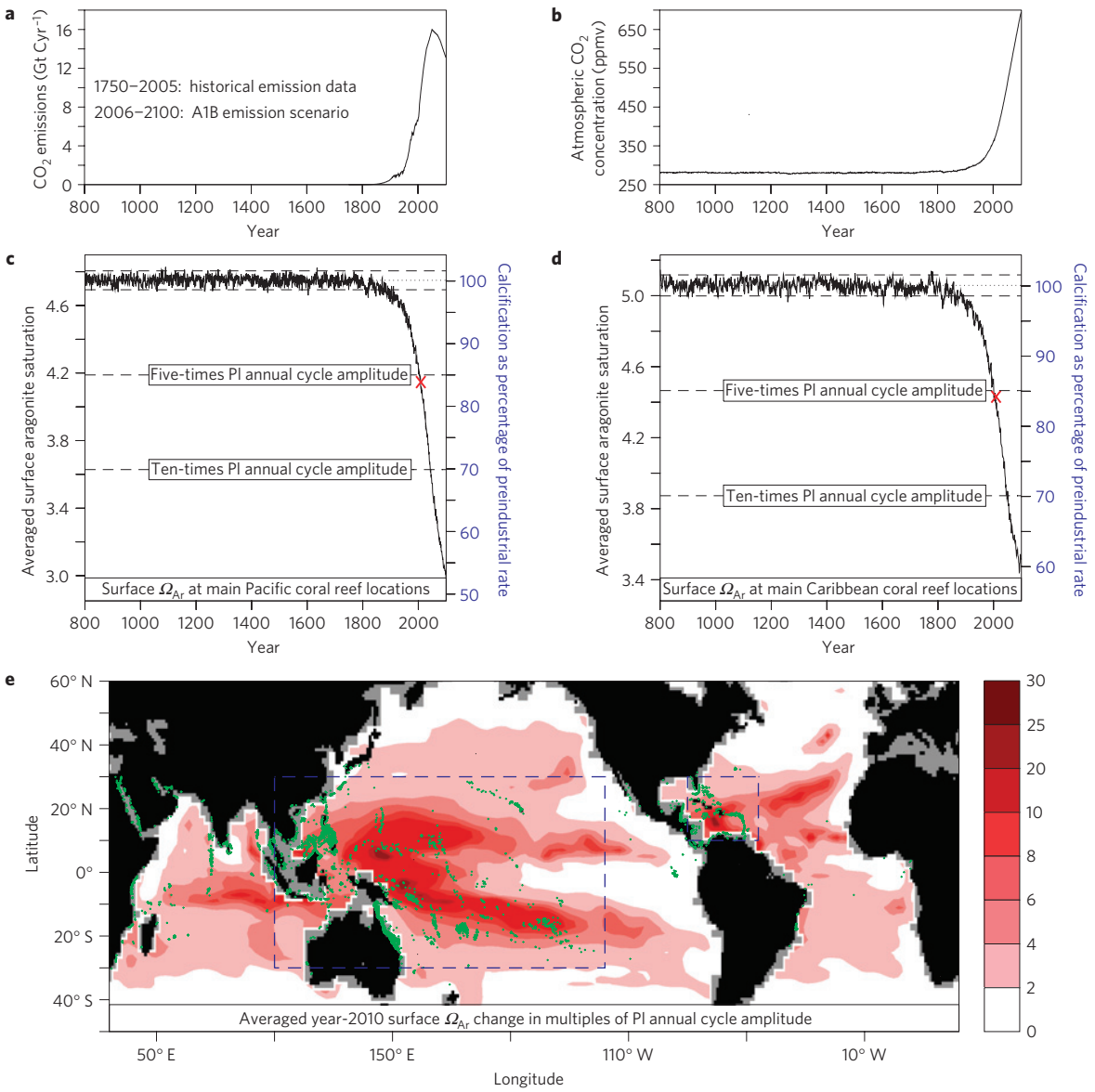


Figure 1 | Regional signal-to-noise ratio of Ω_{Ar}^{surf}. a, Carbon dioxide (CO_2) emissions (Gt Cyr^{-1}) used as forcing of the MPI-ESM. **b**, Atmospheric CO_2 concentration (ppmv) simulated by the MPI-ESM. **c**, Simulated annual-mean Ω_{Ar}^{surf} averaged over the main Pacific coral reef locations (green dots in the blue rectangle in **e**). Dotted line: pre-industrial (PI) average of simulated Ω_{Ar}^{surf}. Dashed lines: simulated amplitude of mean pre-industrial annual cycle of Ω_{Ar}^{surf}. Right axis: calcification rate with respect to pre-industrial level using ref. 8. Red cross indicates year 2010. **d**, Same as **c** for the main Caribbean coral reef regions (green dots in the blue rectangle in **e**). **e**, Simulated year-2010 change in Ω_{Ar}^{surf} with respect to the simulated pre-industrial average in multiples of simulated pre-industrial amplitude of the annual cycle.

The uncertainty of the ecological response to these projected changes is considerable at present.

The pre-industrial amplitude of the local annual cycle in Ω_{Ar}^{surf} can be regarded as a metric of natural variability to which aragonite-calcifying organisms have been exposed to over a long time and to which they have successfully adapted. Any reduction of Ω_{Ar}^{surf} below the minima given by the range of the unperturbed annual cycle will be interpreted here as a stress to these organisms and their associated ecosystems. Past anthropogenic CO_2 emissions have already pushed the aragonite saturation state of seawater far outside the range of natural variability (Fig. 1c,d). The difference between current and pre-industrial Ω_{Ar}^{surf} exceeds the natural annual cycle range already by a factor of five for the Pacific and Atlantic warm pool reefs (Fig. 1c,d).

Overall, the simulated ratio between the anthropogenic change (Ω_{Ar}^{surf} (2010)–Ω_{Ar}^{surf} (pre-industrial)) and the natural variability (expressed in terms of the local, pre-industrial annual cycle range) differs substantially on a regional scale (Fig. 1e). As a result of large natural variability induced by annual to interannual changes in upwelling, equatorial Pacific coral reefs from Galápagos to western Kiribati are projected to experience the most moderate relative decline of the aragonite saturation state due to anthropogenic CO_2 emissions. However, a recent study[11] confirmed a decline in some coral species occurring also in the eastern equatorial Pacific. Further to the west, and in off-equatorial regions of Micronesia, Polynesia and Melanesia, a smaller natural variability in Ω_{Ar}^{surf} (Supplementary Fig. S1) leads to a larger anthropogenic signal-to-noise ratio, attaining values of 6–30. It should be noted here that the western equatorial Pacific is the only region in our simulation in which the pre-industrial interannual variability of Ω_{Ar}^{surf} is slightly larger than its annual cycle (Supplementary Fig. S1). However, even when assuming that the pre-industrial interannual variability is a measure

© 2012 Macmillan Publishers Limited. All rights reserved.

for the corals' 'comfort' zone, present-day values of Ω_{Ar}^{surf} are already exceeding this threshold by a factor of between two and ten.

A large amplitude in the annual cycle corresponds to a smaller signal-to-noise ratio, as documented for higher latitudes in Fig. 1e. However, the combination of a large annual cycle range on top of the anthropogenic signal can lead, in fact, to an earlier undersaturation. On the basis of empirical estimates for the seasonal cycle of carbonate chemistry parameters, it was concluded[12] that undersaturation with respect to aragonite will occur at the surface of the Southern Ocean roughly by the year 2030. Our simulation can confirm this prediction, and it further documents that values of $\Omega_{Ar}^{surf} < 1$ will be found in 30–50% of the ocean poleward of 40° S by the year 2100.

Ocean acidification is anticipated to affect marine ecosystems well beyond the coral reef realm[13–17]. Laboratory and mesocosm experiments show diverse impacts of ocean acidification on different groups of organisms, or even individual species within the same group. Recent studies using open ocean samples indicate that individual coccolithophores mass[18] and foraminfera shell weight[14] decline as CO_2 concentrations increase.

Observations in the North Pacific[19] reported a −0.06 change in surface ocean pH between 1991 and 2006 in the upper 500 m, of which 52% can be attributed to natural variability. Given the irregular sampling of chemical parameters in most parts of the ocean, a detection of ocean acidification and the determination of its local magnitude can be challenging. Our model-based estimates of anthropogenic change in Ω_{Ar}^{surf} reveal that since the mid-twentieth century the anthropogenic signals have exceeded the pre-industrial interannual variability by at least a factor of two in vast areas of the global oceans (Fig. 2). Applying an exceedance factor of two as a detection limit it becomes apparent that the anthropogenic impact on Ω_{Ar}^{surf} is detectable in almost the entire ocean by year 2010, except for the tropical eastern Pacific and the frontal regions near the subpolar gyres. In the Caribbean, a region with very high signal-to-noise ratio, the detection limit was already exceeded at the beginning of the twentieth century. By around 1980, the anthropogenic signal exceeded the natural range in this region by a factor of ten, in accordance with previous observational estimates[20] (Supplementary Fig. S2). According to our modelling results, a robust detection of the anthropogenic signal in Ω_{Ar}^{surf} in the eastern equatorial Pacific against the background variability has been possible for the past 10–20 years. By year 2020–2060, the anthropogenic signal in this region will exceed the natural variability range in aragonite saturation state by at least a factor of five.

In our effort to compare the twentieth century trends of Ω_{Ar}^{surf} with other geochemical trends from the Late Quaternary, we select another benchmark period: the last glacial termination. The concomitant change in atmospheric pCO_2 from ∼190 ppmv to ∼280 ppmv between 17,000 and 11,000 years BP (ref. 21) represents the most recent increase of such magnitude preceding the industrial revolution. Here we study the effect of the deglacial CO_2 rise on the surface aragonite saturation using two different Earth system models: LOVECLIM and MIROC (see Supplementary Information for details). Switching from LGM to pre-industrial equilibrium conditions generates a decrease of the surface aragonite saturation state of 0.88 (0.64) units in LOVECLIM (MIROC), which is in good agreement with reconstructions[18] (Supplementary Fig. S4). These simulated changes (Fig. 3a), although being somewhat larger even than the change from pre-industrial to present-day conditions simulated by the MPI-ESM (Fig. 3f), occurred over a time period that was two orders of magnitude longer than the industrial period.

To compare the simulated Ω_{Ar}^{surf} changes and their rate of change with previous observations, we focus on continuous records of Ω_{Ar}^{surf} from several monitoring sites in the Pacific[22] and the Atlantic[20,23,24] covering the last two to three decades (Fig. 3b–e). The observed decadal changes are dominated by a trend signal, a pronounced

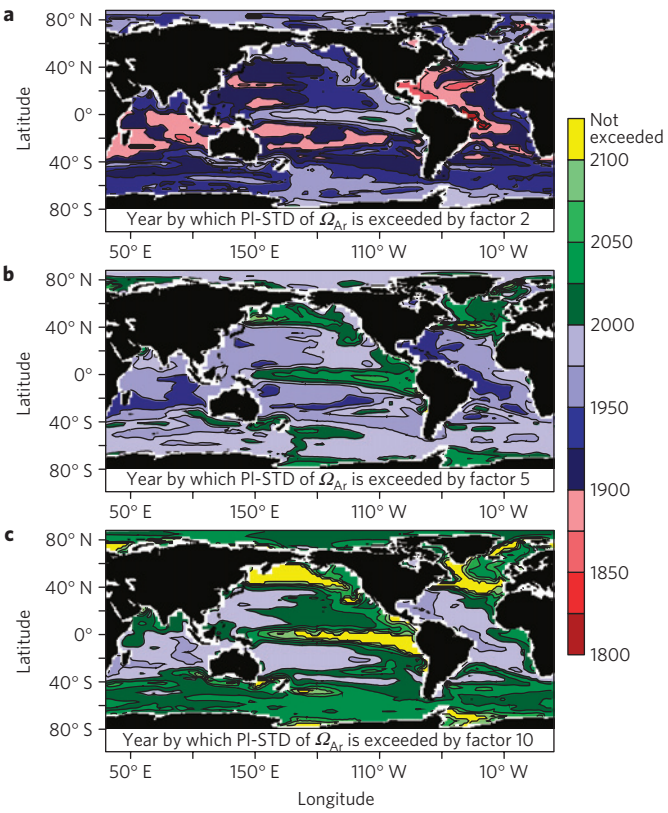


Figure 2 | First year of detectability of anthropogenic Ω_{Ar}^{surf} trend. a, Year by which simulated changes in Ω_{Ar}^{surf} (with respect to the pre-industrial mean) exceed simulated pre-industrial (800–1750 AD) standard deviation (PI-STD, based on annual-mean values, see also Supplementary Fig. S1) by a factor of two. **b**,**c** same as **a** for factors of five and ten respectively. Areas with yellow shading indicate regions where the pre-industrial standard deviation is not exceeded by the respective factor during the course of the model simulation.

annual cycle and interannual variability (Supplementary Fig. S2). The observed trends off the Canary Islands, Bermuda, Hawaii, and in the Caribbean amount to about −0.09, −0.04, −0.08, −0.09 units per decade respectively (Fig. 3i–l). These values are higher than the simulated globally averaged trends over the entire twentieth century, but close to the simulated global values for the twenty-first century (Fig. 3m,n).

The observed present-day, anthropogenic rate of change in Ω_{Ar}^{surf} is one to two orders of magnitude larger than estimated for the last glacial termination (Fig. 3h–l) . Already, the weakest observed rate of change in Bermuda exceeds the glacial–interglacial trend by a factor of 32(56) over the LOVECLIM (MIROC) estimates for the last glacial termination. In the Caribbean, where the largest regional trends are reported, the Ω_{Ar}^{surf} decrease over the past ∼20 years reaches 78(136) times the glacial–interglacial rate of change documented by LOVECLIM (MIROC).

Summarizing, we conclude that it is virtually certain that anthropogenic trends already exceed the natural variability on regional scales and are hence detectable in many areas of the world's ocean. However, the eastern tropical Pacific is an exception and exhibits the weakest signal-to-noise ratio owing to high ENSO-related natural variability in carbonate chemistry.

An unresolved question to address in future studies is how the detectability of anthropogenic Ω_{Ar}^{surf} trends translates into the detectability of the anthropogenic influence on the functionality of marine ecosystems. Marine organisms are exposed to a multitude of other anthropogenic stress factors. Corals, for instance, experience

© 2012 Macmillan Publishers Limited. All rights reserved.

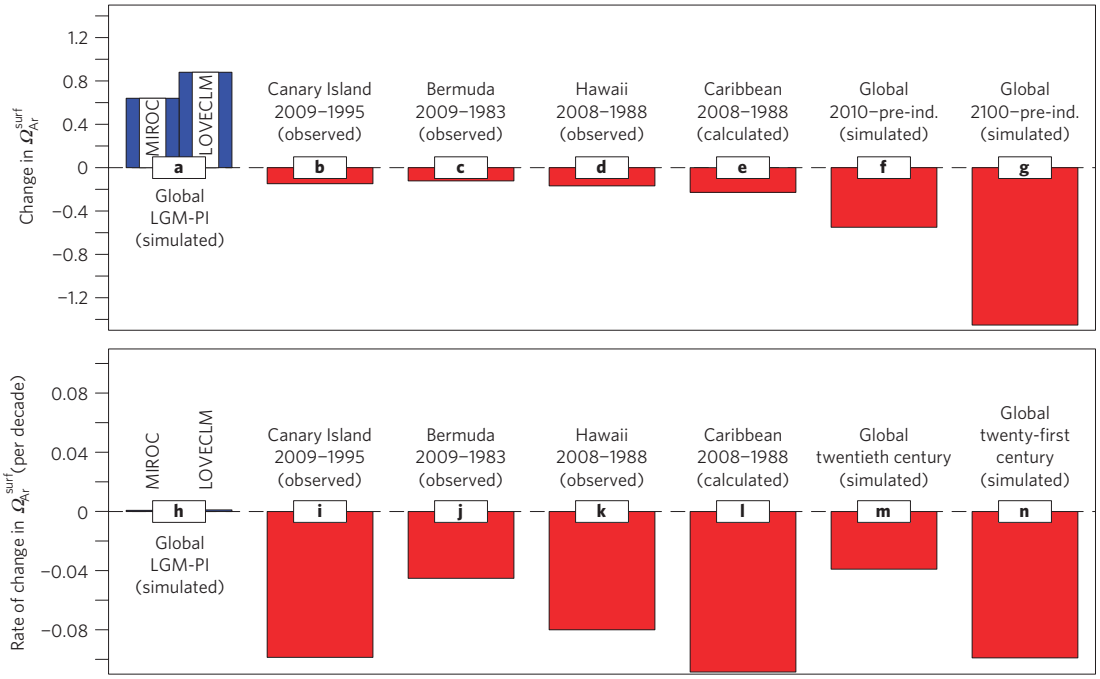


Figure 3 | Current Ω_{Ar}^{surf} trends in the context of the Last Glacial Termination. a, Globally averaged change in Ω_{Ar}^{surf} between pre-industrial (PI) times and the Last Glacial Maximum as simulated by the LOVECLIM and the MIROC model respectively. **b–e**, Trends in Ω_{Ar}^{surf} for the European Station for Time series in the Ocean (1995–2009) (**b**), the Bermuda Atlantic Time-series Study (1983–2009) (**c**), Station ALOHA (1988–2008) (**d**) and the Caribbean region (1988–2008; ref. 20) (**e**). **f**,**g**, Globally averaged change in Ω_{Ar}^{surf} as simulated by the MPI-ESM for the years 2010 (**f**) and 2100 (**g**) respectively (with respect to the pre-industrial average). **h–n**, Rates of change in Ω_{Ar}^{surf} (per decade) for glacial–interglacial as in **a** (**h**), observations as in **b–e** (**i–l**) and the twentieth (**m**) and twenty-first century (**n**), respectively.

increasing stress from ocean acidification, surface warming[25] and coastal pollution[26]. These stress factors probably do not simply add up, but combine in a species-dependent manner[27]. Tropical surface temperatures are projected to increase at a rate that would lead to massive coral bleaching and mortality in the next three to five decades[28]. Combined with a detectable change due to reduced ocean aragonite saturation and the corresponding estimated drop in carbonate accretion of ∼15% since the industrial revolution (Fig. 1c,d), severe reductions are likely to occur in coral reef diversity, structural complexity and resilience by the middle of this century.

Received 29 September 11; accepted 8 December 2011; published online 22 January 2012

References

1. Houghton, R. Revised estimates of the annual net flux of carbon to the atmosphere from changes in land use and land management 1850–2000. *Tellus B* **55,** 378390 (2003).
2. Boden, T., Marland, G. & Andres, R. *Global, Regional, and National Fossil-Fuel CO_2 Emissions* Tech. Rep. http://dx.doi.org/10.3334/CDIAC/00001V2010 (Carbon Dioxide Information Analysis Center, Oak Ridge National Laboratory, US Department of Energy, 2010).
3. Canadell, J. *et al.* Contributions to accelerating atmospheric CO_2 growth from economic activity, carbon intensity, and efficiency of natural sinks. *Proc. Natl Acad. Sci. USA* **104,** 18866–18870 (2007).
4. Raven, J. *et al. Ocean Acidification Due to Increasing Atmospheric Carbon Dioxide* 60, Tech. Rep. (The Royal Society, 2005).
5. Kleypas, J. A. *et al. Impacts of Ocean Acidification on Coral Reefs and Other Marine Calcifiers: A Guide for Future Research*. Tech. Rep. (NSF, NOAA, and the US Geological Survey, 2006).
6. National Research Council Report *Ocean Acidification: A National Strategy to Meet the Challenges of a Changing Ocean* 152 (National Academy of Science, 2010).
7. Riebesell, U. *et al.* Reduced calcification of marine plankton in response to increased atmospheric CO_2. *Nature* **407,** 364–367 (2000).
8. Langdon, C. & Atkinson, M. J. Effect of elevated pCO_2 on photosynthesis and calcification of corals and interactions with seasonal change in temperature/irradiance and nutrient enrichment. *J. Geophys. Res.* **110,** C09S07 (2005).
9. Jungclaus, J. H. *et al.* Climate and carbon-cycle variability over the last millennium. *Clim. Past* **6,** 723–737 (2010).
10. Cooley, S. R., Lucey, N., Kite-Powell, H. & Doney, S. C. Nutrition and income from molluscs today imply vulnerability to ocean acidification tomorrow. *Fish Fish.* http://dx.doi.org/10.1111/j.1467-2979.2011.00424.x (2011).
11. Manzello, D. P. Coral growth with thermal stress and ocean acidification: lessons from the eastern tropical Pacific. *Coral Reefs* **29,** 749–758 (2010).
12. McNeil, B. & Matear, R. Southern Ocean acidification: A tipping point at 450-ppm atmospheric CO_2. *Proc. Natl Acad. Sci. USA* **105,** 18860–18864 (2008).
13. Feely, R. A., Sabine, C., Hernandez-Ayon, J. M., Ianson, D. & Hales, B. Evidence for upwelling of corrosive acidified water onto the continental shelf. *Science* **320,** 1490–1492 (2008).
14. Moy, A. D., Howard, W. R., Bray, S. G. & Trull, T. W. Reduced calcification in modern Southern Ocean planktonic foraminifera. *Nature Geosci.* **2,** 276–280 (2009).
15. Yamamoto-Kawai, M., McLaughlin, F. A., Carmack, E. C., Nishino, S. & Shimada, K. Aragonite undersaturation in the Arctic Ocean: Effects of ocean acidification and sea ice melt. *Science* **326,** 1098–1100 (2009).
16. Ilyina, T. *et al.* Early detection of ocean acidification effects on marine calcification. *Glob. Biogeochem. Cycles* **23,** GB1008 (2009).
17. Atzesu-Scott, K. *et al.* Calcium carbonate saturation states in the waters of the Canadian Arctic Archipelago and the Labrador Sea. *J. Geophys. Res.* **115,** C11021 (2010).
18. Beaufort, L. *et al.* Sensitivity of coccolithophores to carbonate chemistry and ocean acidification. *Nature* **476,** 80–83 (2011).
19. Byrne, R. H., Mecking, S., Feely, R. A. & Liu, X. Direct observations of basin-wide acidification of the North Pacific Ocean. *Geophys. Res. Lett.* **37,** L02601 (2010).
20. Gledhill, D. K., Wanninkhof, R., Millero, F. J. & Eakin, M. Ocean acidification of the Greater Caribbean Region 1996–2006. *J. Geophys. Res.* **113,** C10031 (2008).
21. Lüthi, D. *et al.* High-resolution carbon dioxide concentration record 650,000–800,000 years before present. *Nature* **453,** 379–382 (2008).
22. Dore, J. E., Lukas, R., Sadler, D. W., Church, M. J. & Karl, D. M. Physical and biogeochemical modulation of ocean acidification in the central North Pacific. *Proc. Natl Acad. Sci. USA* **106,** 12235–12240 (2009).
23. Bates, N. R. Interannual variability of the oceanic CO_2 sink in the subtropical gyre of the North Atlantic Ocean over the last two decades. *J. Geophys. Res.* **112,** C09013 (2007).

© 2012 Macmillan Publishers Limited. All rights reserved.

24. Santana-Casiano, J. M., González-Dávila, M., Rueda, M.-J., Llinás, O. & González-Dávila, E. F. The interannual variability of oceanic CO_2 parameters in the northeast Atlantic subtropical gyre at the ESTOC site. *Glob. Biogeochem. Cycles* **21,** GB1015 (2007).
25. Hoegh-Guldberg, O. Coral bleaching, Climate Change and the future of the worlds coral reefs. *Rev. Mar. Freshwat. Res.* **50,** 839–866 (1999).
26. Doney, S. C. The growing human footprint on coastal and open-ocean biogeochemistry. *Science* **328,** 1512–1516 (2010).
27. Fabricius, K. *et al*. Losers and winners in coral reefs acclimatized to elevated carbon dioxide concentrations. *Nature Clim. Change* **1,** 165–169 (2011).
28. McLeod, E. *et al*. Warming seas in the coral triangle: Coral reef vulnerability and management implications. *Coast. Manag.* **38,** 518–539 (2010).

Acknowledgements

This study was funded by The Nature Conservancy (www.nature.org) and National Science Foundation (NSF) grant #0902133. AT is supported by the Japan Agency for Marine-Earth Science and Technology (JAMSTEC) through its sponsorship of the International Pacific Research Center and NSF grant #0902551. We thank S. Lorenz for conducting the MPI-ESM experiments. This is International Pacific Research Center contribution number 829.

Author contributions

The paper was written by T.F. and A.T. Data analysis and interpretation were carried out by T.F., A.T., M.H., D.K.G., N.R.B., M.J.C., J.E.D., M.G-D., J.M.S-C., T.I., J.H.J., M.O.C., E.M. and A.M. Observational data were provided by N.R.B., M.J.C., J.E.D., M.G-D. and J.M.S-C. Data for the Caribbean region were calculated by D.K.G. Last Glacial Maximum modelling data were provided by T.F., M.O.C., A.A-O. and A.M.

Additional information

The authors declare no competing financial interests. Supplementary information accompanies this paper on www.nature.com/natureclimatechange. Reprints and permissions information is available online at http://www.nature.com/reprints. Correspondence and requests for materials should be addressed to T.F. or A.T.

© 2012 Macmillan Publishers Limited. All rights reserved.

nature climate change

LETTERS

PUBLISHED ONLINE: 11 DECEMBER 2011 | DOI: 10.1038/NCLIMATE1324

Severe tissue damage in Atlantic cod larvae under increasing ocean acidification

Andrea Y. Frommel[1]*, Rommel Maneja[1,2], David Lowe[3], Arne M. Malzahn[4,5], Audrey J. Geffen[2], Arild Folkvord[2], Uwe Piatkowski[1], Thorsten B. H. Reusch[1] and Catriona Clemmesen[1]

Ocean acidification, caused by increasing atmospheric concentrations of CO_2 (refs 1–3), is one of the most critical anthropogenic threats to marine life. Changes in seawater carbonate chemistry have the potential to disturb calcification, acid–base regulation, blood circulation and respiration, as well as the nervous system of marine organisms, leading to long-term effects such as reduced growth rates and reproduction[4,5]. In teleost fishes, early life-history stages are particularly vulnerable as they lack specialized internal pH regulatory mechanisms[6,7]. So far, impacts of relevant CO_2 concentrations on larval fish have been found in behaviour[8,9] and otolith size[10,11], mainly in tropical, non-commercial species. Here we show detrimental effects of ocean acidification on the development of a mass-spawning fish species of high commercial importance. We reared Atlantic cod larvae at three levels of CO_2, (1) present day, (2) end of next century and (3) an extreme, coastal upwelling scenario, in a long-term ($2\frac{1}{2}$ months) mesocosm experiment. Exposure to CO_2 resulted in severe to lethal tissue damage in many internal organs, with the degree of damage increasing with CO_2 concentration. As larval survival is the bottleneck to recruitment, ocean acidification has the potential to act as an additional source of natural mortality, affecting populations of already exploited fish stocks.

Present average CO_2 levels in the atmosphere have already exceeded 380 ppm and are predicted to further increase by 0.5% per year throughout this century, a rate 100 times faster than seen in the past 650,000 years[12]. Approximately a third of excess CO_2 in the atmosphere will be dissolved in ocean waters, leading to an estimated drop in pH of 0.4 units ($pCO_2 \sim 1{,}000\,\mu$atm) globally by the year 2100 and up to 0.8 units ($pCO_2 \sim 2{,}000\,\mu$atm) by the year 2300 (refs 1–3). Locally, the effects can be even more severe, especially in coastal regions with upwelling of oxygen-poor, CO_2-rich water, and pCO_2 values above 4,000 µatm in the future could be reached in habitats where cod larvae occur[13].

The Atlantic cod, *Gadus morhua*, has a wide distribution throughout the North Atlantic Ocean. Several eastern Atlantic populations are found from the high Arctic down to North and Baltic seas, where they experience very different conditions in terms of temperature, salinity, oxygen and present pCO_2 levels. For example, in the Baltic Sea pCO_2 concentrations up to 2,300 µatm have recently been measured in the Kiel Fjord[13], close to where the eastern Baltic cod stock spawns, and 1,200 µatm in the deep waters of the Bornholm Basin (Frommel *et al.*, manuscript in preparation), an important spawning ground for the western Baltic cod stock. The Norwegian coastal cod used in this study live and spawn in a large number of fjords along the entire Norwegian coast and near the Lofoten Islands[14]. These high latitudes are assumed to be particularly impacted by future ocean acidification, owing to cold water, high primary productivity and melting of sea ice[15–17], and pH values are predicted to approach 7.7 over most of the coastal Arctic Ocean by 2100 (ref. 18). Recent models even calculate that the decrease in pH could be doubled in some parts of the Arctic Ocean as a result of gas hydrates destabilized by warming ocean temperatures releasing large amounts of methane, which in turn are respired by methanogenic bacteria to CO_2 (ref. 19).

Adult teleost fishes are thought to be relatively robust to changes in ambient pH, as they are able to control their acid–base balance by bicarbonate buffering, mainly across the gills and via the kidneys[20,21]. However, early life-history stages, lacking gills, may not be as competent in regulating their internal acid–base balance[6,7] and are thus predicted to be impacted more heavily by increasing pCO_2 levels.

In this study, we experimentally tested this prediction and exposed larvae of Norwegian coastal cod to three levels of pCO_2 (control: 380 µatm, medium: 1,800 µatm and high: 4,200 µatm) from newly fertilized eggs to seven weeks post-hatch (see Supplementary Information). Cod larvae are difficult to rear in the laboratory and require space, near-natural conditions and live prey to survive. Our large (2,300 l) outdoor mesocosms mimicked natural conditions for the larvae as closely as possible, including flow-through of fresh water and natural zooplankton prey from the fjord. Using such large experimental units allowed only three replicates. It also limited the ability to monitor larval numbers inside the tanks and, because dead larvae decayed before they could be counted, our direct mortality estimates are restricted to the differences in the number of larvae placed into the tanks at the beginning (10,000 per tank) and the ones counted out at the end of the experiment (control: 153 ± 134, medium: 324 ± 513, high: 73 ± 70; mean ± s.d.), which revealed no significant difference. However, a retrospective power analysis informed us that we might have missed a mortality difference as large as 50% (setting $\alpha = 5\%$) because of the small replicate number ($n = 3$) and substantial variation within treatments.

Larval growth was positively affected by high pCO_2 between 25 and 46 days post-hatch (dph) (Fig. 1). At 32 dph cod larvae from the high treatment had attained 59% more dry weight relative to the control whereas the medium treatment was not significantly different (Supplementary Table S1). Interestingly, growth in the control animals stagnated between 25 and 32 dph, indicating that the larvae were re-allocating their energy from growth to development of internal organs. This age coincides

[1]Leibniz-Institute of Marine Sciences IFM-GEOMAR, Duesternbrooker Weg 20, 24105 Kiel, Germany, [2]Department of Biology, University of Bergen, PO Box 7803, N-5020, Bergen, Norway, [3]Plymouth Marine Laboratory, Prospect Place, The Hoe, Plymouth PL1 3DH, UK, [4]Alfred Wegener Institute for Polar and Marine Research, Biologische Anstalt Helgoland, Ostkaje 1118, 27498 Helgoland, Germany, [5]Helmholtz-Zentrum Geesthacht Centre for Materials and Coastal Research, Institute for Coastal Research, Max-Planck-Straße 1, 21502 Geesthacht, Germany. *e-mail: afrommel@ifm-geomar.de.

© 2011 Macmillan Publishers Limited. All rights reserved.

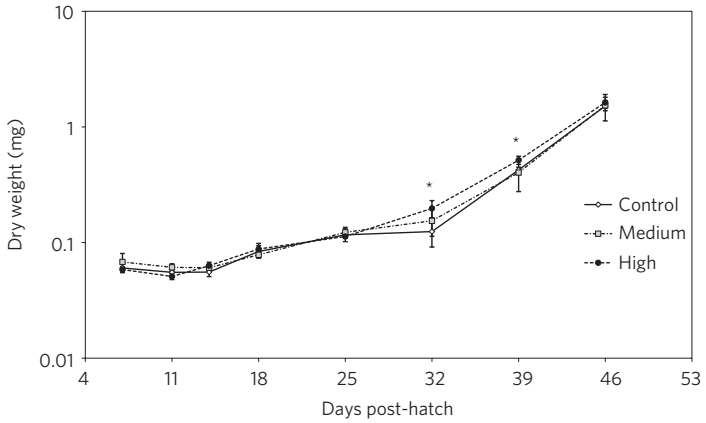


Figure 1 | Larval growth in dry weight over the entire experimental period. Dry weight with standard deviation for each of the three treatments over seven weeks post-hatch on a logarithmic scale. Asterisks indicate significant differences between control and high treatment. $N = 60$ larvae per replicate. For statistics see Supplementary Table S1.

with the developmental stages 8 and 9, a phase of intense transition where critical structural changes take place in all major landmarks[22,23]. Most importantly, the respiratory, feeding and locomotion structures greatly increase their function. As larvae grow in size, they become limited by cutaneous respiration as their body volume to surface ratio decreases and they must switch from cutaneous to branchial respiration. At stage 9 in cod larvae, respiration becomes fully branchial as gill filaments increase in number and secondary lamellae are formed, while the gills become completely covered by the opercular membrane, augmenting unidirectional flow over the gills. The larvae under increased pCO_2, however, continued to allocate energy to growth, at the cost of organ development, and may have outgrown the critical surface-to-volume ratio necessary for effective cutaneous acid–base regulation. Furthermore, the observed increase in growth was based on lipid instead of protein biosynthesis. One effect when larvae cannot extrude protons is respiratory acidosis, which interferes with different metabolic pathways[24] and may cause a shift from aerobic to anaerobic metabolism[25]. This in turn can influence protein biosynthesis[26]. Although the protein content and RNA/DNA ratio—as indicator for the protein synthesis capacity—remained relatively constant, the amount of lipid storage peaked during the same time interval as the growth in the treatment larvae (Fig. 2). At 32 dph, cod larvae from the medium treatment had 61% increase and from the high treatment 97% increase in lipid content compared with the control (Supplementary Table S1). No significant difference in fatty acid composition could be identified (analysis of variance (ANOVA), $p > 0.05$). Higher lipid content, although an energy store, may not necessarily be beneficial to the organism when it accumulates as droplets in specific organs.

Our determination of the critical phase of organ developments and internal re-adjustments coincided with major histological damage observed in the larvae under elevated pCO_2 treatments. Severe tissue damage was found in the liver, pancreas, kidney, eye and the gut of larvae 32 dph (Fig. 3), with the degree of damage significantly increasing with pCO_2 concentration (Fig. 4, Supplementary Table S2). Throughout the livers of CO_2 treated larvae, large lipid vacuoles were observed. Typical lipid vacuoles of control animals were in the order of 3–4 μm in diameter, whereas impacted cod had lipid vacuoles in the order of 7–9 μm in diameter. Furthermore, atypical liver morphology and necrotic hepatocytes were found in the medium and high treatments, respectively. Enlarged lipid vacuoles in the liver result from lysosomal dysfunction and the breakdown of the lysosomal vascular system in the hepatocytes, a response often found in fish from chemical pollution[27], which can lead to the observed necrosis.

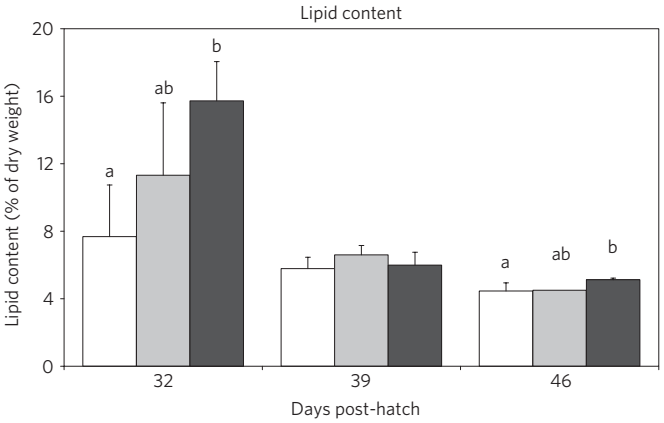


Figure 2 | Lipid content of larval cod for the last three sampling intervals. Mean lipid content of three replicates as a percentage of dry weight with standard deviation for each of the three treatments (control, white bars; medium, grey bars; high, black bars) at 32 dph, 39 dph and 46 dph. Letters indicate significant differences, 'a' is significantly different from 'b', but both are not significantly different from 'ab'. For test statistics, see Supplementary Table S1. $N = 30$ larvae per replicate.

Liver damage as a consequence of high pCO_2 concentrations has previously been found in freshwater fish[28], as well as in isolated Antarctic fish hepatocytes[26]. Similar to the liver, there was evidence of vacuolation in the epithelial cell cytoplasm of the kidney, with changes in the staining characteristics. Furthermore, loss in structural integrity of the pronephric tubules and atrophy was observed. Atrophy may be a reflection of cellular dysfunction or, possibly, a breakdown in the desmosomes that bind adjacent cells together. In the high CO_2 treatment, some of the neck cells of kidney tubules stained darker and showed signs of breaking up, an indication of organ failure. In the pancreas, the damage consisted mainly of alterations to tissue architecture whereby the normal pyramidal exocrine cells sitting on a well-defined basement membrane were replaced by rounded cells on an irregular basement membrane and the rosettes of the acini that form around zymogen granules were absent. Damage to the eyes was mainly visible as vacuoles associated with the choroid layer and between the pigmented layer and the outer layer of the cones. Furthermore, the pigmented layer often had an irregular profile at increased pCO_2 concentrations. In the gut, the connective tissue was found to be highly fragile with the gut epithelium readily detaching from the basement membrane. Furthermore, bacteria were present in the gut

© 2011 Macmillan Publishers Limited. All rights reserved.

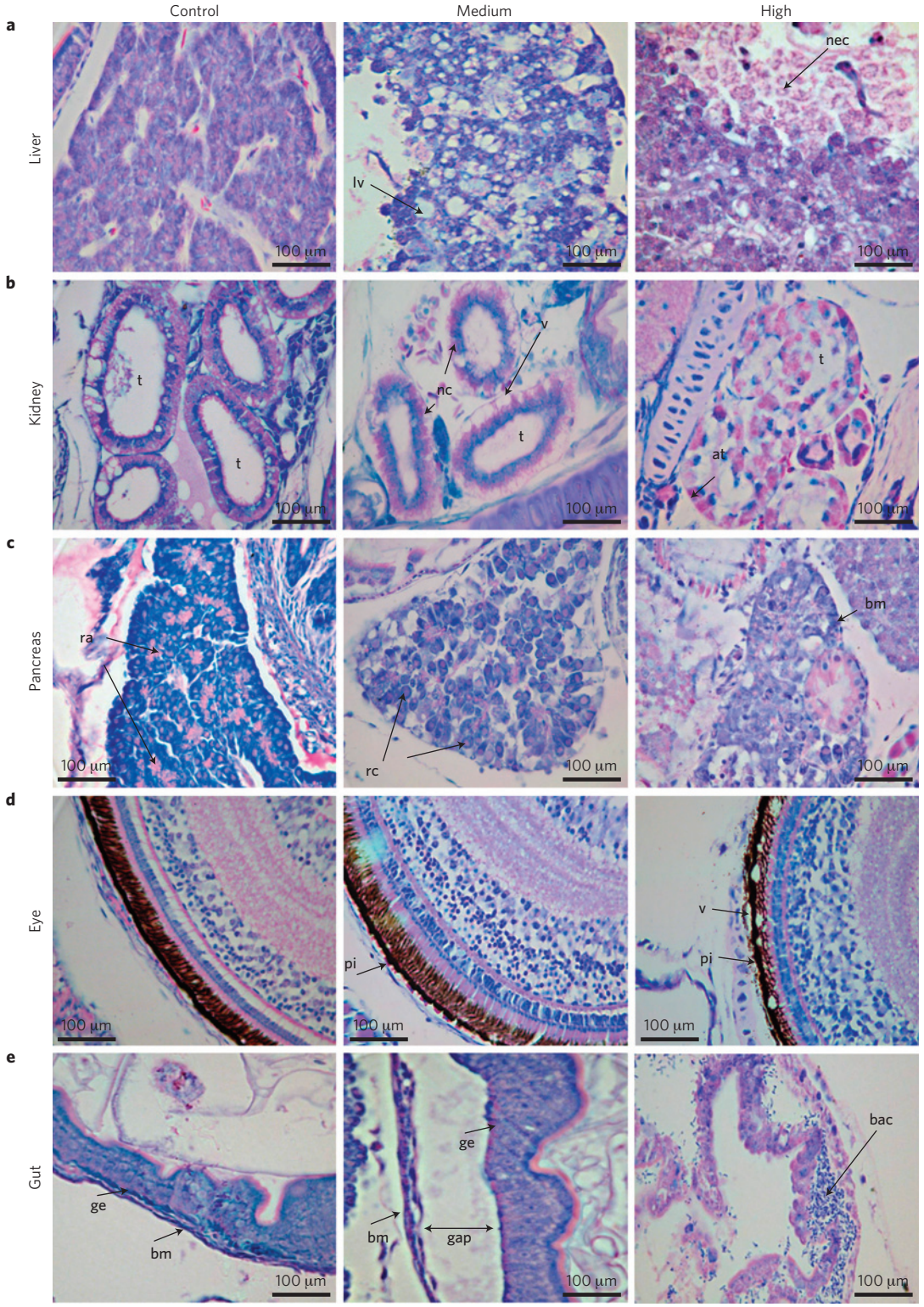
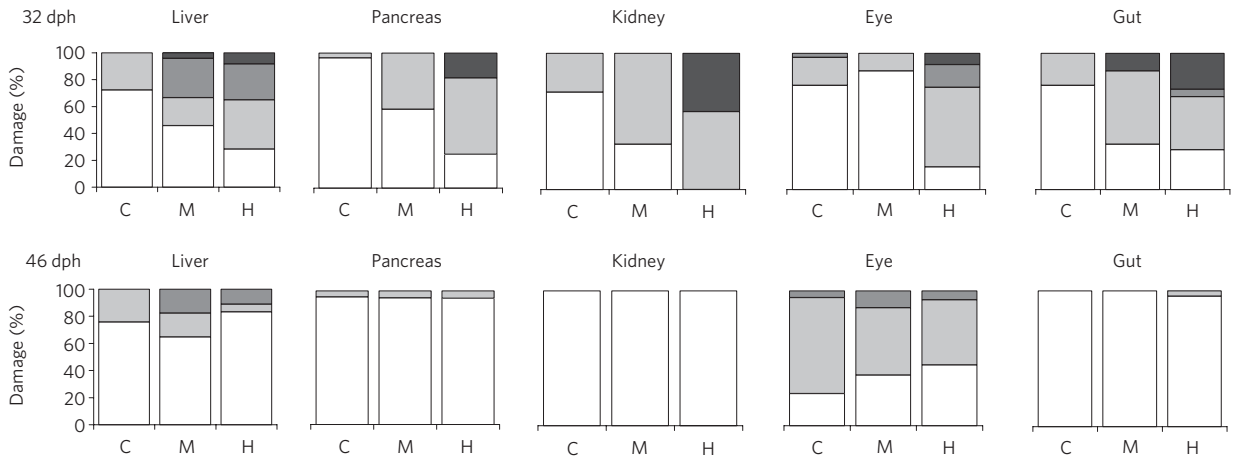


Figure 3 | Tissue damage from histological sections in larvae under increased pCO_2. Histological sections of liver (**a**), kidney (**b**), pancreas (**c**), eye (**d**) and gut (**e**) from cod larvae at three different pCO_2 treatments: control (left panels), medium (centre panels) and high (right panels). Note, gut sections are for control (left) and high (centre) treatments only as the connectivity issue of the gut was only found in the high treatment; bacteria (right) were found in the medium and high treatments. The structures identified in these sections are: liver: enlarged lipid vacuoles (lv), necrotic hepatocytes (nec); kidney: pronephric tubules (t), neck cells breaking up (nc); vacuoles (v), atrophy (at); pancreas: rosettes of acini (ra), rounded cells (rc), basement membrane (bm); eye: pigmented layer (pi), vacuoles (v); gut: gut epithelium (ge), basement membrane (bm), bacteria (bac).

lumen and in the connective tissues lying between the basement membrane and the gut epithelium in many of the samples from 32 dph. Bacterial infection and high parasite load may be an indication of a weakened immune system. There were indications of a possible impact on the structure of the musculature in the high treatment, but as this parameter is highly dependent on the plane of section,

© 2011 Macmillan Publishers Limited. All rights reserved.



Damage	Liver	Pancreas	Kidney	Eye	Gut
0	Normal	Normal	Normal	Normal	Normal
1	Enlarged lipid vacs	Atypical	Vacuoles present	Vacuoles present	Lifting
2	Enlarged lipid vacs abundant	–	–	Vacuoles moderate	Disruption
3	Degenerated tissue	Degenerated tissue	Fragmented tissue	Vacuoles abundant	Bacteria

Figure 4 | Quantification of degree of damage in various organs with increasing pCO_2. Mean percentage of larvae at 32 and 46 dph showing the degree of damage (white bars are normal, with shading increasing with increasing severity of damage) at three different treatment levels (control (C), medium (M) and high (H)) for each different type of tissue. Vacs, vacuoles. $N = 16$–27 larvae per replicate. ($N_{C32} = 26$, $N_{C46} = 27$; $N_{M32} = 24$, $N_{M46} = 19$; $N_{H32} = 16$, $N_{H46} = 24$). For statistical tests, see Supplementary Tables S2 and S3.

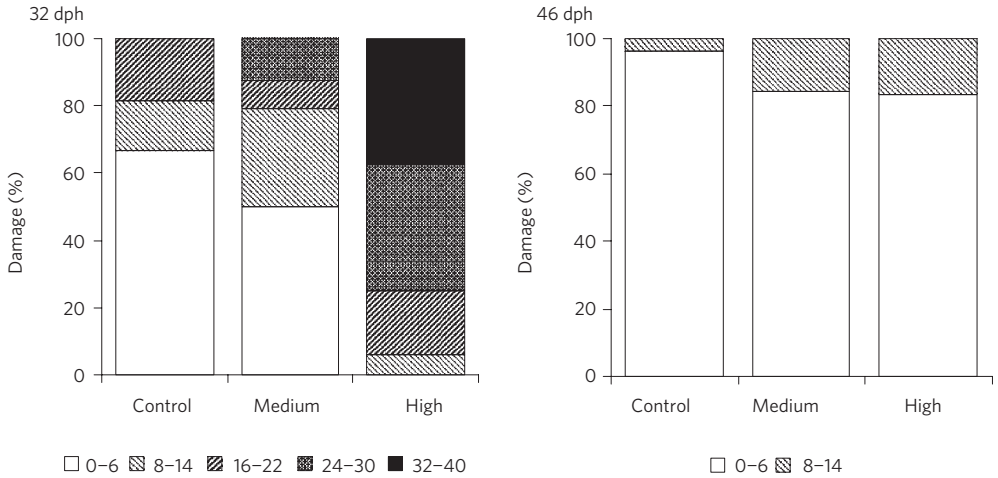


Figure 5 | Quantification of total damage to larvae at increasing pCO_2. Percentage of larvae exhibiting different degrees of total damage (calculated by the total index *Tot-I*) at 32 and 46 dph and three different treatment levels (control, medium and high). The damage is shown as five levels with normal as white bars, and shading increasing with increasing severity of damage.

this parameter was considered unsafe. No effects were found in the heart, gills, skeleton or skin.

Most of the histological damage found was classified as regressive changes that terminate in functional impairment or loss of the organ and involve deposits, architectural and structural alterations, degeneration, atrophy and necrosis (after ref. 29). The health status of each larvae examined, calculated by the total index *Tot-I*, revealed that 12% of the larvae in the medium pCO_2 treatment and 75% of the larvae in the high pCO_2 treatment had severe damage in multiple tissues (Fig. 5). After the onset of gill-mediated acid–base regulation, this damage disappeared and no effect of CO_2 was found at day 46 (for statistics see Supplementary Table S3). This study demonstrates widespread tissue damage as a result of ocean acidification during a critical life-cycle phase within a mass-spawning, commercially important fish. Our data complement other studies that found behavioural effects in response to increased pCO_2 in tropical fish, which have a very different developmental pattern and a much faster developmental rate[8,9,30]. Laboratory experiments are always limited as they simulate increasing CO_2 at a rate much higher than predicted and therefore neglect the potential for genetic adaptation. However, as cod are long-lived fish with a relatively long generation time, the pace of evolutionary adaptation to cope with ocean acidification is probably relatively slow on absolute time scales. Furthermore, like many other commercially exploited fish, cod already experience high selection pressure from fisheries, in addition to other environmental stressors such as pollution, temperature, salinity and oxygen changes. Although we did not directly test for mortality rates, our data on severe tissue damage suggest that ocean acidification will negatively impact the recruitment of mass-spawning fishes because of enhanced mortality

© 2011 Macmillan Publishers Limited. All rights reserved.

rates. Adverse effects of ocean acidification have thus to be added to the growing list of anthropogenic disturbances affecting already exploited fish stocks.

Received 16 May 2011; accepted 9 November 2011; published online 11 December 2011

References

1. Caldeira, K. & Wickett, M. E. Anthropogenic carbon and ocean pH. *Nature* **425,** 365 (2003).
2. IPCC *Climate Change 2007: The Physical Science Basis* (eds Solomon, S. *et al.*) (Cambridge Univ. Press, 2007).
3. Caldeira, K. & Wickett, M. E. Ocean model predictions of chemistry changes from carbon dioxide emissions to the atmosphere and ocean. *J. Geophys. Res.* **110,** C09S04 (2005).
4. Fabry, V. J., Seibel, B. A., Feely, R. A. & Orr, J. C. Impacts of ocean acidification on marine fauna and ecosystem processes. *ICES J. Mar. Sci.* **65,** 414–432 (2008).
5. Pörtner, H. O., Langenbuch, M. & Reipschlaeger, A. Biological impact of elevated ocean CO_2 concentration: Lessons from animal physiology and Earth history. *J. Oceanogr.* **60,** 705–718 (2004).
6. Morris, R. *Acid Toxicity and Aquatic Animals* (Cambridge Univ., 1989).
7. Sayer, M. D. J., Reader, J. P. & Dalziel, T. R. K. Fresh-water acidification—effects on the early-life stages of fish. *Rev. Fish Biol. Fish.* **3,** 95–132 (1993).
8. Munday, P. L. *et al.* Ocean acidification impairs olfactory discrimination and homing ability of a marine fish. *Proc. Natl Acad. Sci. USA* **106,** 1848–1852 (2009).
9. Munday, P. L. *et al.* Replenishment of fish populations is threatened by ocean acidification. *Proc. Natl Acad. Sci. USA* **107,** 12930–12934 (2010).
10. Munday, P. L., Hernaman, V., Dixson, D. L. & Thorrold, S. R. Effect of ocean acidification on otolith development in larvae of a tropical marine fish. *Biogeosciences* **8,** 1631–1641 (2011).
11. Checkley, D. M. *et al.* Elevated CO_2 enhances otolith growth in young fish. *Science* **324,** 1683 (2009).
12. Siegenthaler, U. *et al.* Stable carbon cycle-climate relationship during the late Pleistocene. *Science* **310,** 1313–1317 (2005).
13. Thomsen, J. *et al.* Calcifying invertebrates succeed in a naturally CO_2-rich coastal habitat but are threatened by high levels of future acidification. *Biogeosciences* **7,** 3879–3891 (2010).
14. Nordeide, J. T. Coastal cod and north-east Arctic cod — do they mingle at the spawning grounds in Lofoten? *Sarsia* **83,** 373–379 (1998).
15. Fabry, V. J., McClintock, J. B., Mathis, J. T. & Grebmeier, J. M. Ocean acidification at high latitudes: The bellweather. *Oceanography* **22,** 160–171 (2009).
16. Steinacher, M., Joos, F., Frolicher, T. L., Plattner, G. K. & Doney, S. C. Imminent ocean acidification in the Arctic projected with the NCAR global coupled carbon cycle-climate model. *Biogeosciences* **6,** 515–533 (2009).
17. Bellerby, R. G. J., Olsen, A., Furevik, T. & Anderson, L. G. in *The Nordic Seas: An Integrated Perspective Oceanography, Climatology, Biogeochemistry, and Modeling* (eds Drange, H., Dokken, T., Furevik, T., Gerdes, R. & Berger, W.) 189–197 (Geophysical Monograph Series,Vol. 158, AGU, 2005).
18. Denman, K., Christian, J. R., Steiner, N., Pörtner, H. O. & Nojiri, Y. Potential impacts of future ocean acidification on marine ecosystems and fisheries: Current knowledge and recommendations for future research. *ICES J. Mar. Sci.* **68,** 1019–1029 (2011).
19. Biastoch, A. *et al.* Rising Arctic Ocean temperatures cause gas hydrate destabilization and ocean acidification. *Geophys. Res. Lett.* **38,** L08602 (2011).
20. Gilmour, K. M. & Perry, S. F. Carbonic anhydrase and acid-base regulation in fish. *J. Exp. Biol.* **212,** 1647–1661 (2009).
21. Perry, S. F. & Gilmour, K. M. Acid-base balance and CO_2 excretion in fish: Unanswered questions and emerging models. *Respir. Physiol. Neurobiol.* **154,** 199–215 (2006).
22. Hunt von Herbing, I., Boutilier, R. G., Miyake, T. & Hall, B. K. Effects of temperature on morphological landmarks critical to growth and survival in larval Atlantic cod (*Gadus morhua*). *Mar. Biol.* **124,** 593–606 (1996).
23. Morrison, C. M. Histology of Atlantic cod, *Gadus morhua*: An atlas. Part Four. Eleutheroembryo and larva.. *Can. Spec. Publ. Fish Aquat. Sci.* **119,** 496 (1993).
24. Heisler, N. *Acid Toxicity and Aquatic Animals. Acid-base Regulation in Fishes: 1. Mechanisms* (Cambridge Univ. Press, 1989).
25. Michaelidis, B., Spring, A. & Pörtner, H. O. Effects of long-term acclimation to environmental hypercapnia on extracellular acid–base status and metabolic capacity in Mediterranean fish *Sparus aurata*. *Mar. Biol.* **150,** 1417–1429 (2007).
26. Langenbuch, M. & Pörtner, H. O. Energy budget of hepatocytes from Antarctic fish (*Pachycara brachycephalum* and *Lepidonotothen kempi*) as a function of ambient CO_2: pH-dependent limitations of cellular protein biosynthesis? *J. Exp. Biol.* **206,** 3895–3903 (2003).
27. Köhler, A. Lysosomal perturbations in fish liver as indicators of toxic environmental pollution. *Comput. Biochem. Physiol. C* **100,** 123–127 (2004).
28. Good, C., Davidson, J., Welsh, C., Snekvik, K. & Summerfelt, S. The effects of carbon dioxide on performance and histopathology of rainbow trout *Oncorhynchus mykiss* in water recirculation aquaculture systems. *Aquacult. Eng.* **42,** 51–56 (2010).
29. Bernet, D., Schmidt, H., Meier, W., Burkhardt-Holm, P. & Wahli, T. Histopathology in fish: Proposal for a protocol to assess aquatic pollution. *J. Fish Dis.* **22,** 25–34 (1999).
30. Munday, P. L., Donelson, J. M., Dixson, D. L. & Endo, G. G. K. Effects of ocean acidification on the early life history of a tropical marine fish. *Proc. R. Soc. B* **276,** 3275–3283 (2009).

Acknowledgements

Funding was provided through the European Community's Seventh Framework Programme (FP7/2007-2013) 'European Project on Ocean Acidification' (EPOCA, grant agreement N211384), the European Marie Curie Initial Training Network 'Calcification of Marine Organisms' (CalMarO) and the project by German Ministry for Education and Research (BMBF) 'Biological Impacts of Ocean ACIDification' (BIOACID). The experiments were conducted at the Norwegian National Mesocosm Centre, Espegrend, in cooperation with the University of Bergen. The authors are grateful to R. Bellerby and his lab for assistance with the carbonate chemistry and to H. Otteraa, V. Lokøy, F. Midtøy and C. Eizaguirre for various support.

Author contributions

A.Y.F., R.M., A.J.G., A.F., U.P. and C.C. designed the experiment; A.Y.F., R.M., A.J.G., A.F. and C.C. performed the experiment; D.L. performed the histological analysis and wrote the section on that topic; A.M.M. performed the lipid analysis; A.Y.F, C.C. and T.B.H.R. analysed data; A.Y.F. and T.B.H.R. wrote the main paper; All authors discussed the results and implications and commented on the manuscript at all stages.

Additional information

The authors declare no competing financial interests. Supplementary information accompanies this paper on www.nature.com/natureclimatechange. Reprints and permissions information is available online at http://www.nature.com/reprints. Correspondence and requests for materials should be addressed to A.Y.F.

© 2011 Macmillan Publishers Limited. All rights reserved.

EXTENDED PDF FORMAT
SPONSORED BY
SIGMA
Life Science
sigma.com/knockout

Science
AAAS

The Geological Record of Ocean Acidification

Bärbel Hönisch, *et al.*

Science **335**, 1058 (2012);
DOI: 10.1126/science.1208277

This copy is for your personal, non-commercial use only.

If you wish to distribute this article to others, you can order high-quality copies for your colleagues, clients, or customers by clicking here.

Permission to republish or repurpose articles or portions of articles can be obtained by following the guidelines here.

The following resources related to this article are available online at www.sciencemag.org (this infomation is current as of March 1, 2012):

Updated information and services, including high-resolution figures, can be found in the online version of this article at:
http://www.sciencemag.org/content/335/6072/1058.full.html

Supporting Online Material can be found at:
http://www.sciencemag.org/content/suppl/2012/03/01/335.6072.1058.DC2.html
http://www.sciencemag.org/content/suppl/2012/02/29/335.6072.1058.DC1.html

This article **cites 193 articles**, 62 of which can be accessed free:
http://www.sciencemag.org/content/335/6072/1058.full.html#ref-list-1

This article appears in the following **subject collections:**
Geochemistry, Geophysics
http://www.sciencemag.org/cgi/collection/geochem_phys

Downloaded from www.sciencemag.org on March 2, 2012

Science (print ISSN 0036-8075; online ISSN 1095-9203) is published weekly, except the last week in December, by the American Association for the Advancement of Science, 1200 New York Avenue NW, Washington, DC 20005. Copyright 2012 by the American Association for the Advancement of Science; all rights reserved. The title *Science* is a registered trademark of AAAS.

The Geological Record of Ocean Acidification

Bärbel Hönisch,[1]* Andy Ridgwell,[2] Daniela N. Schmidt,[3] Ellen Thomas,[4,5] Samantha J. Gibbs,[6] Appy Sluijs,[7] Richard Zeebe,[8] Lee Kump,[9] Rowan C. Martindale,[10] Sarah E. Greene,[2,10] Wolfgang Kiessling,[11] Justin Ries,[12] James C. Zachos,[13] Dana L. Royer,[5] Stephen Barker,[14] Thomas M. Marchitto Jr.,[15] Ryan Moyer,[16] Carles Pelejero,[17] Patrizia Ziveri,[18,19] Gavin L. Foster,[6] Branwen Williams[20]

Ocean acidification may have severe consequences for marine ecosystems; however, assessing its future impact is difficult because laboratory experiments and field observations are limited by their reduced ecologic complexity and sample period, respectively. In contrast, the geological record contains long-term evidence for a variety of global environmental perturbations, including ocean acidification plus their associated biotic responses. We review events exhibiting evidence for elevated atmospheric CO_2, global warming, and ocean acidification over the past ~300 million years of Earth's history, some with contemporaneous extinction or evolutionary turnover among marine calcifiers. Although similarities exist, no past event perfectly parallels future projections in terms of disrupting the balance of ocean carbonate chemistry—a consequence of the unprecedented rapidity of CO_2 release currently taking place.

The geological record is imprinted with numerous examples of biotic responses to natural perturbations in global carbon cycling and climate change (Fig. 1), some of which could have been caused by large-scale ocean acidification. By reconstructing past changes in marine environmental conditions, we can test hypotheses for the causes and effects of future-relevant stressors such as ocean acidification on ecosystems (*1*). However, for the fossil record to be of direct utility in assessing future ecosystem impacts, the occurrence and extent of past ocean acidification must be unambiguously identified. In recent years, a variety of trace-element and isotopic tools have become available that can be applied to infer past seawater carbonate chemistry. For instance, the boron isotopic composition ($\delta^{11}B$) of marine carbonates reflects changes in seawater pH, the trace element (such as B, U, and Zn)–to-calcium ratio of benthic and planktic foraminifer shells records ambient [CO_3^{2-}], and the stable carbon isotopic composition ($\delta^{13}C$) of organic molecules (alkenones) can be used to estimate surface ocean aqueous [CO_2] (*2*).

Because direct ocean geochemical proxy observations are still relatively scarce, past ocean acidification is often inferred from a decrease in the accumulation and preservation of $CaCO_3$ in marine sediments, potentially indicated by an increased degree of fragmentation of foraminiferal shells (*3*). However, it is difficult to distinguish between the original calcification responses to chemical changes in the surface ocean and postmortem conditions at the sea floor. For instance, planktic calcifiers may secrete heavier or lighter shells (*4*), but that signal may be modified at the sea floor through dissolution or overgrowth after deposition (*5*, *6*). This duality can introduce controversy over the identification of causes and effects, the drivers of biological change, and

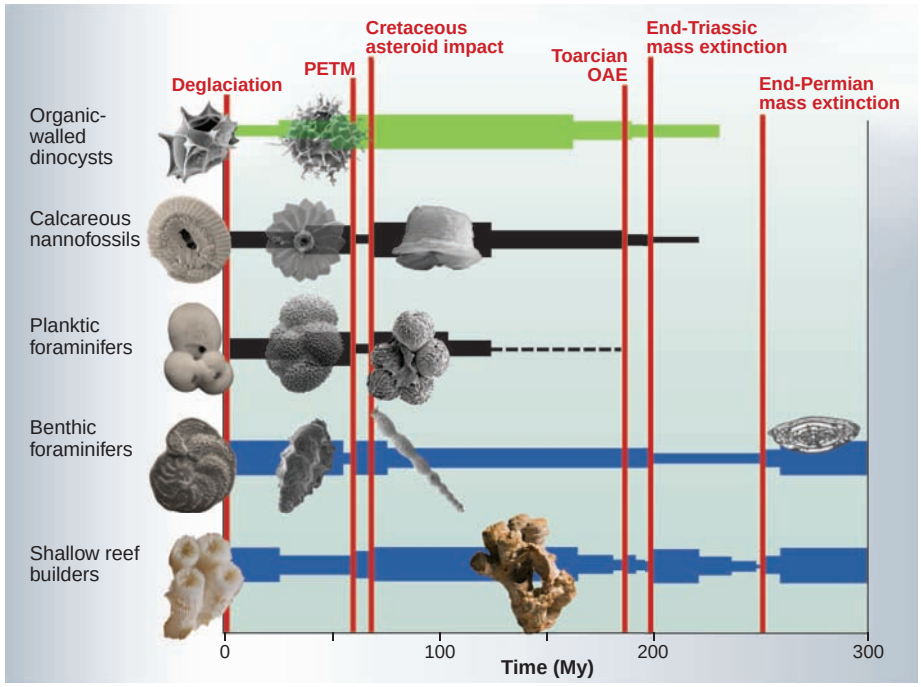


Fig. 1. Idealized diversity trajectories of the calcareous and organic fossil lineages discussed in the text. Extinction and radiation suggest events of major environmental change throughout the past 300 My. Calcareous plankton is shown in black, calcareous benthos in blue, and organic fossils in green, and the line thickness indicates relative and smoothed species richness. Highlighted events (vertical red lines) have been associated with potential ocean acidification events (Fig. 4). Calcareous organisms were not uniformly affected at all times, suggesting the importance of synergistic environmental factors to extinction, adaptation, and evolution as well as different sensitivity due to physiological factors. Identification of a paleo-ocean acidification event therefore requires independent geochemical evidence for ocean chemistry changes. Images of organisms are exemplary. References and further information on the displayed organisms are available in the supporting online material.

[1]Lamont-Doherty Earth Observatory of Columbia University, Palisades, NY 10964, USA. [2]School of Geographical Sciences, University of Bristol, Bristol BS8 1SS, UK. [3]School of Earth Sciences, University of Bristol, Bristol, BS8 1RJ, UK. [4]Department of Geology and Geophysics, Yale University, New Haven, CT 06520, USA. [5]Department of Earth and Environmental Sciences, Wesleyan University, Middletown, CT 06459, USA. [6]Ocean and Earth Science, National Oceanography Centre Southampton, University of Southampton, Southampton SO14 3ZH, UK. [7]Department of Earth Sciences, Utrecht University, 3584 CD Utrecht, Netherlands. [8]School of Ocean and Earth Science and Technology, Department of Oceanography, University of Hawaii at Manoa, Honolulu, HI 96822, USA. [9]Department of Geosciences, Pennsylvania State University, University Park, PA 16802, USA. [10]Department of Earth Sciences, University of Southern California (USC), Los Angeles, CA 90089, USA. [11]Museum für Naturkunde at Humboldt University, 10115 Berlin, Germany. [12]Department of Marine Sciences, University of North Carolina–Chapel Hill, NC 27599, USA. [13]Earth and Planetary Sciences Department, University of California Santa Cruz, CA 95064, USA. [14]School of Earth and Ocean Sciences, Cardiff University, Cardiff CF10 3AT, UK. [15]Department of Geological Sciences and Institute of Arctic and Alpine Research, University of Colorado, Boulder, CO 80309, USA. [16]University of South Florida St. Petersburg, Department of Environmental Science, Policy, and Geography, St. Petersburg, FL 33701, USA. [17]Institució Catalana de Recerca i Estudis Avançats and Department of Marine Biology and Oceanography, Consejo Superior de Investigaciones Científicas, 08003 Barcelona, Catalonia, Spain. [18]Institute of Environmental Science and Technology, Universitat Autònoma de Barcelona, 01893 Barcelona, Spain. [19]Department of Earth Sciences, Vrije Universiteit, 1081HV Amsterdam, Netherlands. [20]W. M. Keck Science Department of Claremont McKenna College, Pitzer College, and Scripps College, Claremont, CA 91711, USA.

*To whom correspondence should be addressed. E-mail: hoenisch@ldeo.columbia.edu

Downloaded from www.sciencemag.org on March 2, 2012

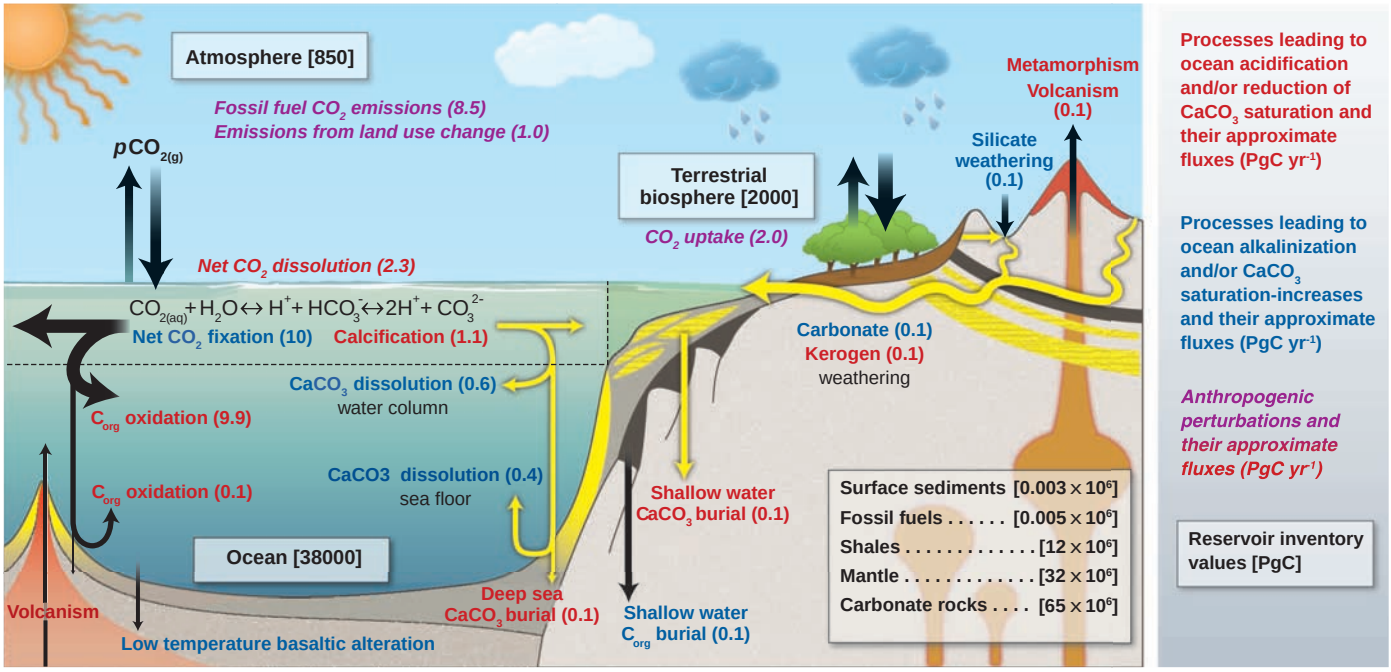


Fig. 2. When CO_2 dissolves in seawater, it reacts with water to form carbonic acid, which then dissociates to bicarbonate, carbonate, and hydrogen ions. The higher concentration of hydrogen ions makes seawater acidic, but this process is buffered on long time scales by the interplay of seawater, seafloor carbonate sediments, and weathering on land. Shown are the major pathways of reduced carbon (black) and of alkalinity (yellow). Processes leading to ocean acidification and/or reduction of $CaCO_3$ saturation are indicated in red, and processes leading to ocean alkalinization and/or $CaCO_3$ saturation increases are indicated in blue. Anthropogenic perturbations are marked in italics. Approximate fluxes are printed in parentheses (PgC year^{-1}), whereas reservoir inventory values are shown in brackets [PgC]. Natural carbon cycle fluxes are from (*70*); anthropogenic fluxes for 2008 are from (*57*), which for the land sink is significantly above its 1990–2000 average of 2.6 PgC year^{-1} due to the 2008 La Niña state (*8*).

whether past intervals of ocean acidification are characterized by environmental conditions relevant for the near future. Coeval changes in ocean circulation will also introduce regional biases in proxy records and hence affect global interpretations.

Here, we review the factors controlling ocean acidification, describe evidence for the occurrence of ocean acidification events in the past, and discuss the potential as well as weaknesses of the geological record in helping us predict future ecosystem changes.

Is Ocean Acidification Primarily a pH-Decline Phenomenon?

The current rate of anthropogenic CO_2 release leads to a surface ocean environment characterized not only by elevated dissolved CO_2 and decreased pH (*7*) but, critically, decreased saturation with respect to calcium carbonate ($CaCO_3$), a compound widely used by marine organisms for the construction of their shells and skeletons (*8*). In contrast, slower rates of CO_2 release lead to a different balance of carbonate chemistry changes and a smaller seawater $CaCO_3$ saturation response, which may induce differential biotic response or even no response at all, invalidating a direct analog. The reason for a smaller saturation response to slow CO_2 release is that the alkalinity released by rock weathering on land must ultimately be balanced by the preservation and burial of $CaCO_3$ in marine sediments (Fig. 2), which itself is controlled by the calcium carbonate saturation state of the ocean (*9*). Hence, $CaCO_3$ saturation is ultimately regulated primarily by weathering on long time scales, not atmospheric partial pressure of CO_2 (P_{CO_2}). While weathering itself is related to atmospheric P_{CO_2} (*10*), it is related much more weakly than ocean pH, which allows pH and $CaCO_3$ saturation to be almost completely decoupled for slowly increasing atmospheric P_{CO_2}.

Using a global carbon cycle model (*2*), we show the progressive coupling between $CaCO_3$ saturation and pH as the rate of CO_2 emissions increases and sources (weathering) and sinks ($CaCO_3$ burial) of alkalinity are no longer balanced. For rapid century-scale and thus future-relevant increases in atmospheric P_{CO_2}, both surface ocean pH and saturation state decline in tandem (Fig. 3). The projected decrease in ocean surface saturation state—here, with respect to aragonite ($\Omega_{aragonite}$)—is an order of magnitude larger for a rapid CO_2 increase than for a slow [100 thousand years (ky)] CO_2 increase. Ultimately, saturation recovers while the pH remains suppressed, reflecting how changes in the oceanic concentrations of dissolved inorganic carbon (DIC) and alkalinity make it possible to have simultaneously both high CO_2 and high carbonate ion concentration saturation ([CO_3^{2-}], which controls saturation), but with the relatively greater increase in [CO_2] causing lower pH. The key to unlocking the geological record of ocean acidification is hence to distinguish between long-term steady states and transient changes. We use the term "ocean acidification event" for time intervals in Earth's history that involve both a reduction in ocean pH and a substantial lowering of $CaCO_3$ saturation, implying a time scale on the order of 10,000 years and shorter (Fig. 3).

Indications of Paleo-Ocean Acidification

With these criteria in mind, we review (in reverse chronological order) the intervals in Earth's history for which ocean acidification has been hypothesized, along with the evidence for independent geochemical and biotic changes. We confine this review to the past ~300 million years (My) because the earlier Phanerozoic (and beyond) lacks the pelagic calcifiers that not only provide key proxy information but also create the strong deep-sea carbonate (and hence atmospheric P_{CO_2}) buffer that characterizes the modern Earth system (*9*). Our criteria for identifying potentially future-relevant past ocean acidification are (i) massive CO_2 release, (ii) pH decline, and (iii) saturation decline. We also discuss evidence for the time scale of CO_2 release, as well as for global warming. Events are given a similarity index that is based on available geochemical data (table S1) and are indicated in Fig. 4A.

Late Pleistocene deglacial transitions. The last deglaciation is the best documented past event associated with a substantive (30%) CO_2 rise: 189 to 265 μatm between 17.8 to 11.6 ky before

Downloaded from www.sciencemag.org on March 2, 2012

the present (B.P.) (*11*). Boron isotope estimates from planktic foraminifers show a 0.15 ± 0.05 unit decrease in sea surface pH (*12*) across the deglacial transition—an average rate of decline of ~0.002 units per 100 years compared with the current rate of more than 0.1 units per 100 years (table S1). Planktic foraminiferal shell weights decreased by 40 to 50% (*4*), and coccolith mass decreased by ~25% (*13*). In the deep ocean, changes in carbonate preservation (*14*), pH [from foraminiferal $\delta^{11}B$ (*15*)] and [CO_3^{2-}] [from foraminiferal B/Ca and Zn/Ca (*16*, *17*)] differed between ocean basins, reflecting covarying changes in deep-water circulation and an internal carbon shift within the ocean. The regional nature of these variations highlights the general need for careful evaluation of regional versus global effects in paleo-studies.

Oligocene–Pliocene. The climate of the Oligocene to Pliocene [34 to 2.6 million years ago (Ma)] contains intervals of elevated temperature and modest deviations of atmospheric P_{CO_2} from modern values (Fig. 4). Of particular interest has been the Pliocene warm period [3.29 to 2.97 Ma (*18*, *19*)], which is characterized by global surface temperatures estimated to be ~2.5°C higher than today (*19*), atmospheric P_{CO_2} between 330 to 400 μatm (Fig. 4C) (*18*, *20*), and sea surface $pH_{(T)}$ ~0.06 to 0.11 units lower (*18*) than the preindustrial. Ecological responses to the warming include migration of tropical foraminifer species toward the poles (*21*), but there are no documented calcification responses or increased nannoplankton extinction rates (*22*). The early to middle Miocene (23 to 11 Ma) and Oligocene (34 to 23 Ma) were also characterized periods of elevated temperatures and slightly higher P_{CO_2} compared with preindustrial values (Fig. 4C) but, because of their long duration, were not associated with changes in $CaCO_3$ saturation (Fig. 3C).

Paleocene–Eocene. Evidence for rapid carbon injection associated with the Paleocene–Eocene Thermal Maximum (PETM, 56 Ma) as well as a number of smaller transient global warming events (hyperthermals) during the late Paleocene and early Eocene (58 to 51 Ma) comes primarily from observations of large [up to –4 per mil (‰)] negative $\delta^{13}C$ excursions (*23*) associated with pronounced decreases in calcium carbonate preservation (*24*). Depending on the assumed source, rate, and magnitude of CO_2 release (*25*), a 0.25 to 0.45 unit decline in surface seawater pH is possible, with a reduction in mean surface ocean aragonite saturation from Ω = 3 down to 1.5 to 2 (*1*). The calcite compensation depth (CCD) (*8*) rose by ~2 km to shallower than 1.5 km in places (*24*) (compared with >4 km today). Although a pH decrease or P_{CO_2} increase remains to be confirmed by geochemical proxies for any of the hyperthermal events, the amount of carbon injected can be modeled on the basis of consistent carbonate $\delta^{13}C$ and CCD changes, yielding between ~2000 and 6000 PgC for the onset of the PETM (*26*, *27*). However, as with the last glacial transition, deep sea geochemistry appears strongly modulated by regional ocean circulation changes (*28*), which adds an additional layer of complexity to global extrapolation and highlights the importance of adequate spatial coverage of the data.

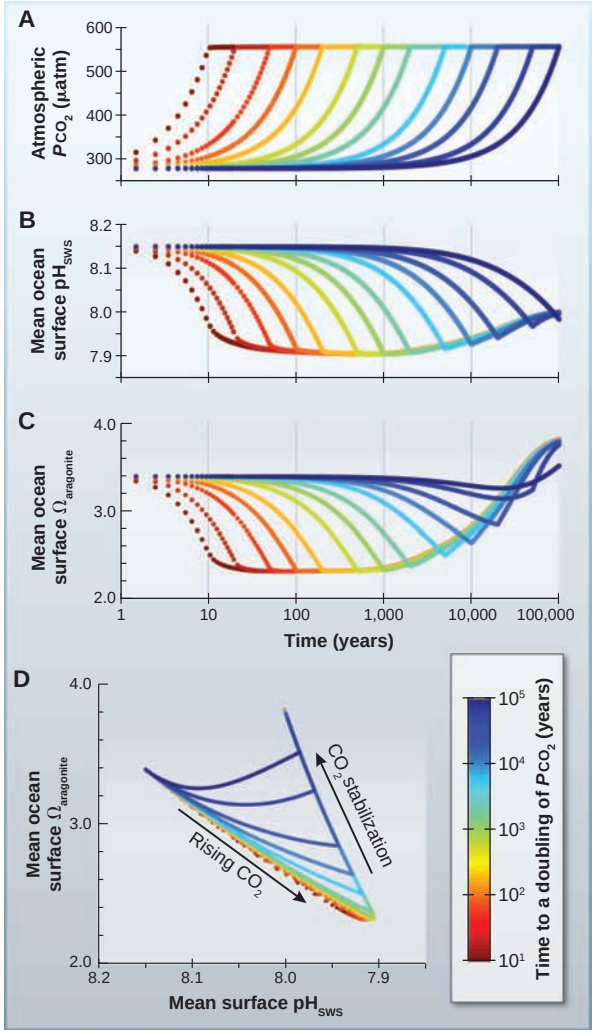


Fig. 3. The trajectories of mean ocean surface pH and aragonite saturation ($\Omega_{aragonite}$) become progressively decoupled as the rate of atmospheric P_{CO_2} change increases. The four panels show the results of a series of experiments in an Earth system model (*2*). (**A**) Prescribed linear increases of atmospheric P_{CO_2} (on a $\log_{10}$ scale) from ×1 to ×2 preindustrial CO_2, with the different model experiments spanning a range of time scales (but experiencing the same ultimate CO_2 change). (**B**) Evolution of mean surface pH in response to rising CO_2. (**C**) Evolution of mean surface $\Omega_{aragonite}$. (**D**) A cross-plot illustrating how $\Omega_{aragonite}$ is progressively decoupled from pH as the rate of P_{CO_2} increase slows, with future-relevant rate of P_{CO_2} increase showing a diagonal trajectory from top left to bottom right, whereas slow P_{CO_2} increases result in an almost horizontal trajectory toward lower pH with very little saturation change. All plots are color-coded from red ("fast") to blue ("slow"). These model results include both climate and long-term (silicate) weathering feedback. See (*2*) and fig. S1 for the role of these and other feedbacks.

PETM sediments record the largest extinction among deep-sea benthic foraminifers of the past 75 My (*29*), and a major change in trace fossils indicates a disruption of the macrobenthic community (*30*). However, the covariation of ocean acidification, warming, and corresponding oxygen depletion (fig. S2) (*23*) precludes the attribution of this extinction to a single cause (*1*, *29*). In shallow water environments, a gradual shift from calcareous red algae and corals to larger benthic foraminifers as dominant calcifiers started in the Paleocene and was completed at the PETM with the collapse of coralgal reefs and larger benthic foraminiferal turnover (*31*). This event is recognized as one of the four major metazoan reef crises of the past 300 My (Fig. 1) (*32*). In marginal marine settings, coccolithophore (*33*) and dinoflagellate cyst (*34*) assemblages display changes in species composition, but these are interpreted to reflect sensitivity to temperature, salinity stratification, and/or nutrient availability (*34*, *35*), not necessarily acidification (fig. S2). In the open ocean, the occurrence of deformities in some species of calcareous nannoplankton has been described (*36*), but despite a strong change in assemblages, there is no bias in extinction or diversification in favor of or against less or more calcified planktic species (*37*).

Cretaceous and Cretaceous-Paleogene. The well-known mass extinction at 65 Ma is generally accepted to have been triggered by a large asteroid impact (*38*). In addition to potential terrestrial biomass or fossil carbon burning, the impact may have caused the emission of SO_2 from vaporized gypsum deposits at the impact site and/or nitric acid aerosols produced by shock heating of the atmosphere, which could have led to acid rain and hence potentially to rapid acidification of the surface ocean (*38*). Although planktic calcifiers exhibited elevated rates of extinction and reduced production (*22*, *39*), reef corals did not experience a major extinction (*32*), and benthic foraminifers were not affected in either shallow or deep waters (*29*). Because multiple environmental changes covaried and proxy data for marine carbonate chemistry are not yet available, unambiguous attribution of the planktic extinctions to any one driver such as ocean acidification is currently not possible.

The earlier Cretaceous (K) (Fig. 4A) is generally a time of massive chalk deposition (mainly

Downloaded from www.sciencemag.org on March 2, 2012

in the form of nannofossil calcite), as well as one of elevated P_{CO_2} (Fig. 4B) and lower pH (Fig. 4D). This association can be misconceived as evidence that marine calcification will not be impaired under conditions of low pH in the future. However, this reasoning is invalid because extended periods of high P_{CO_2} (Fig. 4B) do not necessarily result in a suppressed seawater calcite saturation state (Fig. 3) (*1*, *40*), which exerts an important control on organisms' calcification (*41*).

Cretaceous and Jurassic oceanic anoxic events. The Mesozoic oceanic anoxic events (OAEs) (in particular, OAE 2 ~93 Ma, OAE1a ~120 Ma, and Toarcian OAE ~183 Ma) were intervals during which the ocean's oxygen minimum and deep anoxic zones expanded markedly (*42*). The onsets of these OAEs have been linked to the emplacement of large igneous provinces, degassing large amounts of CO_2 and associated environmental consequences of warming, lower oxygen solubility, and possibly ocean acidification (*42*). Some of the Cretaceous OAEs were associated with turnover in plankton communities (*43*). Deformities and some minor size reduction in coccoliths, as well as a massive increase in the abundance of heavily calcified nannoconids, have been observed (*44*, *45*). However, similar to more recent events, there is difficulty in unequivocally attributing observations to surface water acidification given the covariation of environmental changes (*46*).

Because most old sea floor (~180 Ma or older) is subducted, the sedimentary record of the Toarcian OAE is now restricted to former continental margins. Sedimentary organic and inorganic carbon deposits display initially negative, followed by positive $\delta^{13}C$ excursions, which is consistent with an influx of CO_2 into the atmosphere followed by organic carbon burial (*42*). The negative isotopic transition occurs in distinct negative $\delta^{13}C$ shifts, each estimated to occur in less than 20 ky (*47*) and possibly in as little as 650 years (*48*). The Toarcian OAE is associated with a reef crisis that was particularly selective against corals and hypercalcifying sponges (animals with a large skeletal-to–organic biomass ratio) (Fig. 4B) (*32*) and with a decrease in nannoplankton flux (*49*). Again, these observations could have been a response to any one or combination of a number of different contemporaneous environmental changes.

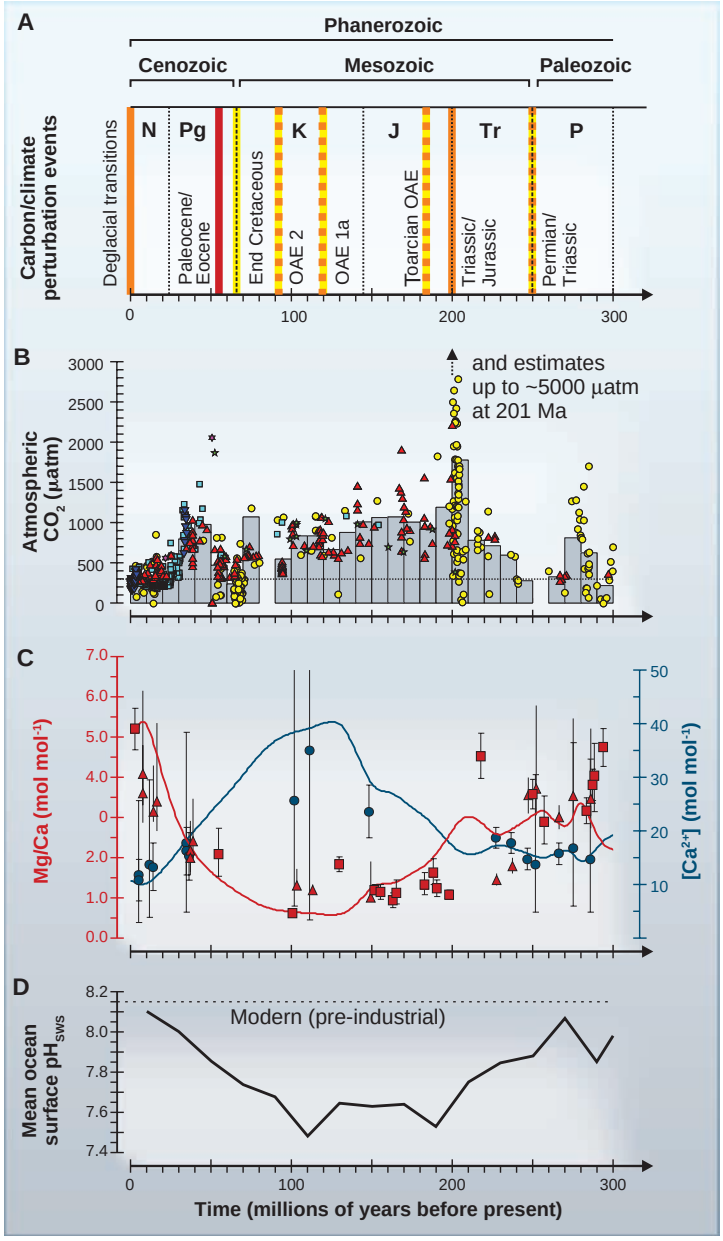


Fig. 4. Compilation of data-based [(B) and (C)] and model-reconstructed [(C) and (D)] indicators of global carbon cycle evolution over the past 300 My together with candidate ocean acidification events (A). (**A**) Summarization of the degree to which events (table S1) have some similarity to modern ocean acidification. The similarity index (table S1) is color-coded, where red indicates 3/most similar, orange indicates 2/partly similar, and yellow indicates 1/unlike. (**B**) Proxy-reconstructed atmospheric P_{CO_2} (*2*) grouped by proxy: yellow circles indicate paleosol $\delta^{13}C$, light blue squares indicate marine phytoplankton $\delta^{13}C$, red triangles indicate stomatal indices/ratios, dark blue inverted triangles indicate planktic foraminiferal $\delta^{11}B$, green five-pointed stars indicate liverwort $\delta^{13}C$, purple six-pointed stars indicate sodium carbonates, with 10-My averages shown by gray bars. For plotting convenience, estimates exceeding 3000 µatm are not shown [primarily paleosol $\delta^{13}C$ from the uppermost Triassic/lowermost Jurassic (*2*)]. (**C**) Ocean Mg/Ca ratios (red triangles, left axis), reconstructed from fluid inclusions (*2*) and echinoderm fossil carbonate [red squares (*71*)] together with the Phanerozoic seawater model of (*72*) (red line). Also shown (blue circles, right axis) is [Ca^{2+}] from fluid inclusions (*2*) and models [blue line (*72*)]. (**D**) Model-reconstructed changes in mean ocean surface pH at 20-My intervals [black line (*73*)].

Triassic–Jurassic. The Triassic–Jurassic (T/J) mass extinction is linked to the coeval emplacement of the Central Atlantic Magmatic Province (*50*). Proxy records across the T/J boundary (~200 Ma) suggest a doubling of atmospheric P_{CO_2} over as little as 20 ky (*51*, *52*), although the absolute P_{CO_2} estimates differ greatly between proxies, with leaf stomata suggesting an increase from 700 to 2000 µatm, whereas pedogenic carbonates indicate an increase from 2000 to 4400 µatm (Fig. 4C) (*2*). Decreased carbonate saturation is inferred from reduced pelagic carbonate accumulation in shelf sediments (*53*), although shallow water carbonate deposition can vary in response to many parameters, not only acidification. A calcification crisis amongst hypercalcifying taxa is inferred for this period (Fig. 4B), with reefs and scleractinian corals experiencing a near-total collapse (*32*). However, the observation that tropical species were more affected than extratropical species suggests that global warming may have been an important contributor or even dominant cause of this extinction (*32*).

Permian–Triassic. The Permo–Triassic (P/T) mass extinction (252.3 Ma) was the most severe of the Phanerozoic Era and coincided, at least in part, with one of the largest known continental eruptions, the Siberian trap basalts. Recent estimates for the total CO_2 release put it at ~13,000 to 43,000 PgC in 20 to 400 ky (*54*–*56*)—an annual carbon release of ~0.1 to 1 PgC [compared with 9.9 PgC in 2008 (*57*)]. There is some observational evidence for carbonate dissolution in shelf settings (*54*), but its interpretation is again debated (*58*). There is abundant evidence for ocean anoxia, photic zone euxinia (enrichment in

Downloaded from www.sciencemag.org on March 2, 2012

hydrogen sulfide) (*59*), and strong warming (*54*), but no direct proxy evidence for pH or carbonate ion changes. Knoll *et al*. (*59*) inferred the preferential survival of taxa with anatomical and physiological features that should confer resilience to reduced carbonate saturation state and hypercapnia (high CO_2 in blood) and preferential extinction of taxa that lacked these traits, such as reef builders (*32*).

Is There a Geologic Analog for the Future?

A number of past ocean carbon-cycle perturbation events share many of the characteristics of anthropogenic ocean acidification (Fig. 4 and table S1), with the notable exception of the estimated rates of CO_2 release. In the general absence of direct proxy evidence for lower pH and reduced saturation before the Pliocene, global carbon cycle models can be used to infer the magnitude of carbon release by fitting observed changes in the $\delta^{13}C$ of calcium carbonates and organic remnants (*60*). However, as well as needing information on the source and isotopic composition of the added carbon, the time scale of $\delta^{13}C$ change is critically important to the estimation of CO_2 fluxes (*25*). Because of the lack of open-ocean sediments and increasingly poor temporal and spatial resolution of the geological record further back in time, it is difficult to place adequate constraints on the duration and rate of CO_2 release. Radiometric dating techniques are not accurate enough to identify Mesozoic intervals of 10-ky duration, although orbital spectral analysis of highly resolved isotope and/or sedimentological records can help to partly overcome this—for example, if a $\delta^{13}C$ excursion is shorter or longer than one precession cycle [21 ky (*51*)]. Even for the well-studied PETM, the duration of the main phase of this carbon injection is still debated (*35*, *61*), and model-inferred peak rates of ≤1 PgC per year (*26*, *61*) could potentially be an underestimate.

Additional complications arise because carbon may not have been released at a uniform rate and, in the extreme, may have occurred in the form of rapid pulses. In such cases, the assumption of an average emissions rate throughout the entire duration of the pulsed release will fail to capture the potential for episodes of intense acidification. For instance, although the total duration of the CO_2 release from the T/J–age Central Atlantic Magmatic Province was estimated to be ~600 ky, pulses as short as ~20 ky have been suggested (*51*, *62*). Similarly, the main phase of OAE1a (excluding the recovery interval) was ~150 ky (*45*) and hence too slow for carbonate saturation to be significantly affected (Fig. 3), but major volcanic eruptions and thus rapid CO_2 release could potentially have produced future-relevant perturbations in the carbon cycle. Substantially improved chronologies and higher-resolution records are needed to refine estimates of rate.

Given current knowledge of the past 300 My of Earth's history (Fig. 4 and table S1), the PETM and associated hyperthermal events, the T/J, and potentially the P/T all stand out as having excellent potential as analog events, although the T/J and P/T are much more poorly constrained because of the absence of deep-sea carbonate deposits. OAEs may also be relevant but were associated with less severe volcanism (CO_2 release) than were the older events (P/T and T/J). The last deglacial transition, although characterized by temperature and CO_2-increase, is two orders of magnitude slower than current anthropogenic change. It is also thought to largely represent a redistribution of carbon within the ocean and to the atmosphere and terrestrial biosphere and hence did not have as potent and globally uniform an acidification effect as an input from geological reserves. Because of the decoupling between pH and saturation on long time scales (Fig. 3), extended intervals of elevated P_{CO_2} such as the middle Miocene, Oligocene, and Cretaceous can be firmly ruled out as future-relevant analogs.

What Are the Perspectives for Using the Geological Record to Project Global Change?

Only rapid or pulsed CO_2 release events can provide direct future-relevant information. Assessment of such events critically depends on independent geochemical quantification of the associated changes in the carbonate system, specifically seawater-pH and $CaCO_3$ saturation. Geochemical proxy estimates are not yet available for the Cretaceous and beyond and need to be obtained to verify whether ocean acidification did indeed happen. This is challenging, because in addition to the potential for increasing post-depositional alteration and reduced stratigraphic exposure, uncertainty over the chemical and isotopic composition of seawater increases and limits our interpretation of these proxies (*63*, *64*). Future studies will have to improve and expand geochemical estimates and their uncertainties of surface and deep-ocean carbonate chemistry associated with carbonate dissolution and ecological changes. This includes finding new archives to study the secular evolution of seawater chemistry but also the laboratory study of living proxy carriers under conditions mimicking past seawater chemistry. An unfortunate aspect of the geological record, however, is the lack of deep-sea carbonates in the Early Jurassic and beyond, which further reduces our ability to reconstruct the carbonate chemistry of those older events.

The sensitivity of ocean chemistry to CO_2 release, and the relationship between induced pH and P_{CO_2} changes, vary through time and further complicate the picture. For instance, seawater calcium and magnesium ion concentrations were different in the past (Fig. 4C). This alters the ocean's carbonate ion buffering capacity and hence sensitivity of the Earth system to carbon perturbation (*65*) because all other things being equal, higher ambient Ca^{2+} concentrations means that a lower carbonate ion concentration is required to achieve the same saturation and hence balance weathering. Varying seawater Mg/Ca ratios may potentially also affect the mineralogy of marine calcifiers, where the more soluble high-Mg calcite predominated Neogene reefs and reefs during the Permian through Early Jurassic, and more resistant low-Mg calcite predominated during the Late Jurassic through Paleogene (*66*). Thus, on this mineralogical basis the response of marine calcifiers to ocean acidification and seawater geochemistry during the P/T and T/J would arguably be closer to the modern than, for example, during the PETM (*67*). Improved estimates of past seawater–Mg/Ca composition are necessary to better evaluate all of this.

Although we have concentrated on the prospects for extracting information from the geological record concerning the impact of ocean acidification, we must question whether it really is necessary to isolate its effect on marine organisms from other covarying factors (*68*). In particular, consequences of increasing atmospheric CO_2 will also be associated with warming in the surface ocean and a decrease in dissolved oxygen concentration (*69*). Massive carbon release, whether future or past, will hence share the same combination and sign of environmental changes. The strength of the geological record therefore lies in revealing past coupled warming and ocean acidification (and deoxygenation) events as an "integrated" analog, with future and past events sharing the same combination and sign of environmental changes. However, in additionally driving a strong decline in calcium carbonate saturation alongside pH, the current rate of (mainly fossil fuel) CO_2 release stands out as capable of driving a combination and magnitude of ocean geochemical changes potentially unparalleled in at least the last ~300 My of Earth history, raising the possibility that we are entering an unknown territory of marine ecosystem change.

References and Notes

1. A. Ridgwell, D. N. Schmidt, *Nat. Geosci.* **3**, 196 (2010).
2. Materials and methods are available as supporting material on *Science* Online.
3. W. H. Berger, *Deep-Sea Res.* **15**, 31 (1968).
4. S. Barker, H. Elderfield, *Science* **297**, 833 (2002).
5. S. Barker *et al*., *Paleoceanography* **19**, PA3008 (2004).
6. S. J. Gibbs, H. M. Stoll, P. R. Bown, T. J. Bralower, *Earth Planet. Sci. Lett.* **295**, 583 (2010).
7. K. Caldeira, M. E. Wickett, *Nature* **425**, 365 (2003).
8. An online associated carbonate chemistry tutorial is available as supporting material on *Science* Online.
9. A. Ridgwell, R. E. Zeebe, *Earth Planet. Sci. Lett.* **234**, 299 (2005).
10. D. Archer, H. Kheshgi, E. Maier-Reimer, *Geophys. Res. Lett.* **24**, 405 (1997).
11. E. Monnin *et al*., *Science* **291**, 112 (2001).
12. B. Hönisch, N. G. Hemming, *Earth Planet. Sci. Lett.* **236**, 305 (2005).
13. L. Beaufort *et al*., *Nature* **476**, 80 (2011).
14. J. W. Farrell, W. Prell, *Paleoceanography* **4**, 447 (1989).
15. B. Hönisch, T. Bickert, N. G. Hemming, *Earth Planet. Sci. Lett.* **272**, 309 (2008).
16. J. Yu *et al*., *Science* **330**, 1084 (2010).
17. T. M. Marchitto, J. Lynch-Stieglitz, S. R. Hemming, *Earth Planet. Sci. Lett.* **231**, 317 (2005).
18. O. Seki *et al*., *Earth Planet. Sci. Lett.* **292**, 201 (2010).
19. A. M. Haywood *et al*., *Global Planet. Change* **66**, 208 (2009).
20. M. Pagani, Z. Liu, J. LaRiviere, A. C. Ravelo, *Nat. Geosci.* **3**, 27 (2010).

Downloaded from www.sciencemag.org on March 2, 2012

21. H. J. Dowsett, M. M. Robinson, *Micropaleontology* **53**, 105 (2007).
22. P. R. Bown *et al.*, in *Coccolithophores—From Molecular Processes to Global Impacts,* H. R. Thierstein, J. R. Young, Eds. (Springer, Berlin, 2004), pp. 481–508.
23. J. P. Kennett, L. D. Stott, *Nature* **353**, 225 (1991).
24. J. C. Zachos *et al.*, *Science* **308**, 1611 (2005).
25. J. C. Zachos, H. McCarren, B. Murphy, U. Röhl, T. Westerhold, *Earth Planet. Sci. Lett.* **299**, 242 (2010).
26. R. E. Zeebe, J. C. Zachos, G. R. Dickens, *Nat. Geosci.* **2**, 576 (2009).
27. K. Panchuk, A. Ridgwell, L. R. Kump, *Geology* **36**, 315 (2008).
28. R. E. Zeebe, J. C. Zachos, *Paleoceanography* **22**, PA3201 (2007).
29. E. Thomas, in *Geological Society of America Special Paper,* S. Monechi, R. Coccioni, M. R. Rampino, Eds. (Geological Society of America, Boulder, CO, 2007), pp. 1–23.
30. F. J. Rodríguez-Tovar, A. Uchman, L. Alegret, E. Molina, *Mar. Geol.* **282**, 178 (2011).
31. C. Scheibner, R. P. Speijer, *Earth Sci. Rev.* **90**, 71 (2008).
32. W. Kiessling, C. Simpson, *Glob. Change Biol.* **17**, 56 (2011).
33. S. J. Gibbs, T. J. Bralower, P. R. Bown, J. C. Zachos, L. M. Bybell, *Geology* **34**, 233 (2006).
34. A. Sluijs, H. Brinkhuis, *Biogeosciences* **6**, 1755 (2009).
35. A. Sluijs *et al.*, *Nat. Geosci.* **2**, 777 (2009).
36. I. Raffi, B. De Bernardi, *Mar. Micropaleontol.* **69**, 119 (2008).
37. S. J. Gibbs, P. R. Bown, J. A. Sessa, T. J. Bralower, P. A. Wilson, *Science* **314**, 1770 (2006).
38. P. Schulte *et al.*, *Science* **327**, 1214 (2010).
39. S. D'Hondt, M. E. Q. Pilson, H. Sigurdsson, A. K. Hanson Jr., S. Carey, *Geology* **22**, 983 (1994).
40. R. E. Zeebe, P. Westbroek, *Geochem. Geophys. Geosyst.* **4**, 1104 (2003).
41. C. Langdon *et al.*, *Global Biogeochem. Cycles* **14**, 639 (2000).
42. H. C. Jenkyns, *Geochem. Geophys. Geosyst.* **11**, Q03004 (2010).
43. R. M. Leckie *et al.*, *Paleoceanography* **17**, 1041 (2002).
44. E. Erba, F. Tremolada, *Paleoceanography* **19**, PA1008 (2004).
45. E. Erba, C. Bottini, H. J. Weissert, C. E. Keller, *Science* **329**, 428 (2010).
46. S. J. Gibbs, S. A. Robinson, P. R. Bown, T. D. Jones, J. Henderiks, *Science* **332**, 175; author reply, 175 (2011).
47. G. Suan *et al.*, *Earth Planet. Sci. Lett.* **267**, 666 (2008).
48. A. S. Cohen, A. L. Coe, D. B. Kemp, *J. Geol. Soc. London* **164**, 1093 (2007).
49. E. Mattioli, B. Pittet, L. Petitpierre, S. Mailliot, *Global Planet. Change* **65**, 134 (2009).
50. J. H. Whiteside, P. E. Olsen, D. V. Kent, S. J. Fowell, M. Et-Touhami, *Palaeogeogr. Palaeoclimatol. Palaeoecol.* **244**, 345 (2007).
51. D. B. Kemp, A. L. Coe, A. S. Cohen, L. Schwark, *Nature* **437**, 396 (2005).
52. M. Ruhl, N. R. Bonis, G. J. Reichart, J. S. Sinninghe Damsté, W. M. Kürschner, *Science* **333**, 430 (2011).
53. A. E. Crne, H. Weissert, S. Gorican, S. M. Bernasconi, *Geol. Soc. Am. Bull.* **123**, 40 (2011).
54. J. L. Payne *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **107**, 8543 (2010).
55. S. V. Sobolev *et al.*, *Nature* **477**, 312 (2011).
56. S. Z. Shen *et al.*, *Science* **334**, 1367 (2011).
57. C. Le Quéré *et al.*, *Nat. Geosci.* **2**, 831 (2009).
58. P. B. Wignall, S. Kershaw, P.-Y. Collin, S. Crasquin-Soleau, *Geol. Soc. Am. Bull.* **121**, 954 (2009).
59. A. H. Knoll, R. K. Bambach, J. L. Payne, S. Pruss, W. W. Fischer, *Earth Planet. Sci. Lett.* **256**, 295 (2007).
60. J. Zachos, M. Pagani, L. Sloan, E. Thomas, K. Billups, *Science* **292**, 686 (2001).
61. Y. Cui *et al.*, *Nat. Geosci.* **4**, 481 (2011).
62. M. Ruhl *et al.*, *Earth Planet. Sci. Lett.* **295**, 262 (2010).
63. D. Lemarchand, J. Gaillardet, E. Lewin, C. J. Allègre, *Nature* **408**, 951 (2000).
64. R. M. Coggon, D. A. Teagle, C. E. Smith-Duque, J. C. Alt, M. J. Cooper, *Science* **327**, 1114 (2010).
65. R. E. Zeebe, A. Ridgwell, in *Ocean Acidification*, J.-P. Gattuso, L. Hansson, Eds. (Oxford Univ. Press, Oxford, 2011), pp. 21–40.
66. S. M. Stanley, L. A. Hardie, *Palaeogeogr. Palaeoclimatol. Palaeoecol.* **144**, 3 (1998).
67. J. B. Ries, *Biogeosciences* **7**, 2795 (2010).
68. C. Turley *et al.*, *Mar. Pollut. Bull.* **60**, 787 (2010).
69. N. Gruber, *Philos. Trans. R. Soc. A-Math. Phys. Eng. Sci.* **369**, 1980 (2011).
70. E. T. Sundquist, K. Visser, Elsevier, in *Treatise on Geochemistry: Biogeochemistry*, W. H. Schlesinger, Ed. (Elsevier, Pergamon, Oxford, 2004), chap. 9.
71. J. A. D. Dickson, *Science* **298**, 1222 (2002).
72. R. V. Demicco, T. K. Lowenstein, L. A. Hardie, R. J. Spencer, *Geology* **33**, 877 (2005).
73. A. Ridgwell, *Mar. Geol.* **217**, 339 (2005).

Acknowledgments: Funding for the "Workshop on Paleocean Acidification and Carbon Cycle Perturbation Events" was provided by NSF OCE 10-32374 and Past Global Changes (PAGES). We thank the workshop participants for stimulating discussions and contributions to this manuscript, and the USC Wrigley Institute on Catalina Island for hosting the workshop. Particular thanks are owed to Thorsten Kiefer of PAGES, who initiated the workshop and supported it at all stages. This work is a contribution to the "European Project on Ocean Acidification" (EPOCA). Data presented in Fig. 4 are presented in tables S2 and S3 (*2*).

Supporting Online Material

www.sciencemag.org/cgi/content/full/335/6072/1058/DC1
SOM Text
Figs. S1 to S3
Tables S1 to S3
References (*74–217*)

10.1126/science.1208277

Downloaded from www.sciencemag.org on March 2, 2012

Effects of Elevated Temperature and Carbon Dioxide on the Growth and Survival of Larvae and Juveniles of Three Species of Northwest Atlantic Bivalves

Stephanie C. Talmage, Christopher J. Gobler*

School of Marine and Atmospheric Sciences, Stony Brook University, Southampton, New York, United States of America

Abstract

Rising CO_2 concentrations and water temperatures this century are likely to have transformative effects on many coastal marine organisms. Here, we compared the responses of two life history stages (larval, juvenile) of three species of calcifying bivalves (*Mercenaria mercenaria*, *Crassostrea virginica*, and *Argopecten irradians*) to temperatures (24 and 28°C) and CO_2 concentrations (~250, 390, and 750 ppm) representative of past, present, and future summer conditions in temperate estuaries. Results demonstrated that increases in temperature and CO_2 each significantly depressed survival, development, growth, and lipid synthesis of *M. mercenaria* and *A. irradians* larvae and that the effects were additive. Juvenile *M. mercenaria* and *A. irradians* were negatively impacted by higher temperatures while *C. virginica* juveniles were not. *C. virginica* and *A. irradians* juveniles were negatively affected by higher CO_2 concentrations, while *M. mercenaria* was not. Larvae were substantially more vulnerable to elevated CO_2 than juvenile stages. These findings suggest that current and future increases in temperature and CO_2 are likely to have negative consequences for coastal bivalve populations.

Citation: Talmage SC, Gobler CJ (2011) Effects of Elevated Temperature and Carbon Dioxide on the Growth and Survival of Larvae and Juveniles of Three Species of Northwest Atlantic Bivalves. PLoS ONE 6(10): e26941. doi:10.1371/journal.pone.0026941

Editor: Brian Gratwicke, Smithsonian's National Zoological Park, United States of America

Received July 20, 2011; **Accepted** October 6, 2011; **Published** October 31, 2011

Copyright: © 2011 Talmage, Gobler. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Funding: This research was supported by the New Tamarind Foundation. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

* E-mail: christopher.gobler@sunysb.edu

Introduction

The combustion of fossil fuels during the past two centuries has caused increases in atmospheric carbon dioxide and global temperatures, trends that are projected to continue in the coming decades [1]. Global temperatures are expected to increase 2 to 5°C this century [2]. Atmospheric CO_2 concentrations that had increased at a rate of 1% per year in the 20th century are now increasing ~3% per year and may exceed 800 ppm by the end of this century [1,3]. Ocean chemistry will be altered by this rising CO_2 as levels of both pH and carbonate ions decline [4]. These changes in ocean chemistry may have transformative effects on ocean life.

Coastal zones are likely to be the first regions to experience high levels of temperature and CO_2 predicted for the open ocean in the future due to both natural and anthropogenic processes, and some regions are already experiencing these increases. For example, upwelling can introduce water with high concentrations of CO_2 (800–1100 ppm) along large sections of the continental shelf [5]. Acidic river water can depress carbonate ion concentrations in coastal marine environments [6]. Furthermore, many coastal regions can be net heterotrophic due to anthropogenic, terrestrial, riverine, and wetland loadings of organic carbon [7,8,9,10], processes that collectively promote supersaturated CO_2 concentrations and lower pH. Coastal water temperatures are more sensitive to extreme and rapid increases in air temperature and increases in estuarine water temperatures have outpaced those observed in the surface ocean [11,12].

Many marine organisms, in particular those with calcified parts, can be negatively affected by acidification of ocean waters [13]. Enrichment of CO_2 can have a negative impacts across a wide range of calcifying marine taxa from coral [14], to coccolithophores [15], echinoderms [16], and coralline algae [17]. Sediments with high levels of CO_2 and low levels of carbonate ion have been shown to promote mortality of juvenile mollusks (*Mercenaria mercenaria* and *Mya arenaria*) [18,19]. Elevated CO_2 can cause decreased calcification in mussels (*Mytilus edulis*) and oysters (*Crassostrea gigas*; [20]), as well as decreased growth in mussels (*M. edulis*; [21]). Seawater enriched in CO_2 can also depress the survival, growth, and metamorphosis of larval stages of calcifying bivalves [19,20,22,23,24,25]. Our previous work has specifically demonstrated that larval hard clams (*M. mercenaria*) and bay scallops (*Argopecten irradians*) reared under the CO_2 conditions representative of the pre-industrial era (250 ppm) experience significantly faster growth and metamorphosis compared to individuals exposed to modern day CO_2 levels (390 ppm) [24,26].

The increases in ocean temperatures projected to occur this century will impact marine life. Higher temperatures in marine ecosystems can alter primary productivity, stratification, and organismal physiology [27]. The current rate of warming in ocean waters will likely apply thermal stress to a wide range of marine organisms as the limits of their temperature tolerances are approached or exceeded [28]. Temperature is a vital factor that influences the spawning and development of invertebrate larvae and most bivalve gametes are spawned at specific temperatures [29,30,31]. While larval bivalves experience maximal growth and

survival rates under ideal temperature conditions (e.g. ~24°C for many northwestern Atlantic species), small increases in temperature beyond that range will depress these rates [32,33,34]. In addition, higher temperatures can make larval bivalves more vulnerable to other environmental stressors such as ocean acidification [35].

Concurrent, future increases in CO_2 and water temperatures in marine environments likely may have synergistic effects on ocean life, in general, and invertebrate larvae in particular. Negative impacts of high CO_2 are often the greatest for early life stages of many organisms, while thermal stress can affect all life stages [36]. For the tropical sea urchin, *Tripneustes gratilla*, higher temperatures increased the growth and size of larvae, while higher CO_2 concentrations reduced calcification and negated the positive effect of higher temperatures when both temperature and CO_2 were increased [16]. For one week old barnacles, *Semibalanus balanoides*, a significant reduction in calcification and survival was estimated under simultaneously elevated temperature and CO_2 [37]. Red abalone larvae, *Haliotis rufescens*, displayed significant reductions in survivorship with increased CO_2 and a brief thermal stress compared to ambient CO_2 levels at the same thermal stress level [38]. The combination of high temperature and CO_2 have had synergistically, negative effects on a species of arctic pteropod [39] but antagonistic impacts on crustose coralline algae [17]. Exposure of two species of oysters (*Saccostrea glomerata* and *Crassostrea gigas*) to high CO_2 and increased temperature caused declines in fertilization success, development of embryos, and the size of larvae, as well as an increase incidence of abnormal morphology [40]. In contrast, the fertilization success of multiple species of marine invertebrates from South East Australia were unaffected by warming and ocean acidification [41]. To date, few studies have examined the simultaneous effects of CO_2 and temperature on any species of North Atlantic marine bivalves.

Here we present experiments investigating the effects of higher seawater temperatures and past, present, and future CO_2 concentrations on the growth and survival of the larvae of two species and juveniles of three species of $CaCO_3$ synthesizing bivalves native to the east coast of North America: the hard clam or northern quahog, *Mercenaria mercenaria (Linnaeus*, 1758), the Eastern oyster, *Crassostrea virginica (Gmelin*, 1791), and the bay scallop, *Argopecten irradians (Lamarck*, 1819). These shellfish are vitally important economic resources and ecosystem engineers in shallow coastal waters [42] and performance of these early life history stages have a profound effect on the population dynamics of these animals [43,44,45]. Simultaneously investigating the impacts of high temperature and increasing CO_2 concentrations permitted an evaluation of the differential vulnerability of larval and juvenile stages of each species to these environmental stressors.

Methods

This study examined the effects of multiple CO_2 and temperature levels on juvenile and larval stages of bivalves. For all experiments, experimental vessels with bivalves (described below) were maintained in water baths set maintained at 24 and 28°C using commercially available aquarium heaters (Aquatic Eco-systems, Inc., Florida, USA). Temperatures were recorded every 6 minutes throughout experiments using in situ data loggers (Onset©) and remained within ±0.7°C of target values. The two experimental temperatures (24 and 28°C) were chosen to represent normal and above average temperatures in Northeast US estuaries during summer months [12,46] when larvae are spawned and juvenile stages are most likely to experience thermal stress. A gas proportionator system (Cole Parmer® Flowmeter system, multitube frame) was used to deliver CO_2 gas to seawater treatments at multiple rates. The gas proportionator mixed appropriate flow rates of 5% CO_2 gas, low CO_2 gas, and pressurized air (~390 ppm CO_2) to yield the concentrations of carbon dioxide desired for experiments at a net flow rate that turned over experimental vessels >100 times daily. We have found that experiments performed with gases mixed via a proportionator as described here generate nearly identical seawater chemistry and larval responses compared to those obtained from tanked gases premixed at specific CO_2 levels [26]. For experiments, the CO_2 gas mixtures from the proportionator system were continuously delivered to the bottom of replicated ($n = 3$ or 4) experimental vessels (detailed below). With continuous bubbling, all treatment carboys remained saturated with respect to oxygen (~8 mg L^{-1}). To quantify precise CO_2 levels attained in experimental treatments, aliquots were removed before addition of larvae as well as at the conclusion of the experiment, and analyzed during experiments using an EGM-4 Environmental Gas Analyzer® (PP Systems) system that quantified total dissolved inorganic carbon levels after separating the gas phase from seawater using a Liqui-Cel® Membrane (Membrana) a standard curve made from sodium bicarbonate. This instrument provided a methodological precision ±3.6% for replicated measurements of total dissolved inorganic carbon and provided full recovery (102±3%) of Dr. Andrew Dickson's (University of California San Diego, Scripps Institution of Oceanography) certified reference material for total inorganic carbon in seawater (Batch 102 = 2013 µmol DIC kg seawater^{-1}). Levels of CO_2 were calculated based on measured levels of total inorganic carbon, pH (mol kg seawater^{-1}, NBS scale;), temperature, salinity, and first and second dissociation constants of carbonic acid in seawater according to [47] using the program CO2SYS (http://cdiac.ornl.gov/ftp/co2sys/). Daily measurements of pH (Thermo Scientific Orion Star Series™ Benchtop pH meter; ±0.002; calibrated prior to each use with NIST traceable standards; equilibrated for ~5 minutes per sample) indicated experimental vessels maintain a constant pH level throughout experiments (<0.5% RSD within treatments). Spectrophotometric measurements of pH made using *m*-cresol purple as described by Dickson *et al.* [48] and corrected for scale [49] were never significantly different from those obtained with the high sensitivity pH microprocessor. The levels of precision for measurements of pH and DIC permitted for the accurate differentiation of CO_2 treatment levels (see below) that differed by hundreds of ppm (250 v 390 v 750 v 1700 ppm).

Larvae experiments

The recommendations of the 'best practices' for small microcosm experiments set forth by European Project on Ocean Acidification (EPOCA) were followed for this project [50]. *M. mercenaria* and *A. irradians* larvae were grown at three levels of CO_2: a high level (~750 ppm CO_2), predicted for the year 2100, a modern level (~390 ppm CO_2), and a near pre-industrial level (~250 ppm CO_2), while at two different temperatures (24 and 28°C). Precise CO_2 levels and complete carbonate chemistry from this experiment appear in Table 1. One-liter, high-density polyethylene beakers were filled with 0.2 µm filtered seawater from eastern Shinnecock Bay, New York, United States. *M. mercenaria* larvae were obtained from Cornell Cooperative Extension, Southold, NY, and *A. irradians* larvae were from the East Hampton Shellfish Hatchery, East Hampton, NY, within hours of fertilization and were distributed to each treatment beaker at a concentration of ~350 L^{-1}, consistent with post-spawning densities in estuaries (Carriker 2001). Twice weekly during experiments, larvae were gently poured onto a 64 µm

Table 1. Mean temperature, pH, carbonate chemistry, alkalinity, and salinity (±1 SD) during the three-level carbon dioxide and two-level temperature experiments with *Mercenaria mercenaria,* and *Argopecten irradians* larvae.

Parameter	Pre-industrial CO_2	Ambient, present day CO_2	Elevated CO_2
Mercenaria mercenaria			
Temperature (°C)	24±0.7	24±0.7	24±0.7
pH	8.210±0.032	8.081±0.042	7.8±0.012
pCO_2 (ppm)*	220.4±24.235	375.3±36.45	771.6±29.113
$\Omega_{calcite*}$	2.86±0.50	2.68±0.51	1.51±0.15
$\Omega_{aragonite*}$	1.84±0.37	1.72±0.36	0.98±0.13
Total DIC (μmol L^1)	1115.3±95.67	1374.1±62.89	1439.4±31.38
CO_3^{2-} (μmol L^{-1})*	112.7±21.24	105.5±26.23	59.7±9.806
Alkalinity (TA) (μmol kg^1)*	1296.8±121.3	1527.3±86.56	1509.3±27.69
Salinity	28.0±1.0	28.0±1.0	28.0±1.0
Argopecten irradians			
Temperature (°C)	24±0.7	24±0.7	24±0.7
pH	8.200±0.026	8.080±0.059	7.810±0.016
pCO_2 (ppm)*	238.4±25.012	373.9±41.540	756.2±19.986
$\Omega_{calcite*}$	2.95±0.16	2.66±0.57	1.55±0.12
$\Omega_{aragonite*}$	1.9±0.42	1.72±0.45	1.00±0.24
Total DIC (umol L^{-1})	1176±56.27	1368.7±36.99	1517±35.45
CO_3^{2-} (μmol L^1)*	133.7±22.32	105.1±28.52	61.3±12.321
Alkalinity (TA) (μmol kg^1)*	1359.6±35.98	1521.4±55.06	1517.1±46.66
Salinity	28.0±1.0	28.0±1.0	28.0±1.0
Mercenaria mercenaria			
Temperature (°C)	28±0.7	28±0.7	28±0.7
pH	8.200±0.040	8.090±0.046	7.8±0.012
pCO_2 (ppm)*	247.4±16.241	379.0±43.12	794.6±29.113
$\Omega_{calcite*}$	3.43±0.53	3.17±0.56	1.75±0.15
$\Omega_{aragonite*}$	2.24±0.99	2.07±0.45	1.14±0.13
Total DIC (μmol L^1)	1196±76.24	1389.2±53.45	1439.6±31.38
CO_3^{2-} (μmol L^{-1})*	133.7±20.34	123.4±36.42	68.1±9.806
Alkalinity (TA) (μmol kg^1)*	1404.3±123.61	1568.2±66.49	1522.8±27.69
Salinity	28.0±1.0	28.0±1.0	28.0±1.0
Argopecten irradians			
Temperature (°C)	28±0.7	28±0.7	28±0.7
pH	8.210±0.029	8.08±0.054	7.810±0.026
pCO_2 (ppm)*	239.8±13.078	386.7±44.23	772.7±29.951
$\Omega_{calcite*}$	3.48±0.17	3.09±0.57	1.78±0.16
$\Omega_{aragonite*}$	2.27±0.78	2.01±0.42	1.16±0.14
Total DIC (umol L^{-1})	1189.1±53.57	1557.1±32.88	1433.6±30.21
CO_3^{2-} (μmol L^1)*	135.7±43.2	120.3±28.46	69.3±12.321
Alkalinity (TA)(μmol kg^1)*	1400.8±65.25	1557.1±70.21	1519.4±36.45
Salinity	28.0±1.0	28.0±1.0	28.0±1.0

*Parameters calculated using CO2SYS.

doi:10.1371/journal.pone.0026941.t001

mesh, and the condition (live or dead) and developmental stage of each larvae (veligers, pediveligers, and metamorphosed) were determined visually under a dissecting microscope; every individual larvae was counted at every water change. Larvae from each beaker ($n = 4$, per treatment) were removed, counted, observed, and transferred into a new beaker with new filtered seawater, food, and antibiotics within a 15 minute period. Percent survivorship of all larvae was determined at each of the bi-weekly water changes when the numbers of larvae in each stage of veligers, pediveligers, and metamorphosed juveniles were quantified. Dead larvae were characterized by a lack of swimming and movement of the velum and, when visible, internal organs, as well as a loss of pigmentation and fully open valves. Experiments were terminated after at least 50% of the surviving larvae in all treatments had metamorphosed,

which averaged three weeks among all experiments. To determine the percentage of individuals that had metamorphosed at each time point, the following equation was employed:

$$((\text{Total beginning larvae} - \text{dead larvae} - \text{non metamorphosed larvae})/\text{Total beginning larvae}) * 100$$

Larvae were fed an ideal food source at a density known to maximize bivalve larval growth and survivorship through metamorphosis [24,34,51]. Cultures of *Isochrysis galbana* (Tahitian strain, T-Iso) were maintained in exponential phase growth using standard culture conditions and added at a density of 2×10^4 mL^{-1} daily to each experimental beaker as a food source. To promote high survivorship, all containers in contact with larvae were never exposed to chemicals or detergents [24]. To discourage the growth of bacteria during experiments, an antibiotic solution (Sigma-Aldrich No. 4083, 5000 units of Penicillin, 5 mg of Streptomycin, and 10 mg of Neomycin per milliliter of solution) was added to each beaker at 1% its original concentration at the beginning of each experiment and at the time of each water change (approximately 2 times weekly). This antibiotic mixture at this concentration has been shown to have no negative effects on the growth and survivorship of shellfish larvae [24]. Experiments presented here were repeated without antibiotic treatments and yielded no difference in bivalve larval survival suggesting that neither the antibiotics nor the bacteria in seawater altered the results presented here. To meet the assumption of normality and homogeneity, survival and percent metamorphosed data were arc-sin square root transformed after which a two-way ANOVAs was performed where temperature and CO_2 were the main effects. Sizes of larvae were also examined via two-way ANOVAs. Post-hoc Tukey multiple comparison tests were performed to examine the differences among percent survival, percent metamorphosis, and sizes at each temperature and CO_2 level. Statistical analyses were performed with SYSTAT 13 © Copyright, 2009, Systat Software, Inc.

To estimate the relative lipid content of larvae, Nile Red dye was used to bind to neutral lipids and fluoresce under an FITC filter on an epifluorescent microscope [51,52]. A Nile Red stock solution was made of 1.25 mg of Nile Red crystals in 100 ml of acetone. Randomly selected larvae ($n = 15$) from each replicated treatment bottle ($n = 12$) were stained with a 1:9 dilution of the stock solution and 0.2 μm filtered seawater. Larvae were exposed to the stain for ~1.5 hours during which larval motion ceased, permitting the uniform, planar orientation of each individual for image analyses. Larvae were digitally photographed with a Roper Scientific Photometrics CoolSNAP ES camera mounted to an epiflorescent microscope. Digital images of each larva were analyzed for the area of lipid accumulation and the diameter and the area of individuals using Image J® software. Diameters were measured on randomly selected larvae ($n = 15$) from each replicated treatment vessel ($n = 12$). A lipid index was estimated by dividing the area of the larvae containing the fluorescing lipids by the total larval area thereby allowing for direct comparisons among treatments. Two-way ANOVAs and post-hoc Tukey multiple comparison tests were performed to examine the differences among larval lipid indexes, as well as shell length at each CO_2 level.

Juvenile experiments

Juvenile bivalves were obtained during early summer from the East Hampton Shellfish Hatchery, East Hampton, NY. Starting mean lengths and ash-free, dry weights (± standard deviation) of individuals were 6.09±0.65 mm and 1.36±0.048 g for *M. mercenaria*, 11.48±3.60 mm and 1.48±0.221 g for *C. virginica*, and 15.93±1.59 mm and 1.74±0.172 g for *A. irradians*. Ten individuals of each species were placed into triplicate, 10-liter, high-density polyethylene vessels that were maintained in water baths of 24 or 28°C (Table 2). CO_2 was continuously delivered as described above at ~400 and 1700 ppm representing ambient, pelagic CO_2 found today and a high concentration that our atmosphere may approach in the future [53], but within the range of levels found in and near the benthos which is frequently undersaturated with regarding to carbonate [6,18,19]. The range of CO_2 used in this experiment (~400–1700 ppm) is also commonly found in nearshore and estuarine marine environments [7,8,9,10]. Experimental vessels were bubbled with appropriate CO_2 levels for 24 h prior to commencing experiments. Precise CO_2 levels and complete carbonate chemistry from this experiment appear in Table 2. Each juvenile introduced into each treatment was identified with colored paint, allowing growth of individuals to be assessed through the 45 day experiment, a duration matching peak, hot, summer temperature in temperate estuaries [12,46]. Every three days, water was exchanged with ambient sea water from Old Fort Pond, Southampton, NY, USA, or Northwest Harbor in East Hampton, NY, USA (salinities = 28±3). Newly collected water was bubbled for 12 h prior to transferring individual bivalves to new vessels. Nutrients (10 μM nitrate and 0.63 μM orthophosphate) were added immediately and daily to experimental vessels that were held under a bank of fluorescent lights that were on an ~12:12 h light:dark cycle and delivered a light intensity of ~10 μmol quanta m^{-2} s^{-1} to encourage phytoplankton growth. Consequently, chlorophyll *a* measured using standard methods at the start and end of each water change during experiments [54] averaged 9.8±3.7 μg L^{-1} and never fell below 5 μg L^{-1}, a level generally deemed adequate for maximal growth rate of juvenile bivalves [55,56,57].

Tissue and shell weight of juvenile bivalves was quantified by drying individuals for 72 hours at 60°C followed by combustion for 4 hours at 450°C. Individuals that did not survive the duration of the experiment were removed immediately, frozen, and then weighed with individuals surviving the duration of the experiment. The post-combustion weight represented the shell weight whereas the difference between the dry and combusted weights represented organic tissue weight. Tissue and shell weight-based growth rates were calculated by dividing the change in weight by the duration of the experiment in days. Growth rates were compared by means of two-way ANOVAs where temperature and CO_2 were the main effects. Post-hoc Tukey multiple comparison tests were performed to examine the differences among juvenile growth at each temperature and CO_2 level. Survival of individuals was assessed daily and dead individuals (*A. irradians* only during the final weeks of the experiment) were removed in <24 hr of expiring. The percent mortality of *A. irradians* within each treatment was arc-sin square root transformed after which a two-way ANOVA was performed where temperature and CO_2 were the main effects.

Results

Carbon dioxide and temperature both significantly affected larval metamorphosis ($p<0.001$; two-way ANOVA, Table S1), survival ($p<0.001$; two-way ANOVA, Table S1), growth ($p<0.001$; two-way ANOVA, Table S1) and lipid synthesis ($p<0.001$; two-way ANOVA, Table S1). In *M. mercenaria* larvae, temperature and CO_2 had a significant, slightly antagonistic, interactive effect on *M. mercenaria* metamorphosis ($p<0.001$; two-

Table 2. Mean temperature, pH, carbonate chemistry, alkalinity, and salinity (±1 SD) during the two-level carbon dioxide and two-level temperature experiments with *Mercenaria mercenaria, Crassostrea virginica,* and *Argopecten irradians* juveniles.

Parameter	Ambient, present day CO_2	Elevated CO_2
Mercenaria mercenaria, Crassostrea virginica, and *Argopecten irradians* juveniles		
Temperature (°C)	24±0.65	24±0.65
pH	8.091±0.001	7.620±0.060
pCO_2 (ppm)*	400±12.34	1665±25.60
$\Omega_{calcite*}$	2.99±0.10	1.42±0.18
$\Omega_{aragonite*}$	1.93±0.07	0.92±0.12
Total DIC (μmol L^1)	1502±48.47	2023±29.01
CO_3^{2-} (μmol L^{-1})*	117.9±3.95	56.1±7.16
Alkalinity (TA)(μmol kg^1)*	1667.5±52.15	2052.2±19.39
Salinity	28.0±3.0	28.0±3.0
Temperature (°C)	28±0.65	28±0.65
pH	8.092±0.002	7.617±0.047
pCO_2 (ppm)*	399.5±1.68	1737±18.71
$\Omega_{calcite*}$	3.38±0.04	1.64±0.19
$\Omega_{aragonite*}$	2.20±0.03	1.07±0.12
Total DIC (μmol L^1)	1473±11.61	2039±4.10
CO_3^{2-} (μmol L^{-1})*	131.5±1.64	64.0±7.16
Alkalinity (TA) (μmol kg^1)*	1659.4±13.52	2080.8±17.67
Salinity	28.0±3.0	28.0±3.0

*Parameters calculated using CO2SYS.

doi:10.1371/journal.pone.0026941.t002

way ANOVA, Table S1). The percentage of individuals that had metamorphosed and survived, as well as individual grow rates were all highest for individuals grown under 250 ppm and at 24°C and were lowest for individuals grown at 750 ppm CO_2 and 28°C (Fig. 1, S1). For example, 18 days post-fertilization, 45±2.6, 16±2.0, and 8±5.3% of individuals (± standard deviation) at 24°C had metamorphosed under ~250, 390, 750 ppm CO_2, where as 27±0.6, 13±0.4, and 5±0.3% had done so at ~250, 390, 750 ppm CO_2 and 28°C (Fig. 1a, S1). With increasing CO_2 values (~250, ~390, and ~750 ppm), larval survival decreased from 44±3.1 to 30±2.1 and 20±0.3% at 24°C compared to 20±0.9 to 14±0.5 and 8±1.5% at 28°C ($p<0.05$ for all, Fig. 1b.). For *M. mercenaria* larvae, there was a synergistic interaction ($p<0.001$; two-way ANOVA, Table S1) between CO_2 and temperature, as survival percentages in this combined treatment were lower than expected from the individual treatments. Regarding size, *M. mercenaria* larvae at 24°C and ~250 ppm CO_2 had mean diameters of 553±38 µm while increasing temperatures and CO_2 level progressively depressed sizes with individuals grown at 28°C and ~750 ppm CO_2 having mean diameters of 325±22 μm (Fig. 1c). Lipid indices for *M. mercenaria* were always higher at 24°C (0.23±0.09) compared to larvae grown at 28°C (0.15±0.07; $p<0.001$, two-way ANOVA; Table S1, Fig. 1d). The lipid content for *M. mercenaria* larvae also decreased with increasing CO_2 levels ($p<0.001$, two-way ANOVA; Table S1, Fig. 1d). While there was no significant differences in lipid indices between ~250 and ~390 ppm at either temperature, there was a significant decrease in lipid indices when the CO_2 level was enriched from ~250 or ~390 to ~750 ppm for both temperatures (Fig. 1d).

Responses of *A. irradians* larvae to temperature and CO_2 levels were similar to *M. mercenaria* and in some cases were more dramatic. There was a significant decrease in the percent of individual *A. irradians* larvae that had developed into metamorphosed juveniles with increasing CO_2 and increasing temperature ($p<0.001$; two-way ANOVA, Table S1), as well as a synergistic interaction between both temperature and CO_2 concentrations for larval metamorphosis ($p<0.001$; two-way ANOVA, Table S1). While 87±0.8 and 71±0.9 and 53±2.3% individuals had metamorphosed after 20 days at 24°C and ~250, ~390, and ~750 ppm, respectively, fewer than 10% of individuals did so at 28°C with fewer than 0.5% metamorphosed at 28°C and ~750 ppm CO_2 (Fig. 2a, S2). There was also a significant decline in larval survival with each increased CO_2 and temperature level ($p<0.001$; two-way ANOVA; Table S1, Fig. 2b). There was also a slightly antagonistic interactive effect of CO_2 and temperature on the percentage of *A. irradians* larval survival ($p<0.001$; two-way ANOVA, Table S1). At 24°C, 91±0.9, 74±1.1, and 54±2.3% of individuals survived at 250, ~390, and ~750 ppm, respectively, whereas at 28°C, 45±0.8, 35±0.4, and 27±0.9±% of individuals survived, respectively (Fig. 2b). Higher CO_2 and temperature depressed the size attained by *A. irradians* larvae ($p<0.001$; two-way ANOVA, Table S1). Mean diameters of *A. irradians* larvae at 24°C and ~250 ppm were 530±33 μm while sizes progressively decreased with higher temperature and CO_2 levels to 309±33 µm at 28°C and ~750 ppm ($p<0.05$, Tukey for all; Fig. 2c). For *A. irradians* larvae, there were significant differences in lipid indices among CO_2 levels ($p<0.001$, two-way ANOVA, Table S1), and between the two temperatures ($p<0.05$, two-way ANOVA, Table S1). At both temperatures, lipid indices in *A. irradians* larvae decreased from 0.21±0.04 to 0.18±0.03 to 0.08±0.01 as CO_2 levels increased from ~250 to ~390 and ~750 ppm ($p<0.001$ for ~250 or ~390 compared to ~750 ppm CO_2; Fig. 2d). At 28°C, lipid indices decreased from 0.19±0.007

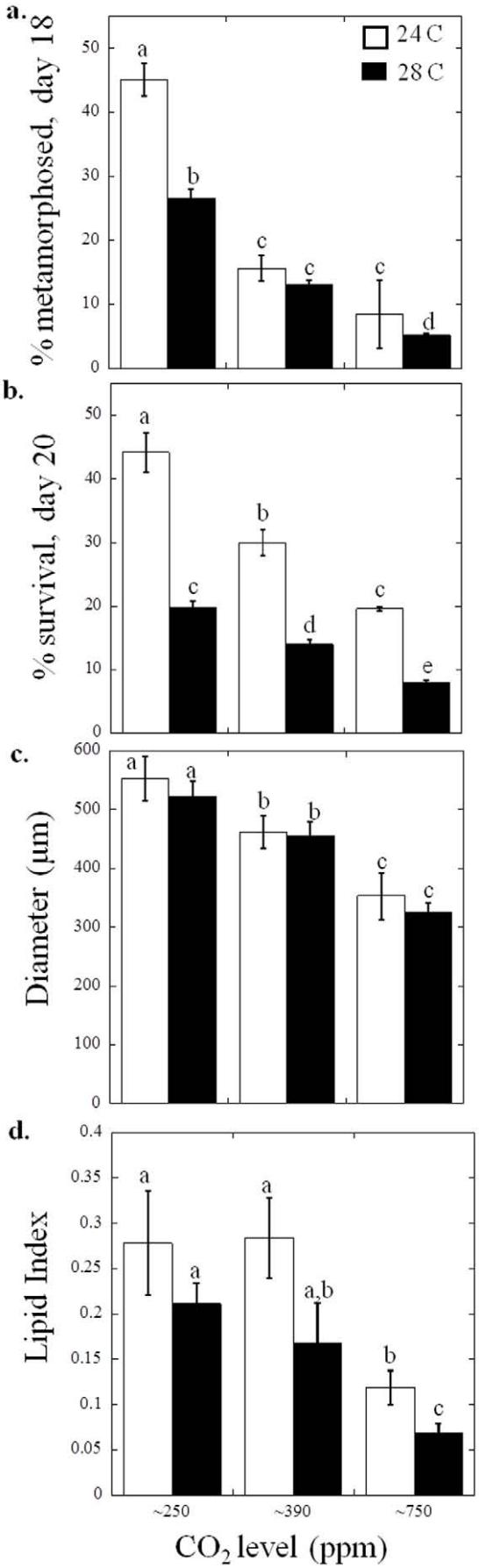


Figure 1. Performance of *Mercenaria mercenaria* larvae grown under three levels of CO_2, approximately 250, 390, and 750 ppm, and two temperatures 24°C (white bars) and 28°C (black bars; see Table 1 for carbonate chemistry). a. Percent metamorphosed of individuals 18 days post- fertilization, b. Percent larval survival (20 days post-fertilization), c. Diameters of larvae (20 days post-fertilization), and d. Lipid index (lipid area/total area) (20 days post-fertilization). Error bars represent standard deviation of replicated vessels per treatment ($n=4$ per treatment), and for Tukey multiple comparisons, $p \leq 0.05$. Statistical results were based on arcsine square root transformations of the % data for a. and b.
doi:10.1371/journal.pone.0026941.g001

to 0.15±0.01 to 0.07±0.004 as CO_2 levels increased from ~250 to ~750 pm (Fig. 2d).

Unlike the larvae, juvenile *M. mercenaria* were unaffected by even higher levels of CO_2, but were affected by temperature differences. For example, the shell growth of juvenile *M. mercenaria* was significantly greater at 24°C (1.03±0.06 mg d^{-1}) compared to 28°C ($p<0.01$, two-way ANOVA; Table S1, Fig. 3a). Tissue growth for *M. mercenaria* juveniles was not significantly altered by temperature and CO_2 did not significant alter shell or tissue growth of *M. mercenaria* juveniles (Table S1, Fig. 3b).

Unlike *M. mercenaria*, shell growth of *C. virginica* juveniles was significantly lower at 1700 ppm CO_2 (2.88±0.10 mg d^{-1}) compared to 400 ppm CO_2 (4.57±0.17 mg d^{-1}; $p<0.05$; two-way ANOVA; Table S1, Fig. 4a). Tissue growth for *C. virginica* juveniles was not significantly affected by temperature or CO_2 (Table S1, Fig. 4b).

Juvenile *A. irradians* were sensitive to both elevated CO_2, and elevated temperatures treatments used in this study. With increasing temperature from 24 to 28°C, *A. irradians* juvenile shell growth decreased from 4.75±0.17 mg d^{-1} to 3.30±0.13 mg d^{-1} while tissue growth decreased from 0.14±0.02 mg d^{-1} to 0.03±0.002 mg d^{-1} ($p<0.05$; two-way ANOVA; Table S1, Fig. 5a). Although CO_2 did not significantly alter shell- or tissue-based growth in juvenile *A. irradians* (Table S1), the higher CO_2 and temperature yielded a significant interactive, decline in juvenile *A. irradians* survival from 73.3±15% and 53.3±15.3% for 24 and 28°C, respectively, at 400 ppm CO_2, to 43.3±5.8% and 33.3±13.0% for 24 and 28°C, respectively, at 1700 ppm ($p<0.05$; two-way ANOVA for CO_2 only, Table S1, Fig. 5c). Survival of juvenile *M. mercenaria* and *C. virginica* juveniles was very high (97±6% and 93±6%, respectively) and was not significantly altered by temperature or CO_2 (Table S1).

Discussion

Global climate change has acidified and warmed the oceans, trends that are projected to continue this century. Anthropogenic processes, proximity to terrestrial carbon sources, and the shallow nature of coastal ecosystems make them currently vulnerable to temperatures and CO_2 increases that may not occur in open ocean waters for many decades. This study demonstrates, for the first time, that elevated levels of CO_2 and temperature negatively impact both juvenile and larval stages of bivalves. Larvae were found to be more sensitive to elevated levels of CO_2 and temperature than juvenile stages and unlike two of the three juvenile species investigated, the effects of CO_2 and temperature were additive for larvae. The high temperature (28°C) and high CO_2 (~750 ppm) treatment yielded the lowest survival, growth, metamorphosis, and lipid accumulation for both larval species. Collectively, these results provide novel insight regarding the effects of CO_2 and temperature on the survival and development of multiple bivalves in coastal ecosystems.

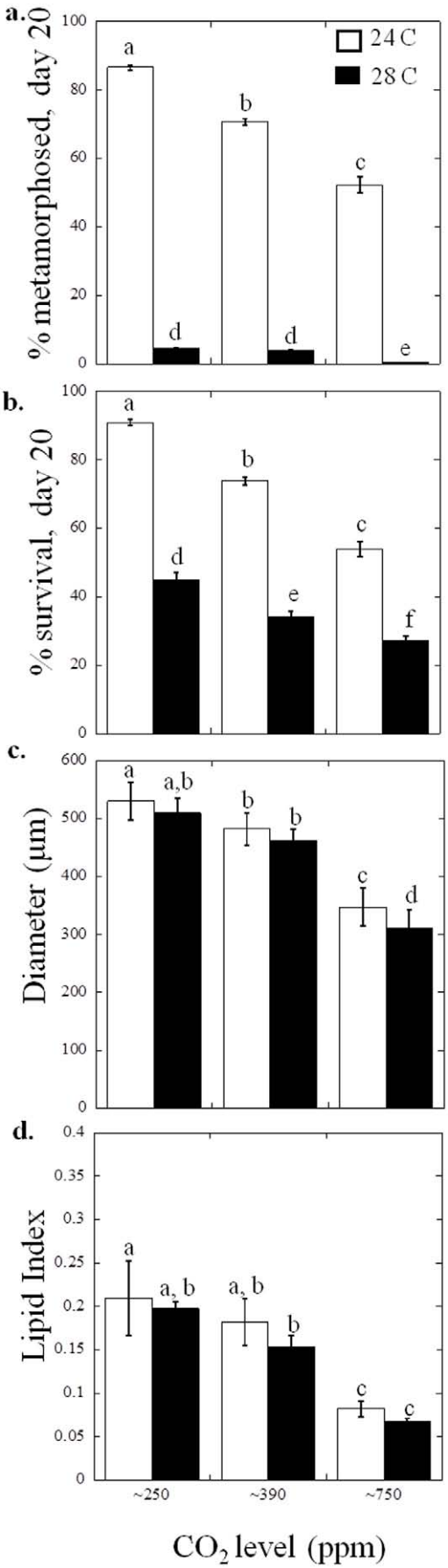


Figure 2. Performance of *Argopecten irradians* larvae grown under three levels of CO_2, approximately 250, 390, and 750 ppm, and two temperatures 24°C (white bars) and 28°C (black bars; see Table 1 for carbonate chemistry). a. Percent metamorphosed of individuals 18 days post- fertilization, b. Percent larval survival (20 days post-fertilization), c. Diameters of larvae (20 days post-fertilization), and d. Lipid index (lipid area/total area) (20 days post-fertilization). Error bars represent standard deviation of replicated vessels per treatment ($n = 4$ per treatment), and for Tukey multiple comparisons, $p \leq 0.05$. Statistical results were based on arcsine square root transformations of the % data for a. and b.
doi:10.1371/journal.pone.0026941.g002

Larvae represent a critical life stage for shellfish populations as reductions in the growth and survival of larvae have the potential to translate into substantial declines in adult populations [44,45,58,59]. Temperature has a primary influence on the spawning, growth, and development of bivalve larvae. *M. mercenaria* adults from New York to Connecticut waters are known to spawn when summer water temperatures reach 23–25°C [30] while in temperate populations of *A. irradians* spawning is typically triggered by water temperatures close to 23°C [29,60]. Resultant larvae grow optimally at temperatures around 24–25°C, but may experience slowed growth and even enhanced mortality at higher temperatures [32]. Consistent with this finding, *M. mercenaria* and *A. irradians* larvae experienced significant declines in survival, growth, and metamorphosis at 28°C compared to 24°C during this study. In the future, hotter summer water temperatures (I.P.C.C. 2007; Fussel 2009) may present bivalves with a smaller window of opportunity for optimal larval growth.

Prior studies have demonstrated that increases in CO_2 concentrations beyond levels found in today's surface oceans have negative impacts on juvenile [18,20] and larval bivalves [22,24,26,61]. The present study revealed similar trends and confirmed our prior finding that pre-industrial CO_2 levels provide maximal performance in larval hard clams and bay scallops [24,26] as there were declines in survival, metamorphosis, diameter, and lipid indices for both *M. mercenaria* and *A. irradians* larvae at CO_2 concentrations above ~250 ppm (Fig. 1 and 2). Exposure of shellfish larvae to higher temperatures can make them more vulnerable to other stressors such as pollutants [35] and consistent with this, the simultaneous increase in temperature and CO_2 depressed survival, metamorphosis, growth and lipid content of larvae beyond the effect of either individual treatment (Fig. 1 and 2). This was most dramatically represented by *A. irradians* larvae that displayed only 10% mortality under 24°C and 250 ppm CO_2 compared to >70% morality of individuals exposed to 28°C and ~750 ppm CO_2 (Fig. 2). *A. irradians* populations are known to display boom and bust cycles that have been previously attributed to disease [62], overfishing [63], and/or harmful algae [64]. Our results demonstrate that interannual variability in temperature and CO_2 are also likely to promote such cycles. Within an ecosystem setting the net effects of higher temperature and CO_2 on bivalve larval survival may be more profound than measured during our experiments since larvae with extended metamorphosis times, that are smaller, and/or that accumulate fewer lipids, all symptoms of larvae reared at high temperature and CO_2, are more likely to perish once settled [34,52,65,66]. In the past decade, temperate coastal waters have experienced periods of high temperatures (three weeks >28°C in NY in 2010; C. Flagg, Stony Brook University, unpublished data) that match the duration of typical bivalve larval development periods [30,34]. Since such high temperatures can be coupled with CO_2 levels exceeding 1,000 ppm [5,24] the negative effects of high

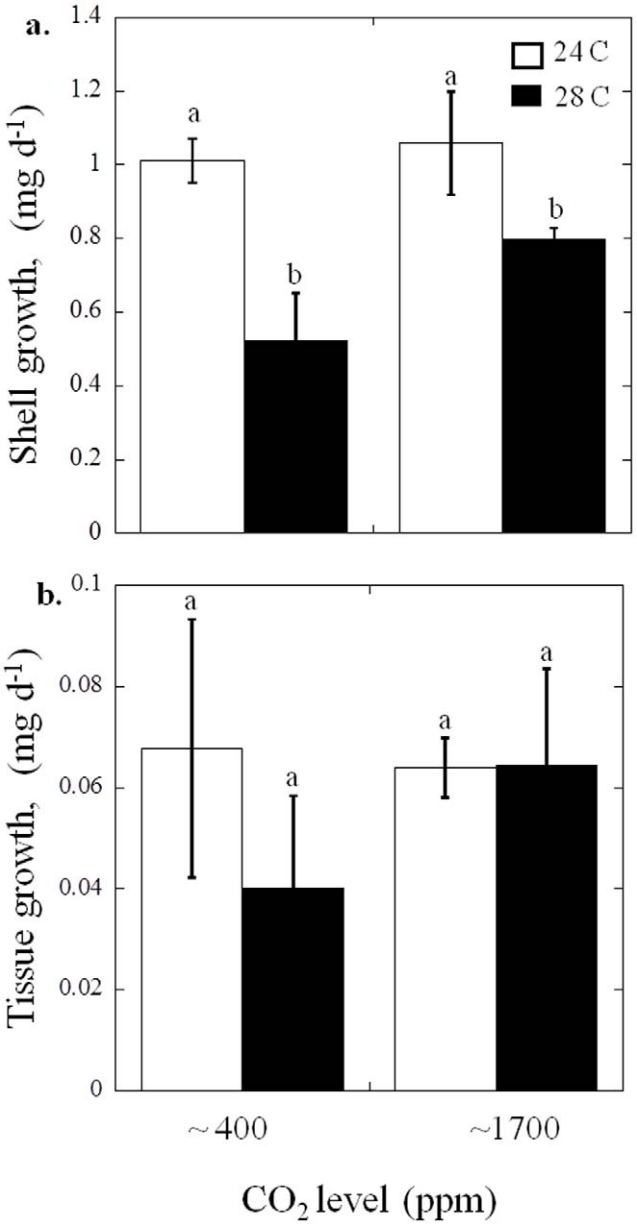


Figure 3. Growth of *Mercenaria mercenaria* juveniles at two levels of CO_2, approximately 400 and 1700 ppm, and two temperatures 24°C (white bars) and 28°C (black bars; see Table 2 for carbonate chemistry). a. Shell growth and b. Tissue growth. Error bars represent standard deviation of replicated vessels per treatment ($n = 3$ per treatment).
doi:10.1371/journal.pone.0026941.g003

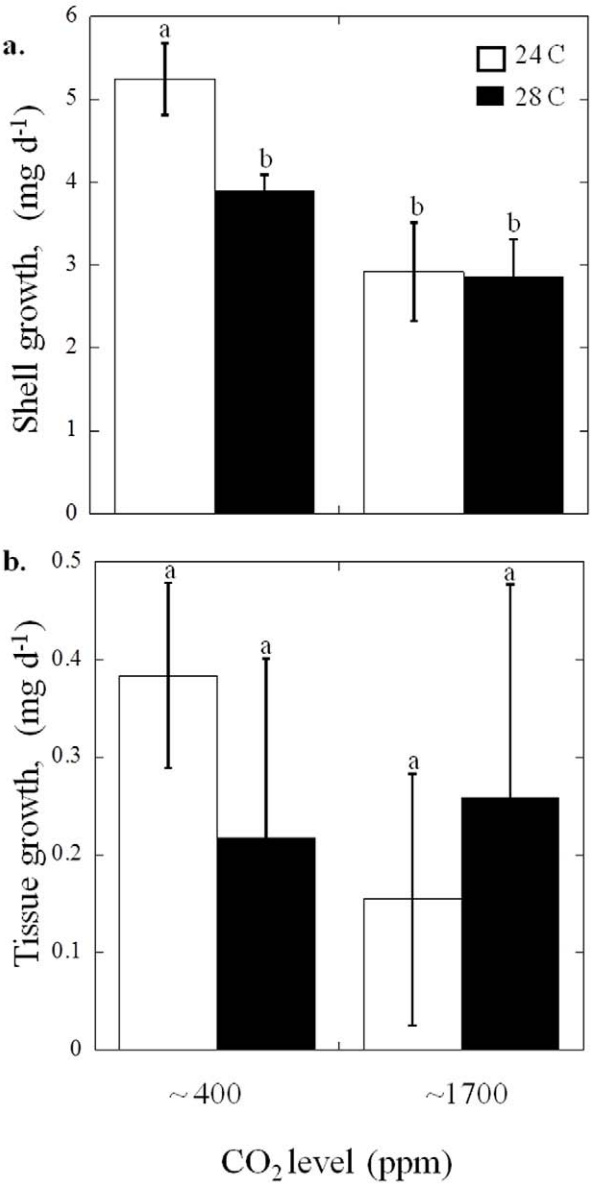


Figure 4. Growth of *Crassostrea virginica* juveniles at two levels of CO_2, approximately 400 and 1700 ppm, and two temperatures 24°C (white bars) and 28°C (black bars; see Table 2 for carbonate chemistry). a. Shell growth and b. Tissue growth. Error bars represent standard deviation of replicated vessels per treatment ($n = 3$ per treatment).
doi:10.1371/journal.pone.0026941.g004

temperature and high CO_2 may already be impacting coastal marine bivalve populations [26].

Adult populations of the three bivalve species examined in this study exist over a wide range of temperatures. *C. virginica* adults are tolerant of temperatures from −2 to 36°C and the geographical distribution of this species extends from the Gulf of St. Lawrence to the Gulf of Mexico [67] with growth being most rapid in the warmer waters found at its southern extent [68]. *M. mercenaria* distributions extend from the Gulf of St. Lawrence south to the Florida Keys, and this species can survive from 0 to 30°C [69]. Water temperatures between 20 and 24°C, however, have proven to provide maximal growth rates for *M. mercenaria* [70,71] with levels above 24°C yielding reduced pumping rates [72] and depressed growth rates of juvenile populations [57]. *A. irradians* and populations of *A. irradians* subspecies can be found from Cape Cod, Massachusetts into the Gulf of Mexico [73] and prolonged exposure of all life stages to 30°C can promote mortality in this species [74,75]. With global warming, shallow water habitats are experiencing extended periods of high temperature that heightens physiological stress for bivalves [76]. During this study, temperatures of 28°C decreased the growth and survival of juvenile *M. mercenaria* and *A. irradians*, respectively (Fig. 3 and 5), a temperature known to be detrimental to juvenile stages of these species [72,73]. In contrast, juvenile *C. virginica* growth was not reduced at 28°C (Fig. 4), a finding consistent with this species' ability to thrive in warmer waters [68].

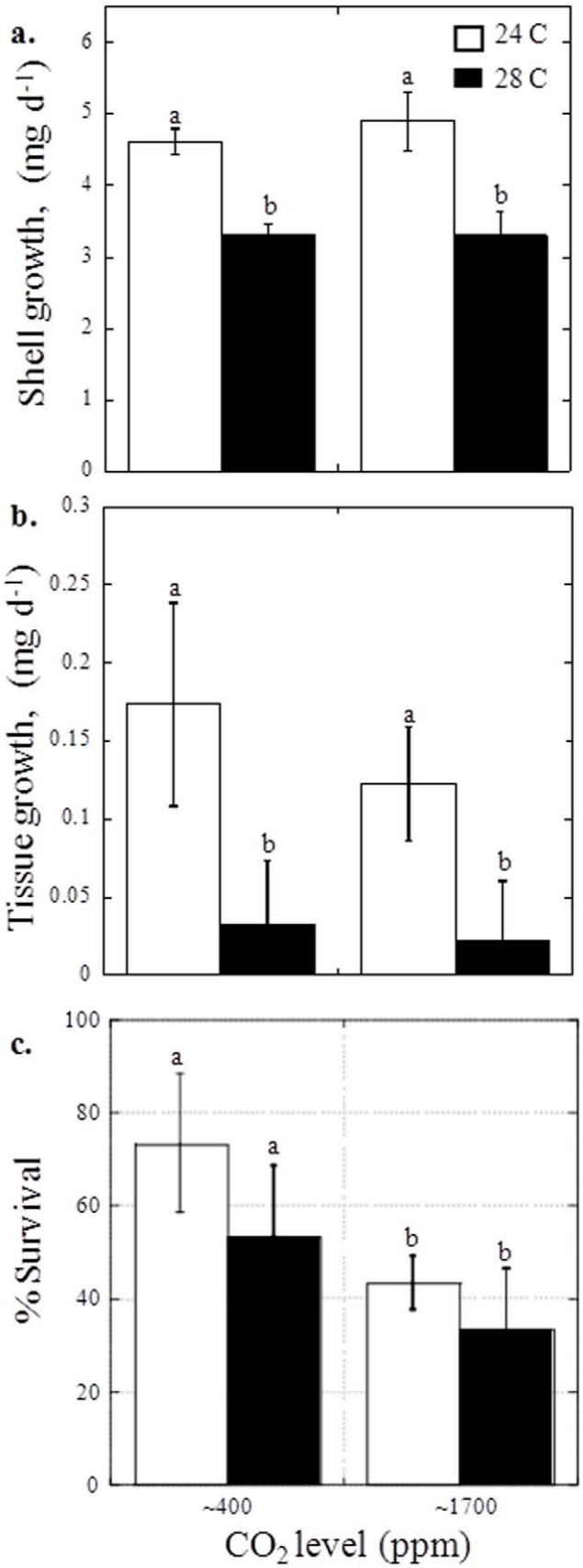


Figure 5. Growth and survival of *Argopecten irradians* juveniles at two levels of CO_2, approximately 400 and 1700 ppm, and two temperatures 24°C (white bars) and 28°C (black bars; see Table 2 for carbonate chemistry). a. Shell growth, and b. Tissue growth, c. Percent survival of individuals after 45 days. Error bars represent standard deviation of replicated vessels per treatment ($n = 3$ per treatment).
doi:10.1371/journal.pone.0026941.g005

Some of the differential susceptibility to high CO_2 among bivalve species seemed consistent with their position in the benthos. *M. mercenaria* juveniles and adults are infaunal being commonly burrowed in sediments that can be undersaturated with respect to carbonate [18]. Consistent with being well-adapted to such exposure, juvenile *M. mercenaria* growth was unaffected by the high levels of CO_2 administered during our experiment, despite aragonite being slightly undersaturated during the experiment ($\Omega = 0.92$). In contrast, high CO_2 significantly depressed the growth and survival of juvenile *C. virginica* and *A. irradians*, respectively (Figs. 4 and 5), two epifaunal species that are less likely to encounter sediments undersaturated with respect to calcium carbonate compared to infaunal species. Recent studies indicate many epifaunal species will encounter or already have encountered environments undersatruated with respect to calcium carbonate that may already be altering bivalve population structure [6,77,78]. In addition, two other epifaunal species (blue mussels, *Mytilus edulis*, and the Pacific oyster, *Crassostrea gigas*) have experienced decreased calcification and survival under high CO_2 concentrations [20,21]. Similar to these epifaunal bivalves, *C. virginica* may also be calcifying less under increased concentrations of CO_2 leading to the depressed growth rates observed during this study.

With regard to temperature, it may be hypothesized that epifaunal species are less sensitive to high temperatures since they are commonly exposed to warmer temperatures within shallow estuaries whereas infaunal species may avoid high temperatures by burrowing into cooler in sediment [79]. This could partly account for the significant decline in growth for the normally infaunal *M. mercenaria* at higher temperatures but an absence of a temperature affect on the epibenthic eastern oysters, *C. virginica*. We observed a different trend, however, for juvenile *A. irradians*, which were highly sensitive to prolonged exposure to both high temperature and high CO_2. Consistent with this finding, the early development the epifaunal oyster, *Saccostrea glomerata*, was negatively affected by both high temperature and high CO_2 while *Crassostrea gigas* was more resistant to these stressors [40,80]. Furthermore, a study of *C. virginica* juveniles native to Chesapeake Bay reported that higher temperatures and CO_2 additively decreased calcification rates [78]. Therefore, it would seem factors beyond life history-facilitated adaptations influence the vulnerability of bivalves to high temperature with and without high CO_2 and that mollusks are differentially adapted to these environmental stressors.

For *M. mercenaria* and *A. irradians*, the responses of the larval and juvenile stages to increased temperature and increased CO_2 concentrations may be compared. For *M. mercenaria* larvae, survival declined by 82% as conditions changed from low temperature and CO_2 to 28°C and 750 ppm CO_2. In contrast, survival juvenile *M. mercenaria* was unaffected by 28°C and even higher levels of CO_2 (~1700 ppm). *A. irradians* larval survival declined by 70% as conditions changed from low temperature and CO_2 to 28°C and 750 ppm CO_2 while juvenile *A. irradians* displayed a 50% reduction in survival when 24°C, ~400 ppm CO_2 treatments were compared to 28°C, and ~1700 ppm CO_2 treatments, a level more than two-fold higher than the concentration larvae were exposed to. Therefore, the larval stages

of both species were substantially more sensitive to high temperature and CO_2 than juvenile stages. The greater sensitivity of bivalve larvae compared to juveniles to higher CO_2 may be partly related to the types of $CaCO_3$ each stage synthesizes. The first $CaCO_3$ secreted by bivalve larvae is likely amphorous calcium carbonate, a precursor to aragonite or calcite that is 50-fold more susceptible to carbonate dissolution compared to the forms of $CaCO_3$ (aragonite, calcite) primarily found in juvenile stage bivalves [81]. Regarding *A. irradians*, juveniles and particularly larvae were highly sensitive to elevated temperature and somewhat less affected by CO_2. As such, the future success of this species may be highly dependent on the ability of all developmental stages to cope with temperature stress.

Many coastal ecosystems already experience elevated levels of CO_2 [5,6,24], in part due to decomposition of naturally and anthropogenically derived organic matter [7,8,9,10]. As these systems experience warming in the coming decades, a positive feedback loop may be established whereby increasing temperatures increase microbial remineralization rates of organic matter leading to further increases in CO_2 concentrations. As such, further studies that concurrently examine the effects of increasing temperatures and CO_2 concentrations on calcifying organisms in coastal marine ecosystems are certainly warranted.

Experimental research that seeks to mimic natural phenomena is inherently prone to limitations and this study was not an exception. For example, our delivery of a static level of CO_2 and temperature during experiments may have elicited a more extreme response than those displayed by individuals in coastal ecosystems where natural variations in temperature and CO_2 concentration may provide periods of stress and recovery that might permit some physiological compensation. In addition, like many other studies (e.g. [18,19,20,21,78]), our experiments with juvenile bivalves introduced animals reared under ideal conditions into experimental treatments, a procedure that does not mimic future ocean acidification but may be characteristic of some present day, coastal ocean acidification. In contrast, since the internal pH of adult bivalves is osmotically regulated and relatively static, developing gametes persist under ideal chemical conditions until spawned [29,30,31,34]. Once spawned, larvae suddenly enter a new chemical environment that differs from the biochemical stability offered by their parent. Similarly our experiments introduced bivalve larvae into experimental vessels within hours of fertilization. As coastal oceans acidify over the next two centuries, there may be selection pressure on bivalves to become more resistant to high CO_2 but it seems less likely that selection will alter homeostatic processes that regulate internal pH of adult bivalves [34]. As such, in the future, bivalve larvae may experience elevated CO_2 in a manner similar to our experiment design: Persisting under ideal conditions as gametes and then thrust into a new, high temperature or CO_2 environment as larvae. Therefore, while some aspects of this research had limitations, introducing hours-old larvae into a new environment may be one of the more realistic experimental approaches attempting to mimic future ocean acidification.

The sum of environmental stressors that may affect marine organisms in the coming decades, particularly in coastal ecosystems, is substantial. Exactly how increased temperature and CO_2 concentrations will combine to affect bivalve populations is still not entirely understood. This study demonstrates the negative consequences of developing in a thermally stressed and acidified environment for larval and juvenile bivalves. These effects may have serious implications for the future of these bivalves and other marine calcifying organisms faced with global climate change.

Supporting Information

Figure S1 Percent metamorphosed of *M. mercenaria* larvae grown under three levels of CO_2, approximately 250, 390, and 750 ppm, and two temperatures 24°C and 28°C (Table 1). Error bars represent standard deviation of replicated vessels per treatment ($n = 4$ per treatment).
(TIF)

Figure S2 Percent metamorphosed of *A.irradians* larvae grown under three levels of CO_2, approximately 250, 390, and 750 ppm, and two temperatures 24°C and 28°C (Table 1). Error bars represent standard deviation of replicated vessels per treatment ($n = 4$ per treatment).
(TIF)

Table S1 Two-way analysis of variance tables for all experiments.
(DOC)

Acknowledgments

We are grateful for our supply of *Mercenaria mercenaria* larvae from Cornell Cooperative Extension, Southold, NY and *Argopecten irradians* larvae from the East Hampton Shellfish Hatchery, East Hampton, NY. Juveniles of all three species were provided from the East Hampton Shellfish Hatchery.

Author Contributions

Conceived and designed the experiments: SCT CJG. Performed the experiments: SCT CJG. Analyzed the data: SCT CJG. Contributed reagents/materials/analysis tools: SCT CJG. Wrote the paper: SCT CJG.

References

1. IPCC (2007) Intergovernmental Panel on Climate Change. Summary for Policymakers. Cambridge University Press, Cambridge, 2007.
2. Houghton JT, Ding Y, Griggs DJ, Noguer M, Linden PJ, et al. (2001) Climate Change 2001: The Scientific Basis: Cambridge Univeristy Press. 881 p.
3. Fussel HM (2009) An updated assessment of the risks from climate change based on research published since the IPCC Fourth Assessment Report. Climatic Change 97: 469–482.
4. Cao L, Caldeira K, Jain AK (2007) Effects of carbon dioxide and climate change on ocean acidification and carbonate mineral saturation. Geophysical Research Letters 34: 5.
5. Feely RA, Sabine CL, Hernandez-Ayon JM, Ianson D, Hales B (2008) Evidence for upwelling of corrosive "acidified" water onto the continental shelf. Science 320: 1490–1492.
6. Salisbury J, Green M, Hunt C, Campbell J (2008) Coastal acidification by rivers: a new threat to shellfish? Eos, Transactions, American Geophysical Union 89: 513.
7. Gattuso JP, Frankignoulle M, Wollast R (1998) Carbon and carbonate metabolism in coastal aquatic ecosystems. Annual Review of Ecology and Systematics 29: 405–434.
8. Koch F, Gobler CJ (2009) The effects of tidal export from salt marsh ditches on estuarine water quality and plankton communities. Estuaries and Coasts 32: 261–275.
9. Paerl HW, Pinckney JL, Fear JM, Peierls BL (1998) Ecosystem responses to internal and watershed organic matter loading: consequences for hypoxia in the eutrophying Neuse river estuary, North Carolina, USA. Marine Ecology-Progress Series 166: 17–25.
10. Thomas H, Bozec Y, Elkalay K, de Baar HJW (2004) Enhanced open ocean storage of CO_2 from shelf sea pumping. Science 304: 1005–1008.
11. Levitus S, Antonov JI, Boyer TP, Stephens C (2000) Warming of the world ocean. Science 287: 2225–2229.
12. Nixon SX, Granger S, Buckley BA, Lamont M, Rowell B (2004) A one hundred and seventeen year coastal water temperature record from Woods Hole, Massachusetts. Estuaries 27: 397–404.
13. Doney SC, Fabry VJ, Feely RA, Kleypas JA (2009) Ocean acidification: the other CO_2 problem. Annual Review of Marine Science 1: 169–192.
14. Hoegh-Guldberg O, Mumby PJ, Hooten AJ, Steneck RS, Greenfield P, et al. (2007) Coral reefs under rapid climate change and ocean acidification. Science 318: 1737–1742.

15. De Bodt C, Van Oostende N, Harlay J, Sabbe K, Chou L (2010) Individual and interacting effects of pCO(2) and temperature on Emiliania huxleyi calcification: study of the calcite production, the coccolith morphology and the coccosphere size. Biogeosciences 7: 1401–1412.
16. Brennand HS, Soars N, Dworjanyn SA, Davis AR, Byrne M (2010) Impact of Ocean Warming and Ocean Acidification on Larval Development and Calcification in the Sea Urchin Tripneustes gratilla. Plos ONE 5: 7.
17. Martin S, Gattuso JP (2009) Response of Mediterranean coralline algae to ocean acidification and elevated temperature. Global Change Biology 15: 2089–2100.
18. Green MA, Jones ME, Boudreau CL, Moore RL, Westman BA (2004) Dissolution mortality of juvenile bivalves in coastal marine deposits. Limnology and Oceanography 49: 727–734.
19. Green MA, Waldbusser GG, Reilly SL, Emerson K, O'Donnell S (2009) Death by dissolution: sediment saturation state as a mortality factor for juvenile bivalves. Limnology and Oceanography 54: 1037–1047.
20. Gazeau F, Quiblier C, Jansen JM, Gattuso JP, Middelburg JJ, et al. (2007) Impact of elevated CO2 on shellfish calcification. Geophysical Reserach Letters 34: doi:10.1029/2006GL028554.
21. Berge JA, Bjerkeng B, Pettersen O, Schaanning MT, Oxnevad S (2006) Effects of increased sea water concentrations of CO2 on growth of the bivalve *Mytilus edulis* L. Chemosphere 62: 681–687.
22. Kurihara H, Kato S, Ishimatsu A (2007) Effects of increased seawater pCO_2 on early development of the oyster *Crassostrea gigas*. Aquatic Biology 1: 91–98.
23. Miller AW, Reynolds AC, Sobrino C, Riedel GF (2009) Shellfish face uncertain future in high CO_2 world: Influence of acidification on oyster larvae and growth in estuaries. Plos ONE 4: e5661. doi:5610.1371/journal.pone.0005661.
24. Talmage SC, Gobler CJ (2009) The effects of elevated carbon dioxide concentrations on the metamorphosis, size, and survival of larval hard clams (*Mercenaria mercenaria*), bay scallops (*Argopecten irradians*), and Eastern oysters (*Crassostrea virginica*). Limnology and Oceanography 54: 2072–2080.
25. Gazeau F, Gattuso JP, Dawber C, Pronker AE, Peene F, et al. (2010) Effect of ocean acidification on the early life stages of the blue mussel Mytilus edulis. Biogeosciences 7: 2051–2060.
26. Talmage SC, Gobler CJ (2010) Effects of past, present, and future ocean carbon dioxide contrations on the growth and survival of larval shellfish. Proceedings of the National Academy of Sciences of the United States of America.
27. Scavia D, Field JC, Boesch DF, Buddemeier RW, Burkett V, et al. (2002) Climate change impacts on US coastal and marine ecosystems. Estuaries 25: 149–164.
28. Hoffman GE, Todgham AE (2010) Living in the now: Physiological mechanisms to tolerate a rapidly changing environment. Annual Review of Physiology 72: 127–145.
29. Barber BJ, Blake NJ (2006) Reproductive physiology. In: Shumway SE, Parsons GJ, eds. Scallops: Biology, Ecology, and Aquaculture: Elsevier. pp 357–416.
30. Eversole AG (2001) Reproduction in *Mercenaria mercenaria*. In: Kraeuter JN, Castagna M, eds. Biology of the Hard Clam: Elsevier. pp 221–260.
31. Thorson G (1950) Reproductive and larval ecology of marine bottom invertebrates. Biological Reviews of the Cambridge Philosophical Society 25: 1–45.
32. Fritz LW (2001) Shell Structure and Age Determination. In: Kraeuter JN, Castagna M, eds. Biology of the Hard Clam: Elsevier Science. pp 53–76.
33. Loosanoff VL (1965) The American or Eastern oyster. United States Dept of the Interior Circular 205: 1–36.
34. Cragg SM (2006) Development, Physiology, Behaviour, and Ecology of Scallop Larvae. In: Shumway SE, Parsons GJ, eds. Scallops: Biology, Ecology, and Aquaculture: Elsevier. pp 45–122.
35. Cherkasov AS, Taylor C, Sokolova IM (2010) Seasonal variation in mitochondrial responses to cadmium and temperature in eastern oysters Crassostrea virginica (Gmelin) from different latitudes. Aquatic Toxicology 97: 68–78.
36. Pörtner HO (2008) Ecosystem effects of ocean acidification in times of ocean warming: a physiologist's view. Marine Ecology-Progress Series 373: 203–217.
37. Findlay HS, Kendall MA, Spicer JI, Widdicombe S (2010) Post-larval development of two intertidal barnacles at elevated CO2 and temperature. Marine Biology 157: 725–735.
38. Zippay ML, Hofmann GE (2010) Effect of pH on gene expression and thermal tolerance of early life history stages of red abalone (*Haliotis rufescens)*. Journal of Shellfish Research 29: 429–439.
39. Comeau S, Jeffree R, Teyssie JL, Gattuso JP (2010) Response of the Arctic Pteropod Limacina helicina to Projected Future Environmental Conditions. Plos ONE 5: 7.
40. Parker LM, Ross PM, O'Connor WA (2010) Comparing the effect of elevated pCO_2 and temperature on the fertilization and early development of two species of oysters. Marine Biology 157: 2435–2452.
41. Byrne M, Soars NA, Ho MA, Wong E, McElroy D, et al. (2010) Fertilization in a suite of coastal marine invertebrates from SE Australia is robust to near-future ocean warming and acidification. Marine Biology 157: 2061–2069.
42. Newell RIE (2004) Ecosystem influences of natural and cultivated populations of suspension-feeding bivalve molluscs: A review. Journal of Shellfish Research 23: 51–61.
43. Andre C, Rosenberg R (1991) Adult-larval interactions in the suspension feeding bivalves *Cerastoderma edule* and *Mya arenaria*. Marine Ecology Progress Series 71: 227–234.
44. Arnold WS (2008) Application of larval release for restocking and stock enhancement of coastal marine bivalve populations. Reviews in Fisheries Science 16: 65–71.
45. Caley MJ, Carr MH, Hixon MA, Hughes TP, Jones GP, et al. (1996) Recruitment and the local dynamics of open marine populations. Annual Review of Ecology and Systematics 27: 477–500.
46. S.C.D.H.S (1976–2008) Suffolk County Department of Health Services, Annual Report of Suface Water Quality.
47. Roy RN, Roy LN, Vogel KM, Porter-Moore C, Pearson T, et al. (1993) The dissociation constants of carbonic acid in seawater at salinities 5 to 45 and temperatures 0 to 45 C. Marine Chemistry 44: 249–267.
48. Dickson AG, Sabine CL, Christian JR, eds. Guide to best practises for ocean CO_2 measurements: PICES Special Publication, 3, 191pp. 191 p.
49. Dickson AG (1993) pH buffers for sea-water media based on the total hydorgen-ion concentration scale. Deep-Sea Research Part I-Oceanographic Research Papers 40: 107–118.
50. Riebesell U, Fabry VJ, Hansson L, Gattuso JP (2010) Guide to best practices for ocean acidification reserach and data reporting. , Luxembourg: Publications Office of the European Union.
51. Castell LL, Mann R (1994) Optimal staining of lipids in bivalve larvae with Nile Red. Aquaculture 119: 89–100.
52. Phillips NE (2002) Effects of nutrition-mediated larval condition on juvenile performance in a marine mussel. Ecology 83: 2562–2574.
53. Moss RH, Edmonds JA, Hibbard KA, Manning MR, Rose SK, et al. (2010) The next generation of scenarios for climate change research and assessment. Nature 463: 747–756.
54. Welschmeyer NA (1994) Fluorometric analysis of chlorophyll A in the presence of chlorophyll B and pheopigments. Limnology and Oceanography 39: 1985–1992.
55. Carmichael RH, Shriver AC, Valiela I (2004) Changes in shell and soft tissue growth, tissue composition, and survival of quahogs, Mercenaria mercenaria, and softshell clams, Mya arenaria, in response to eutrophic-driven changes in food supply and habitat. Journal of Experimental Marine Biology and Ecology 313: 75–104.
56. Nixon SW, Buckley BA (2002) "A strikingly rich zone" - Nutrient enrichment and secondary production in coastal marine ecosystems. Estuaries 25: 782–796.
57. Weiss MB, Curran PB, Peterson BJ, Gobler CJ (2007) The influence of plankton composition and water quality on hard clam (*Mercenaria mercenaria* L.) populations across Long Island's south shore lagoon estuaries (New York, USA). Journal of Experimental Marine Biology and Ecology 345: 12–25.
58. Gosselin LA, Qian PY (1997) Juvenile mortality in benthic marine invertebrates. Marine Ecology Progress Series 146: 265–282.
59. Schneider DW, Stoeckel JA, Rehmann CR, Blodgett KD, Sparks RE, et al. (2003) A developmental bottleneck in dispersing larvae: implications for spatial population dynamics. Ecology Letters 6: 352–360.
60. Tettelbach ST, Smith CF, Smolowitz R, Tetrault K, Dumais S (1999) Evidence for fall spawning of northern bay scallops Argopecten Irradians irradians (Lamarck 1819) in New York. Journal of Shellfish Research 18: 47–58.
61. Kurihara H, Asai T, Kato S, Ishimatsu A (2008) Effects of elevated pCO_2 on early development in the mussel *Mytilus galloprovincialis*. Aquatic Biology 4: 225–233.
62. McGladdery SE, Bower SM, Getchell RG (2006) Diseases and parasites of scallops. In: Shumway SE, Parsons GJ, eds. Scallops: Biology, Ecology, and Aquaculture: Elsevier. pp 595–639.
63. Orensanz JM, Parma AM, Turk T, Valero J (2006) Dynamics, assessment and management of exploited natural populations. In: Shumway SE, Parsons GJ, eds. Scallops: Biology, Ecology, and Aquaculture: Elsevier. pp 765–867.
64. Gobler CJ, Lonsdale DJ, Boyer GL (2005) A review of the causes, effects, and potential management of harmful brown tide blooms caused by *Aureococcus anophagefferens* (Hargraves et Sieburth). Estuaries 28: 726–749.
65. Wacker A, von Elert E (2002) Strong influences of larval diet history on subsequent post-settlement growth in the freshwater mollusc *Dreissena polymorpha*. Proceedings of the Royal Society of London Series B-Biological Sciences 269: 2113–2119.
66. Wikfors GH, Ferris GE, Smith BC (1992) The relationship between gross biochemical composition of cultured algal foods and growth of the hard clam *Mercenaria mercenaria* (L.). Aquaculture 108: 135–154.
67. Carlton JT, Mann R (1996) Transfers and world-wide introductions. In: Kennedy VS, Newell RIE, Eble AE, eds. The Eastern Oyster, *Crassostrea virginica*. College ParkMaryland: Maryland Sea Grant Book.
68. Shumway SE (1996) Natural environmental factors. In: Kennedy VS, Newell RIE, Eble AE, eds. The Eastern Oyster: *Crassostrea virginica*. College ParkMaryland: Maryland Sea Grant.
69. Harte ME (2001) Systematics and Taxonomy. In: Kraeuter JN, Castagna M, eds. Biology of the Hard Clam: Elsevier. pp 3–43.
70. Ansell AD (1968) Rate of growth of the hard clam *Mercenaria mercenaria* (L) throughout the geographical range. Journal Du Conseil 31: 364–409.
71. Pratt DM, Campbell DA (1956) Environmental factors affecting growth in *Venus Mercenaria*. Limnology and Oceanography 1: 2–17.
72. Grizzle RE, Bricelj VM, Shumway SE (2001) Physiological ecology of *Mercenaria mercenaria*. In: Kraeuter JN, Castagna M, eds. Biology of the Hard Clam: Elsevier.

73. Brand AR (2006) Scallop ecology: distributions and behaviour. In: Shumway SE, Parsons GJ, eds. Scallops: Biology, Ecology, and Aquaculture: Elsevier. pp 651–713.
74. Brun NT, Bricelj VM, MacRae TH, Ross NW (2008) Heat shock protein responses in thermally stressed bay scallops, Argopecten irradians, and sea scallops, Placopecten magellanicus. Journal of Experimental Marine Biology and Ecology 358: 151–162.
75. Castagna M (1975) Culture of bay scallop, *Argopecten irradians*, in Virginia Marine Fisheries Review 37: 19–24.
76. Helmuth B, Harley CDG, Halpin PM, O'Donnell M, Hofmann GE, et al. (2002) Climate change and latitudinal patterns of intertidal thermal stress. Science 298: 1015–1017.
77. Beck MW, Brumbaugh RD, Airoldi L, Carranza A, Coen LD, et al. (2011) Oyster Reefs at Risk and Recommendations for Conservation, Restoration, and Management. Bioscience 61: 107–116.
78. Waldbusser GG, Voigt EP, Bergschneider H, Green MA, Newell RIE (2011) Biocalcification in the Eastern Oyster (*Crassostrea virginica*) in Relation to Long-term Trends in Chesapeake Bay pH. Estuaries and Coasts 34: 221–231.
79. Carriker MR (2001) Functional morphology and behavior of shelled veligers and early juveniles. In: Kraeuter JN, Castagna M, eds. Biology of the Hard Clam: Elsevier. pp 283–300.
80. Parker LM, Ross PM, O'Connor WA (2009) The effect of ocean acidification and temperature on the fertilization and embryonic development of the Sydney rock oyster Saccostrea glomerata (Gould 1850). Global Change Biology 15: 2123–2136.
81. Weiss IM, Tuross N, Addadi L, Weiner S (2002) Mollusc larval shell formation: Amorphous calcium carbonate is a precursor phase for aragonite. Journal of Experimental Zoology 293: 478–491.

Estuaries and Coasts
DOI 10.1007/s12237-010-9307-0

NOTE

Biocalcification in the Eastern Oyster (*Crassostrea virginica)* in Relation to Long-term Trends in Chesapeake Bay pH

George G. Waldbusser · Erin P. Voigt · Heather Bergschneider · Mark A. Green · Roger I. E. Newell

Received: 22 October 2009 / Revised: 2 February 2010 / Accepted: 7 May 2010
© Coastal and Estuarine Research Federation 2010

Abstract Anthropogenic carbon dioxide (CO_2) emissions reduce pH of marine waters due to the absorption of atmospheric CO_2 and formation of carbonic acid. Estuarine waters are more susceptible to acidification because they are subject to multiple acid sources and are less buffered than marine waters. Consequently, estuarine shell forming species may experience acidification sooner than marine species although the tolerance of estuarine calcifiers to pH changes is poorly understood. We analyzed 23 years of Chesapeake Bay water quality monitoring data and found that daytime average pH significantly decreased across polyhaline waters although pH has not significantly changed across mesohaline waters. In some tributaries that once supported large oyster populations, pH is increasing. Current average conditions within some tributaries however correspond to values that we found in laboratory studies to reduce oyster biocalcification rates or resulted in net shell dissolution. Calcification rates of juvenile eastern oysters, *Crassostrea virginica*, were measured in laboratory studies in a three-way factorial design with 3 pH levels, two salinities, and two temperatures. Biocalcification declined significantly with a reduction of ~0.5 pH units and higher temperature and salinity mitigated the decrease in biocalcification.

Keywords Biocalcification · Bivalve · Chesapeake Bay · Estuarine acidification · Oyster · pH

G. G. Waldbusser (✉) · H. Bergschneider
College of Oceanic and Atmospheric Sciences,
Oregon State University,
104 COAS Administration Building,
Corvallis, OR 97331, USA
e-mail: waldbuss@coas.oregonstate.edu

E. P. Voigt
Southeast Coast Inventory and Monitoring Network,
Fort Sumter National Monument,
Sullivan's Island, SC 29482, USA

M. A. Green
Division of Natural Sciences, St. Joseph's College of Maine,
278 Whites Bridge Road,
Standish, ME 04084, USA

R. I. E. Newell
Horn Point Laboratory,
University of Maryland Center for Environmental Science,
2020 Horns Point Rd,
Cambridge, MD 21613, USA

Introduction

Rapidly increasing atmospheric carbon dioxide (CO_2) concentrations has the potential to lower the pH of natural waters due to the absorption and hydrolysis of CO_2 resulting in the formation of carbonic acid (i.e., Doney et al. 2009). Acidification results in the titration of a portion of carbonate (CO_3^{2-}) ions to bicarbonate (HCO_3^-) thereby lowering the availability of carbonate ions to calcifying organisms (Kleypas et al. 2006). When enough carbonate ions are titrated, waters become thermodynamically unstable for calcium carbonate minerals, or undersaturation with respect to calcium carbonate occurs and dissolution follows. It is less well-recognized that such adverse pH effects may be more pronounced in estuarine waters and interact with other variables affected by global climate change such as salinity and temperature (Pörtner 2008; O'Donnell et al. 2009; Najjar et al. 2010). Since many ecologically and commercially important shell forming organisms reside in or rely on estuarine habitats (Dame 1996) and estuaries are typically less buffered than oceans due to lower absolute concentrations of carbonate as well as proportionally less carbonate relative to the total dissolved

Published online: 29 May 2010

inorganic carbon pool (Cai and Wang 1998), acidification may occur sooner in coastal zones than in the open oceans.

Dissolution and hydrolysis of atmospheric CO_2 into estuarine waters is only one of many processes affecting pH in estuaries (Blackford and Gilbert 2007; Soetaert et al. 2007; Miller et al. 2009). Eutrophication and net heterotrophy (Kemp et al. 2005; Borges and Gypens 2010), watershed inputs (Dove and Sammut 2007; Salisbury et al. 2008), and dry deposition of other acid forming compounds such as sulfur and nitrogen (Doney et al. 2007), all can alter the pH of estuarine waters. The complex interaction of salinity, temperature, and pH on physiological processes makes it difficult to predict potential effects of increased atmospheric CO_2 on calcifying biota in estuaries that are often considered already degraded due to multiple anthropogenic impacts such as overfishing, eutrophication, and hydrological alteration (Lotze et al. 2006).

Molluscs are dominant components of the estuarine benthos; they serve as agents of benthic-pelagic coupling, provide food and habitat for other organisms, and are also harvested and cultivated commercially (Dame 1996). These diverse estuarine species may be especially vulnerable to long-term declines in baseline pH because they occupy highly variable habitats that often present conditions at the limits of their physiological tolerance. Therefore, even modest changes in pH may present conditions that are corrosive to shells and/or have physiological impacts on larval and adult oysters (Dove and Sammut 2007; Kurihara et al. 2007; Gazeau et al. 2007; Miller et al. 2009; Parker et al. 2009; Talmage and Gobler 2009).

The once abundant oyster populations in Chesapeake Bay have been decimated by multiple factors, including inadequate fisheries management, habitat decline, and disease (Rothschild et al. 1994; Kennedy et al. 1996; Smith et al. 2005). Ongoing stock enhancement projects often capitalize on the reduced virulence of these diseases in lower salinity (<10) waters to provide refugia (Paynter 1999). However, these lower salinity waters may now constitute unsuitable habitat for oysters, given the typically reduced availability of calcium carbonate for shell growth at lower salinities, coupled with often reduced pH values. For example, Ringwood and Keppler (2002) found that hard clam growth was significantly hampered by reduced pH values, and these effects were more pronounced in lower salinity waters.

The deposition of calcium carbonate in the form of an oyster shell is a complex and biologically controlled process that occurs on the internal shell surface. Shell growth initiates with the formation of a periostracum (outer coating) in the mantle folds, followed by the deposition of an organic matrix, and then calcium carbonate within this organic matrix (Wilber and Saleuddin 1983; Carriker 1996; Addadi et al. 2006). Proton pumps are used to precipitate mineral calcium carbonate (dominantly calcite in post-larval individuals; Fan et al. 2007) into the interior organic matrix of the shell (Levi-Kalisman et al. 2001). However, some recent work highlights the possibility of direct mineral deposition by the mantle tissue, rather than super-saturating the calcifying fluid (Addadi et al. 2006). The high level of physiological control over this process is evidenced by the small but significant role of the organic components, the periostracum and matrix, as well the deviation of carbon isotope ratios in some bivalve shells from seawater indicating both seawater bicarbonate and respired CO_2 being incorporated into the mineral phase (Gillikin et al. 2007; McConnaughey and Gillikin 2008). Therefore, it is important to recognize the potential effects that acidification of estuarine waters have on shell formation, and ultimately growth, are due to a combination of dissolution of the external shell surface and physiological changes to internal acid-base balance affecting the rate of new shell deposition.

Although reduced pH has many adverse physiological effects on aquatic organisms (Pörtner et al. 2004; Pörtner 2008) our study focuses on linking experimental measurements of oyster shell deposition in response to salinity, temperature, and pH with long-term changes in Chesapeake Bay conditions.

Methods

Chesapeake Bay Data Analyses

Water quality data from the Chesapeake Bay Program's Data Hub http://www.chesapeakebay.net/dataandtools.aspx were analyzed with linear regression analyses. Only water temperature, salinity, and pH, data from 1985 onward were used. Pre-1985 data were removed due to a limited sampling coverage. Mean values for each year, season, and salinity used in the analysis were based on ~100-2,000 data points for whole bay evaluations, and 10-50 data points for the oyster ground analyses. The measurements of pH by the Chesapeake Bay program are made with a CTD system equipped with pairs of glass silver chloride electrodes calibrated with National Bureau of Standards (NBS) buffers. Quality control and assurance for these publically available data is detailed at: http://www.chesapeakebay.net/qualityassurance.aspx; details about equipment calibration, field procedures, and data recording may be found at: http://archive.chesapeakebay.net/pubs/quality_assurance/MainstemTrib_QAPP07_Draft1.pdf.

Further quality control/quality assurance steps were taken in conducting these analyses by visual inspection of

plots of data for extreme outliers. Out of the 32,525 observations used in the original data set, only 53 extreme outliers were removed, one for having a salinity of over 40, and the other 52 for having pH above 9.5. The original data were arranged by the following arguments: data prior to 1985 were removed, the months of April, May, and June were assigned to the spring, and June, July, August, and September were assigned to summer. Data were grouped into these two seasons as they coincide with important growth periods for adult eastern oysters (spring) and juvenile oysters (summer). Observations with salinity between 5 and 18 were grouped as mesohaline, above 18 were polyhaline, and all observations deeper than 1 m were removed. Once the above filters were applied to the data set, mean values of pH were calculated for each season, salinity, and year. These values were then used in a simple linear regression model of pH by year for the four salinity-season combinations to determine the overall patterns of pH change within Chesapeake Bay, resulting in an $n=24$ for each linear regression.

Regression analyses and 5-year means for pH, water temperature, and salinity on historically important oyster grounds were carried out for Tangier Sound and the Chester, Choptank, Patuxent, Rappahannock, and James Rivers (Fig. 1). With the exception of Tangier Sound, sites within each river were identified using the Chesapeake Bay Program's analytical segmentation scheme to analyze mesohaline sites within each river. The segmentation scheme is a spatial delineation of the Chesapeake Bay and tidal tributaries based on areas with similar natural features used for organizational and management purposes. Sites within Tangier Sound were delineated by maps of oyster distributions (Smith et al. 2001). All observations within a tributary were averaged by season and year as noted above for regression analyses. However, for this analysis, all available depth data were averaged with the exception of observations below 9 m to avoid including data from water that may be hypoxic. Regression analyses were run for pH, water temperature, and salinity change over year from 1985 to 2008 ($n=24$). Mean values for each of these tributaries were also determined as above, except data were pooled from 2003 to 2008. All statistical analyses were conducted using SAS version 9.1 (SAS Institute Inc., Cary, NC, USA).

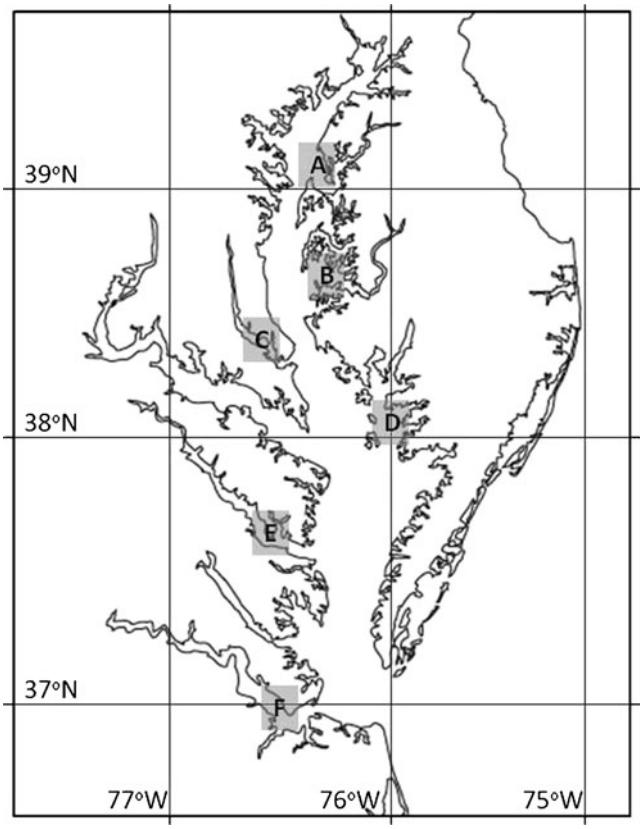


Fig. 1 Map of Chesapeake Bay indicating areas of historically important oyster grounds. Letters correspond to mesohaline areas in which pH data were analyzed in Table 1, as follows: **a** Chester River, **b** Choptank River, **c** Patuxent River, **d** Tangier Sound, **e** Rappahannock, and **f** James River

Calcification Rates

Oysters were reared at a salinity of 32. The low salinity treatment (16) oysters were acclimated to the new salinity by diluting the seawater with river water in the holding tank to reduce the salinity at a rate of 2 d^{-1}. During acclimation and holding periods, oysters were fed a diet of cultured *Isochrysis* spp. (Tiso) strain CCMP1324, raised in similar salinities to that of the oysters, and supplemented with Shellfish Diet 1800 (Reed Mariculture, Campbell, CA, USA). The high salinity water was collected from the Indian River inlet, lower Delaware Bay and the low salinity water was collected from the Patuxent River, near Solomons Island, MD, USA.

Shell calcification rates were measured on groups of approximately 300 juvenile oysters that were 1-2 mm shell height and totaled roughly 1-2 g live weight. During experimental measurements, oysters were held in 55-ml flasks containing 50 ml of water at a constant temperature, salinity, and pH. Two levels of temperature and two levels of salinity were used resulting in four experimental runs, each run having three pH treatments (Table 1) with four replicate flasks per pH treatment. In the mid and low pH treatment flasks, pH was controlled by injecting CO_2 into an airstream bubbled into each flask whilst measuring pH within flasks. The high pH treatment was bubbled with only air. One control flask per treatment combination was used with no oysters to monitor pH and titration alkalinity (TA) over the course of the experiment. We did not try to modify the pH in the high pH treatment across salinity-temperature combinations, and therefore, pH in the high

Table 1 Experimental conditions for each experimental run

Date Run	Temp.	Salinity	pH Treatment	pH	T. Alkalinity	Ω Calcite
3 Oct 08	20	32	High	8.29	2.052	4.62
			Mid	7.66	2.063	1.32
			Low	7.56	2.041	1.05
8 Oct 08	30	32	High	8.14	2.120	4.74
			Mid	7.85	2.148	2.78
			Low	7.43	2.115	1.13
10 Oct 08	30	16	High	7.92	1.248	1.45
			Mid	7.66	1.246	0.83
			Low	7.46	1.227	0.53
15 Oct 08	20	16	High	7.76	1.198	0.70
			Mid	7.52	1.155	0.39
			Low	7.41	1.160	0.31

Temperature and salinity were constant throughout the experiment; pH and titration alkalinity (T. Alkalinity) are initial conditions in the experimental flasks. Titration alkalinity is in millimoles L^{-1}, temperature is in °C, and saturation state (Ω calcite) was calculated with CO2SYS using salinity dependent carbonic acid dissociation constants of Millero et al.

pH-high salinity treatments is higher than the high pH-low salinity treatments. It is however important to note that the average of all pH treatments between salinities was not significantly different, and other than the high pH-high salinity treatments, all pH values fell within the same range of values between salinity treatments.

Immediately following the addition of oysters to flasks, a water sample was taken from each control flask to determine the initial TA (t=0). Water samples (4 ml) were taken by syringe from all flasks every 5 h for periods up to 15 h, resulting in four sample points per flask over time. Titration alkalinity data were examined immediately following each determination, and measurements were terminated if the rate of change had appeared to slow due to container effects. Rates of titration alkalinity change would often appear to slow by 10 h (depending on how fast initial rates were), and this corresponded with titration alkalinity changes on the order of 100-200 μmol L^{-1}. Therefore, we excluded these later data points to ensure the slopes of titration alkalinity change over time were in fact linear and provided estimates of calcification based on initial conditions.

Water samples were passed through a 0.2-μm filter cartridge and diluted with a 0.7-M NaCl solution in order to have enough sample for a two-point titration to measure total alkalinity (Edmond 1970). Temperature and pH were recorded using an Orion 938007MD micro temperature probe, Thermo-scientific 8103BN combination semimicro pH electrode and Thermo-scientific five-star pH meter (Thermo Fisher Scientific Inc., Waltham, MA, USA). The NBS scale was used for calibrating the pH probe with a three-point calibration curve, and pH standards were checked over the course of each experimental run to verify the calibration. Additionally, at each sample time, an alkalinity standard was measured to ensure analytical consistency. The change in TA over time in each flask was used to calculate the calcification rates of the oysters (Smith and Key 1975), assuming that any other processes contributing to TA change in the flask were negligible. Due to the small volume of water in the flasks, relative to the volume of samples removed, we converted the TA concentrations to an absolute alkalinity within each flask and corrected these values for the amount of TA removed at each sample time. The volume of water removed at each sample time was determined from weight and density. The average pH change (±1 SD) in all experimental flasks over the course of the experiments was −0.02±0.12.

Saturation state with respect to calcite was calculated by the program CO2SYS in Excel 2007 using Millero (1986) constants for K1 and K2, Dickson's KSO_4 constants, and pH entered on the NBS scale.

Statistical Analysis of Calcification Measurements

Calcification data were analyzed with a three-way analysis of variance including interaction effects. The three-way interaction of temperature, salinity, and pH was not significant (α=0.05) and dropped from the model. Since all two-way interactions were significant, the estimated least-squares means for each of these two-way combinations are presented. Differences among treatment combinations within each two-way interaction were evaluated using t tests with a Bonferroni correction for multiple pairwise comparisons at α=0.05. Assumptions of normality and heteroscedasticity were verified by visual inspection of residual distributions as well as Shapiro-Wilk's test for normality, and Hatley's f-max test for heteroscedasticity.

Results

Our analysis of monitoring data from 1985 to 2008 from the Chesapeake Bay Program revealed statistically significant declines in seasonally averaged daytime pH within polyhaline surface waters (>18; Fig. 2). The rate of

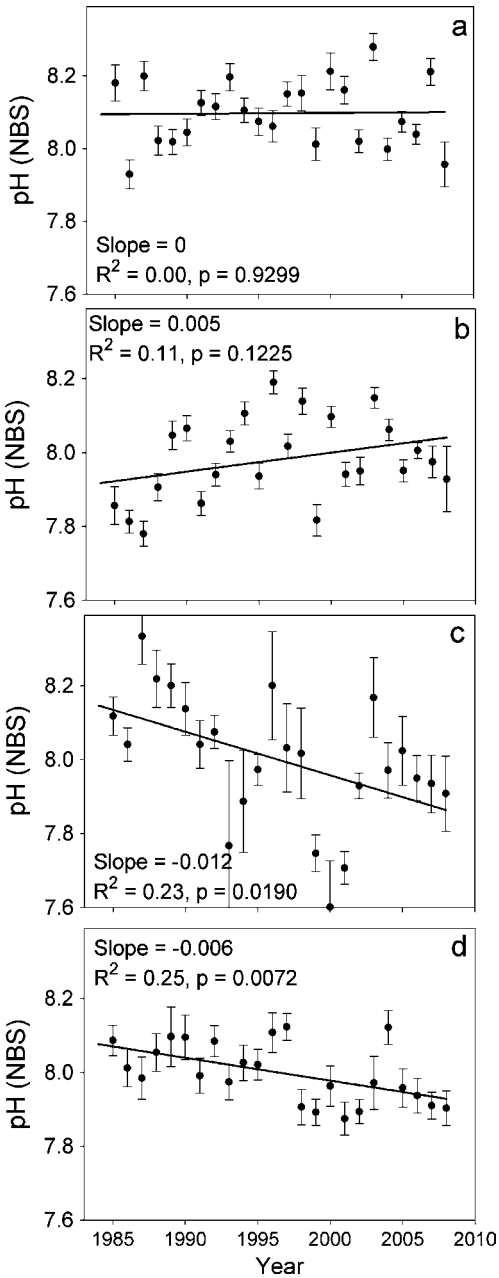


Fig. 2 Annual trends in Chesapeake Bay surface water pH in the **a** Spring-Mesohaline, **b** Summer-Mesohaline, **c** Spring-Polyhaline, and **d** Summer-Polyhaline. Mesohaline includes salinities of 5–18, and polyhaline are salinities >18. Mean values are based on surface waters of 1 m or less, averaged over season. Spring is defined as April, May, June, and summer June, July, August, September, corresponding to important growth periods for adults and juvenile oysters, respectively. *Error bars* are the 95% confidence interval for the mean values. Data originally obtained from Chesapeake Bay Program Water Quality Database (1985-2008)

decrease, in both spring and summer, exceeded those found in the open Pacific Ocean (Doney et al. 2009), where pH decrease is attributed solely to increasing atmospheric CO_2. Although no statistically significant trends were found in the mesohaline (five to 18) region of the mainstem Bay, a general increasing trend in pH was seen in the summer months, with some locations reporting pH values well above 9.0. Furthermore, we found that many mesohaline tributaries that once supported historically productive oyster grounds, exhibited a statistically significant increasing pH (Table 2).

Although there were broad trends of pH change across Chesapeake Bay (Fig. 2), we highlight here average values and trends in mesohaline portions of tributaries that once supported important oyster grounds (Fig. 1, Table 2). We have divided these data into seasons with April, May, and June being most important to new shell growth of adult oysters, and June, July, August, and September being important to larval development as well as settlement and growth of juveniles (Kennedy et al. 1996). In general, these tributaries showed increasing pH with the spring and summer pH values being similar within oyster grounds, with the exception of the Patuxent River (Table 2).

In laboratory studies, we found a complex change in biocalcification rates by juvenile *Crassostrea virginica* in response to variations in temperature, salinity, and pH (Figs. 3 and 4, Table 3). The three-way interaction of salinity, temperature, and pH was not significant, however the two-way interactions of salinity with pH and temperature with pH were significant (Table 3). We therefore estimated calcification rates by least squares means and made pairwise comparisons for each two-way interaction (Figs. 3 and 4). The absolute pH values within a treatment level (e.g., high pH) varied among experimental runs of different salinity-temperature combinations (Table 1). This discrepancy was due to the fact that initial pH values for the lower salinity waters were lower, and we did not try to increase the pH to match the higher salinity water. The high pH treatments, therefore, are unamended natural waters, or may be considered a control treatment with no addition of CO_2. Despite the significant two-way factorial interactions, our results clearly showed that under both lower salinity (=16) and lower temperatures (20°C) calcification rates steadily decreased as pH dropped. Conversely, at higher temperature (30°C) and salinity (=32), calcification rates did not significantly change as pH declined from high to mid-pH; however, calcification rates dropped significantly at the lowest pH. Additionally, calcification rates were not significantly affected by salinity at higher temperatures, although at lower temperatures there was a large salinity effect (Fig. 4).

Discussion

If small decreases in calcification results in a weaker or smaller shell over time, juvenile bivalves will be increasingly susceptible to predation or other possible mortality

Table 2 Mean seasonal pH, salinity, and surface water temperature (wtemp) and significant annual trends at historically important oyster grounds in Chesapeake Bay

Tributary	Season	pH	ΔpH	Salinity	ΔSalinity	Wtemp	ΔWtemp
Chester	Spring	7.67	0.025	8.7	n/s	16.9	0.2
	Summer	7.63	0.018	9.6	−0.1	25.2	0.1
Choptank	Spring	7.98	n/s	10.9	n/s	17.1	−0.1
	Summer	7.90	n/s	11.5	n/s	25.4	n/s
Patuxent	Spring	7.92	n/s	8.9	n/s	18.2	n/s
	Summer	7.76	0.010	10.2	n/s	26.0	n/s
Rappahannock	Spring	7.88	n/s	10.8	n/s	17.9	n/s
	Summer	7.82	0.011	13.1	n/s	25.9	n/s
James	Spring	7.66	n/s	12.6	n/s	19.2	n/s
	Summer	7.65	0.007	15.8	n/s	26.0	n/s
Tangier	Spring	7.84	n/s	12.6	n/s	18.7	n/s
	Summer	7.80	0.005	13.0	n/s	26.3	n/s

Spring and summer defined in text. Water temperature is in °C. Mean values are averages for 2003-2008 and changes in parameters are change in units per year, from significant regression slopes from 1985 to 2008

n/s non-significant regression at $\alpha=0.05$

factors (Kennedy et al. 1996; Newell et al. 2000, 2007). Indeed, recent work shows that shell dissolution is likely an important mortality factor for juvenile infaunal bivalves in marine muds (Green et al. 2009). Mollusk shell growth depends on the animal's ability to precipitate $CaCO_3$ into an internal organic matrix that provides the framework for their shell (Wilber and Saleuddin 1983; Levi-Kalisman et al. 2001). Since $CaCO_3$ deposition and the thermodynamics of this calcifying fluid is regulated to a large degree by internal physiological processes (Fan et al. 2007; Pörtner 2008), it is possible for shell growth to occur in waters that are undersaturated with respect to calcium carbonate. Green et al. (2009) note that juvenile infaunal hard clams, *Mercenaria mercenaria*, can survive corrosive conditions for several weeks (and well into adulthood) upon reaching what appears to be an escape size, or a size at which external corrosive conditions no longer affect survival. In our experiments, the relationship between calcification rates and calcite saturation state deviates from what may be expected if seawater thermodynamics were strictly controlling calcification rates (Fig. 5). Since the shells of many molluscs are covered to varying degrees by the periostracum, an organic based outer shell layer, the $CaCO_3$ shell material is protected from exposure to surrounding conditions. Therefore, our result showing net calcification occurring at undersaturated conditions is not surprising (Fig. 5); we also found that higher salinity (and thus higher saturation state) only increases calcification at reduced pH (Fig. 3a). Interestingly, calcification was also higher at the low salinity-high pH treatment than high salinity-low pH (Fig. 5), even though these treatments had similar saturation states with respect to calcite, indicating the sensitivity of calcification to pH. More generally, the importance of pH versus saturation state versus pCO_2 on calcification will likely vary with species, life stage, mode of calcification, and the degree of departure from what are currently poorly quantified thresholds to changes in carbonate variables. It is also important to acknowledge that our pH treatments were not identical between low and high salinity waters (Table 1), due to the natural differences in these waters. Therefore, we present the interpretation of Fig. 4 as a starting point to develop a more comprehensive understanding of the complex response of marine calcifiers to acidification.

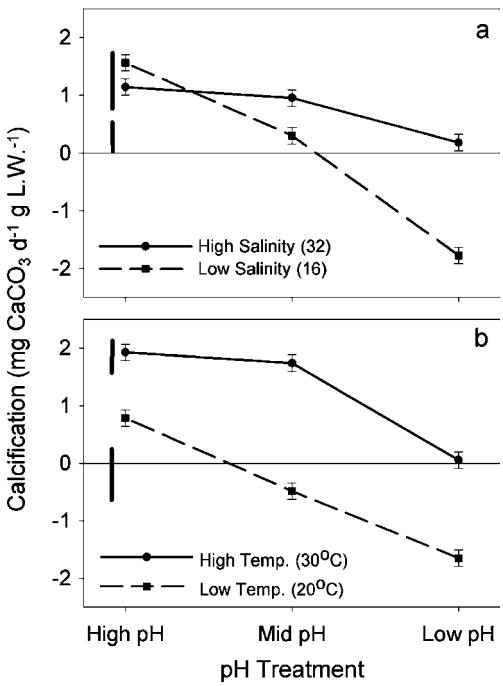


Fig. 3 Biocalcification rates of *C. virginica* for treatment combinations of **a** salinity by pH and **b** temperature by pH estimated by least squares means from each two-way ANOVA. Units of calcification are milligram of calcium carbonate per gram oyster whole live weight per day. Treatment levels of salinity, temperature, and pH are listed in Table 1. *Error bars* are standard errors of the least squares means estimates. Non-significant differences among estimated least squares means by pairwise comparisons with Bonferroni correction are noted by values falling within the *black vertical bars next to the left y-axis* at $\alpha=0.05$

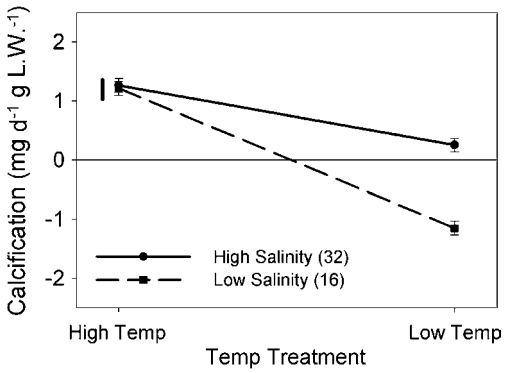


Fig. 4 Biocalcification rates of *C. virginica* for treatment combinations of temperature and salinity estimated by least squares means from the two-way ANOVA. Units of calcification are milligram of calcium carbonate per gram oyster whole live weight per day. Treatment levels of salinity, temperature, and pH are listed in Table 1. *Error bars* are standard errors of the least squares means estimates. Non-significant differences among estimated least squares means by individual pairwise comparisons with Bonferroni correction are noted by values falling within the *black vertical bars next to the left y-axis* at $\alpha=0.05$

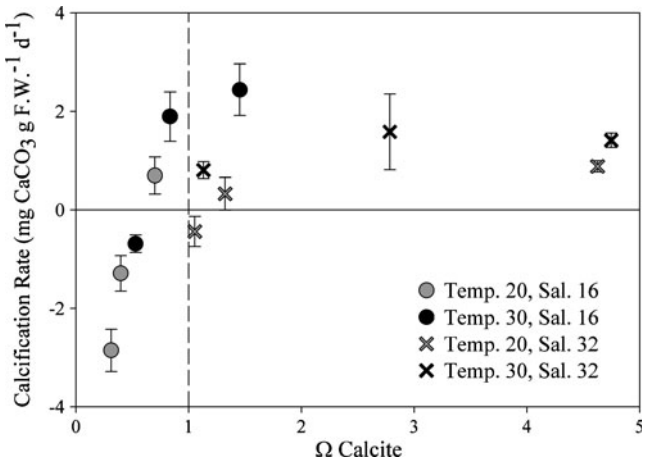


Fig. 5 Biocalcification rates of *C. virginica* plotted against saturation state of calcite. Units of calcification are mg of calcium carbonate per gram oyster whole live weight per day. Temperature and salinity treatments are noted as follows: 20°C is in *gray*, 30°C in *black*, salinity 16 (*filled square*) and salinity 32 (*multiplication symbol*). Values are the means and standard deviations from the measured rates and therefore differ slightly from the estimated least squares and standard errors derived from the ANOVA and presented in Figs. 3 and 4

Concurrent with anthropogenic acidification of natural waters, climate change impacts to local weather patterns has the potential to alter salinity and temperature dynamics of estuarine waters. Within Chesapeake Bay, expected changes to temperature and salinity coupled with our experimental data highlight the important role these environmental parameters may play in the overall bivalve response to an anthropogenically altered carbonate system in this estuary. Najjar et al. (2010) predict that temperature will continue to increase in Chesapeake Bay waters over coming decades, with the strongest warming trends in the northern portion of the Bay. Our laboratory measurements indicate that moderate increases in temperature (within the range of tolerance of this species) would allow oysters to calcify more rapidly, however temperature had less effect on calcification in the low pH treatment (Fig. 3b). Also at the higher temperature, differences in calcification due to salinity were non-significant (Fig. 4), regardless of pH. Chesapeake Bay watershed precipitation is forecast to increase during winter and spring. This increased precipitation coincides with peak freshwater input into Chesapeake Bay, lowering salinity of the bay in these important adult growth and reproductive development seasons (Najjar et al. 2010 and references therein). Additionally, through summer and fall, increasingly variable trends in precipitation and therefore salinity are also predicted (Najjar et al. 2010), coinciding with larval and post-larval growth periods. Our experimental data found that higher salinity helped juvenile oysters overcome low pH effects on calcification (Fig. 3a), although calcification rates did not increase directly due to salinity across all pH treatments. Salinity has been previously noted as the most important climatological variable on recruitment strength in Chesapeake Bay oyster populations (Ulanowicz et al. 1980; Kimmel and Newell 2007), with generally stronger recruitment at higher salinity. Our findings suggest the increased saturation state of higher salinity waters may provide the ability to overcome decreases in pH during the sensitive post-metamorphosis stage, as one of many possible reasons why increased salinity may increase rates of oyster recruitment. Coupled with long-term increases in eutrophication, primary production, and hypoxia (i.e metabolic uptake and production of CO_2) (Hagy et al. 2004; Kemp et al. 2005) a complex picture of future carbonate system status and impacts on estuarine bivalves emerges. Determining the future carbonate system in estuaries, therefore, evades simple forecasts of estuarine $CaCO_3$ saturation state by increasing atmospheric CO_2 alone (Blackford and

Table 3 Significant results from the three-way ANOVA on the biocalcification experiments

Effect	DF	*F* value	*P* value
Salinity	1, 38	39.24	<0.0001
Temperature	1, 38	210.43	<0.0001
pH	2, 38	117.91	<0.0001
Salinity × pH	2, 38	34.89	<0.0001
Temperature × pH	2, 38	7.24	0.0022
Salinity × temperature	1, 38	33.92	<0.0001

The three-way interaction is not presented, as this was found to be non-significant and was removed from the model

DF degrees of freedom

Gilbert 2007; Salisbury et al. 2008; Borges and Gypens 2010).

Another important component to the differential response of calcification to pH in different salinities is the role of disease and management. Oyster restoration in Maryland has centered on lower salinity waters (Paynter 1999) due to the decreased incidence of oyster diseases in these habitats (Kennedy et al. 1996). In our experiments, the salinity-pH interactions (Fig. 3a) indicate that low pH will most adversely affect eastern oysters inhabiting lower salinity waters. Importantly, several of these lower salinity sites had past 5-year average pH-salinity combinations that resulted in decreased shell growth or even dissolution in our experimental treatments (Tables 1 and 2, Fig. 3a). Although lower salinity habitats may provide refuge from disease, they also prove a more difficult geochemical habitat for shell formation/deposition.

Oysters require a hard substrate for recruitment (Kennedy et al. 1996), and in natural populations this is primarily the calcium carbonate shells of living and dead oysters. The loss of shell from oyster reefs is a major impediment to achieving oyster restoration goals in Chesapeake Bay (Smith et al. 2001; Mann and Powell 2007; Schulte et al. 2009) and in other estuaries (Powell et al. 2006). The dissolution of shell materials by declines in pH, or an inability of live oysters to grow new shell faster than it is lost, may alter available substrate and shell budgets of oyster reefs. Our analyses of pH data suggest that polyhaline portions of Chesapeake Bay will become increasingly corrosive to oyster shell (Fig. 2) and that average pH conditions in many oyster grounds are already unsuitable for shell preservation (Table 2). This conclusion is based on pH, and by extension mineral thermodynamics, alone; the fate of oyster shells in the estuary however is also controlled by other factors such as sedimentation, bioerosion, and harvesting (Smith et al. 2005; Mann and Powell 2007; Schulte et al. 2009). As evidenced by Powell et al. (2006), the highest rates of shell loss occurred in mid-salinity regions, with higher preservation in lower and higher salinity waters, suggesting a decoupling between strict thermodynamics and mineral preservation in this estuary. Although a rich literature exists on taphonomy and fate of biogenic calcium carbonate, little is known regarding the role of pH and biogeochemistry on shell budgets of estuarine oyster reefs. Our study highlights the potential threat of estuarine acidification to oyster calcification and reefs, and reinforces concerns of others with regards to shell material as an important resource of the estuary (Gutierrez et al. 2003; Powell et al. 2006; Powell and Klinck 2007).

Our laboratory measurements of oyster calcification combined with field data of pH change over time in Chesapeake Bay are suggestive of potential effects of changing estuarine dynamics on juvenile oyster calcification. However, our findings are tempered by the limited extrapolation of laboratory measurements to in situ conditions and concerns of environmental pH measurements in estuarine waters, discussed below. Two concerns of short-term calcification experiments using the alkalinity anomaly method are the metabolic contribution to total alkalinity change and the acclimation time for organisms to pH change. Based on ammonia excretion rates for the eastern oyster (Srna and Baggaley 1976), ammonia excretion over the course of our experiments accounted for less than 1% of the observed total alkalinity change in experimental flasks. The potential effect of respired CO_2 on the water chemistry was also not an issue as the flasks were not a closed system and were continuously bubbled with a CO_2/air mixture. However, with regard to short-term experiments such as these, acclimation time may be of some concern. Our treatment range was well within range of diurnal changes often found in estuarine waters and static conditions for weeks to months do not represent in situ estuarine conditions either. Future studies of long-term acclimated oysters that can be compared to our short-term non-acclimated oysters may allow us to better understand calcification responses of estuarine organisms due to different processes that occur across different time scales.

Calcification in eastern oyster responded rapidly to changes in pH, although the cumulative effect of diel variations in pH on longer-term shell growth remains unknown. An integral question to understanding calcification responses to acidification is whether chronic or acute changes in carbonate parameters are more significant to oyster population dynamics. While it is clear that below certain threshold pH (and hence saturation state) values shell dissolution occurs (e.g., Green et al. 2009) and there are shifts in internal acid-base physiology (e.g., Michaelidis et al. 2005) the animal may be able to recover depending on exposure magnitude, length of exposure, and life stage. An analogy is the periodic dissolution of internal shell during aerial exposure by decreased pallial cavity pH induced by the accumulation of anaerobic metabolites (Burnett 1988), which is reversed once the animal is submerged again. These concerns constitute critical questions that need to be addressed regarding the impact of acidification on economically and ecologically important shell forming species in coastal and estuarine habitats.

The statistically significant and compelling trends in the Chesapeake Bay pH data we found (Fig. 2) are likely due to increased primary production (from eutrophication) (Hagy et al. 2004) in the mesohaline region of the Bay and resulting increased respiration of this material with transport down Bay to the polyhaline waters. Borges and Gypens (2010) note that eutrophication was a larger driver of decadal pH trends in the Belgium Coastal Zone than increased atmospheric CO_2. Whether CO_2 is derived from

enhanced respiration due to eutrophication or hydrolysis of atmospheric CO_2 into natural waters, the ultimate outcome is the same, lower pH. The increasing concentration of atmospheric CO_2 presents the possibility of a shifting baseline (Jackson et al. 2001) that should not be ignored simply because other processes impact pH more rapidly on shorter timescales. In fact, given the highly variable nature of estuarine and coastal ecosystems and several anthropogenic acidity sources, it is likely that even gradual decreases in baseline pH could alter estuarine carbonate dynamics in important ways sooner than open ocean environments.

The results of our Chesapeake Bay pH analysis should however be viewed cautiously due to substantive and justified concerns with the measurement of pH in estuarine waters with glass potentiometric electrodes. The difference in ionic strength of calibration and measurement solutions can lead to drift and error in pH measurements (e.g., Millero 1986). However, the large number of pH measurements used to calculate each mean value (n=~100-2000) in the regression analyses should smooth out random measurement errors. Systematic bias over time is still possible. One additional concern with discrete monitoring data, such as these, is the timing of sampling in relation to diurnal cycles in production and respiration which lead to significant pH variability even in well buffered systems (Yates et al. 2007). Daytime sampling could impart significant positive bias in pH measurements and the monitoring program's measurement of pH occurs primarily during daylight hours. For example, our findings of increased pH in several mesohaline tributaries (Table 2), if due to increased primary production, would likely be accompanied by larger respiratory signal during night and decreased pre-dawn pH. We therefore caution the reader to recognize the shortcomings of our Chesapeake Bay data analyses, and acknowledge that more work is needed to better constrain what appears to be long-term trends in pH and resulting carbonate chemistry of Chesapeake Bay.

Ocean acidification has been referred to the "other CO_2 problem" (Doney et al. 2009) because it is another consequence of increasing atmospheric CO_2. The capacity of the world's oceans to absorb CO_2 and the resulting hydrolysis of carbonic acid is well understood and may be predicted relatively easily. Responses of calcifying organisms, scales and timing of pH and total alkalinity variability, and the role of other biogeochemical processes and environmental variables in regulating estuarine pH are less well understood but represent critical problems. As research advances, it is vital to recognize that the biogeochemical effects of anthropogenic increases in CO_2 on carbonate chemistry are the same regardless if it is derived from the burning of fossil fuels or from net heterotrophy associated with eutrophication (Frankignoulle et al. 1998). The magnitude and variability of estuarine pH due to eutrophication enhanced respiration will certainly be larger over short time scales than the gradual increase from atmospheric CO_2 (Borges and Gypens 2010). However, increasing atmospheric CO_2 may indirectly contribute to greater primary production in regions that may be carbon limited during blooms, by leading to higher respiratory production of CO_2 and consequently larger variability in pH in these regions. Although pH is highly variable in estuarine waters, our analyses of long-term Chesapeake Bay pH data and laboratory experiments with eastern oysters highlight the importance in recognizing possible shifting baselines (Jackson et al. 2001) especially in relation to physiological thresholds for calcifying organisms. Accounting for the interacting climate related effects of temperature, salinity, and pH is vital to understanding the potential impact of acidification to estuaries and their living resources.

Acknowledgments This research was supported by a National Science Foundation award #OCE-0622999 to MAG and GGW and The Geneva Boone Endowed Award Fund to EPV by St. Mary's College of Maryland. We thank two anonymous reviewers for helpful and critical feedback on a previous version of this manuscript. We thank M. Luckenbach and S. Bonniwell of the Virginia Institute of Marine Science-Eastern Shore Laboratory for providing juvenile oysters. Components of this research were conducted as an undergraduate senior research thesis by EPV in partial fulfillment of requirements for a B.A. GGW would also like to thank Chesapeake Biological Laboratory, where this work was conducted.

References

Addadi, L., D. Joester, F. Nudelman, and S. Weiner. 2006. Mollusk shell formation: a source of new concepts for understanding biomineralization processes. *Chemistry-a European Journal* 12: 981–987.

Blackford, J.C., and F.J. Gilbert. 2007. pH variability and CO2 induced acidification in the North Sea. *Journal of Marine Systems* 64: 229–241.

Borges, A.V., and N. Gypens. 2010. Carbonate chemistry in the coastal zone responds more strongly to eutrophication than to ocean acidification. *Limnology and Oceanography* 55: 346–353.

Burnett, L.E. 1988. Physiological responses to air exposure: acid-base balance and the role of branchial water stores. *American Zoologist* 28: 125–135.

Cai, W.J., and Y. Wang. 1998. The chemistry, fluxes, and sources of carbon dioxide in the estuarine waters of the Satilla and Altamaha Rivers, Georgia. *Limnology and Oceanography* 43: 657–668.

Carriker, M.R. 1996. The shell and ligament. In *The eastern oyster Crassostrea virginica*, ed. V.S. Kennedy, R.I.E. Newell, and A. Eble, 734. College Park: Maryland Sea Grant.

Dame, R.F. 1996. *Ecology of marine bivalves, an ecosystem approach*. Boca Raton: CRC Press.

Doney, S.C., N. Mahowald, I. Lima, R.A. Feely, F.T. Mackenzie, J.F. Lamarque, and P.J. Rasch. 2007. Impact of anthropogenic atmospheric nitrogen and sulfur deposition on ocean acidification and the inorganic carbon system. *Proceedings of the National*

Academy of Sciences of the United States of America 104: 14580–14585.

Doney, S.C., V.J. Fabry, R.A. Feely, and J.A. Kleypas. 2009. Ocean acidification: the other CO2 problem. *Annual Review of Marine Science* 1: 169–192.

Dove, M.C., and J. Sammut. 2007. Impacts of estuarine acidification on survival and growth of Sydney rock oysters *Saccostrea Glomerata* (gould 1850). *Journal of Shellfish Research* 26: 519–527.

Edmond, J.M. 1970. High precision determination of titration alkalinity and total carbon dioxide content of sea water by potentiometric titration. *Deep-Sea Research* 17: 737.

Fan, W.M., C.Z. Li, X. Wang, N.P. Gong, L.P. Xie, and R.Q. Zhang. 2007. Cloning, characterization and expression analysis of calcium channel beta subunit from pearl oyster (*Pinctada fucata*). *Journal of Bioscience and Bioengineering* 104: 47–54.

Frankignoulle, M., G. Abril, A. Borges, I. Bourge, C. Canon, B. DeLille, E. Libert, and J.M. Theate. 1998. Carbon dioxide emission from European estuaries. *Science* 282: 434–436.

Gazeau, F., C. Quiblier, J.M. Jansen, J.P. Gattuso, J.J. Middelburg and C.H.R. Heip. 2007. Impact of elevated CO2 on shellfish calcification. Geophysical Research Letters 34: doi:10.1029/2006GL028554

Gillikin, D.P., A. Lorrain, L. Meng, and F. Dehairs. 2007. A large metabolic carbon contribution to the delta C-13 record in marine aragonitic bivalve shells. *Geochimica et Cosmochimica Acta* 71: 2936–2946.

Green, M.A., G.G. Waldbusser, S.L. Reilly, K. Emerson, and S. O'Donnell. 2009. Death by dissolution: sediment saturation state as a mortality factor for juvenile bivalves. *Limnology and Oceanography* 54: 1037–1047.

Gutierrez, J.L., C.G. Jones, D.L. Strayer, and O.O. Iribarne. 2003. Mollusks as ecosystem engineers: the role of shell production in aquatic habitats. *Oikos* 101: 79–90.

Hagy, J.D., W.R. Boynton, C.W. Keefe, and K.V. Wood. 2004. Hypoxia in Chesapeake Bay, 1950-2001: long-term change in relation to nutrient loading and river flow. *Estuaries* 27: 634–658.

Jackson, J.B.C., M.X. Kirby, W.H. Berger, K.A. Bjorndal, L.W. Botsford, B.J. Bourque, R.H. Bradbury, R. Cooke, J. Erlandson, J.A. Estes, T.P. Hughes, S. Kidwell, C.B. Lange, H.S. Lenihan, J. M. Pandolfi, C.H. Peterson, R.S. Steneck, M.J. Tegner, and R.R. Warner. 2001. Historical overfishing and the recent collapse of coastal ecosystems. *Science* 293: 629–638.

Kemp, W.M., W.R. Boynton, J.E. Adolf, D.F. Boesch, W.C. Boicourt, G. Brush, J.C. Cornwell, T.R. Fisher, P.M. Glibert, J.D. Hagy, L.W. Harding, E.D. Houde, D.G. Kimmel, W.D. Miller, R.I.E. Newell, M.R. Roman, E.M. Smith, and J.C. Stevenson. 2005. Eutrophication of Chesapeake Bay: historical trends and ecological interactions. *Marine Ecology-Progress Series* 303: 1–29.

Kennedy, V.S., R.I.E. Newell, and A. Eble. 1996. *The eastern oyster, Crassostrea virginica*. College Park: Maryland Sea Grant.

Kimmel, D.G., and R.I.E. Newell. 2007. The influence of climate variation on eastern oyster (*Crassostrea virginica*) juvenile abundance in Chesapeake Bay. *Limnology and Oceanography* 52: 959–965.

Kleypas, J.A., R.A. Feely, V.J. Fabry, C. Langdon, C.L. Sabine, and L. L. Robbins, 2006. Impacts of ocean acidification on coral reefs and other marine calcifiers: a guide for future research, report of a workshop held 18–20 April 2005, St. Petersburg, FL, sponsored by NSF, NOAA, and the U.S. Geological Survey, 88 pp.

Kurihara, H., S. Kato, and A. Ishimatsu. 2007. Effects of increased seawater pCO(2) on early development of the oyster *Crassostrea gigas*. *Aquatic Biology* 1: 91–98.

Levi-Kalisman, Y., G. Falini, L. Addadi, and S. Weiner. 2001. Structure of the nacreous organic matrix of a bivalve mollusk shell examined in the hydrated state using Cryo-TEM. *Journal of Structural Biology* 135: 8–17.

Lotze, H.K., H.S. Lenihan, B.J. Bourque, R.H. Bradbury, R.G. Cooke, M.C. Kay, S.M. Kidwell, M.X. Kirby, C.H. Peterson, and J.B.C. Jackson. 2006. Depletion, degradation, and recovery potential of estuaries and coastal seas. *Science* 312: 1806–1809.

Mann, R., and E.N. Powell. 2007. Why oyster restoration goals in the Chesapeake Bay are not and probably cannot be achieved. *Journal of Shellfish Research* 26: 905–917.

McConnaughey, T.A., and D.P. Gillikin. 2008. Carbon isotopes in mollusk shell carbonates. *Geo-Marine Letters* 28: 287–299.

Michaelidis, B., C. Ouzounis, A. Paleras, and H.O. Portner. 2005. Effects of long-term moderate hypercapnia on acid-base balance and growth rate in marine mussels *Mytilus galloprovincialis*. *Marine Ecology-Progress Series* 293: 109–118.

Miller, A.W., A.C. Reynolds, C. Sorbino, and G.F. Riedel. 2009. Shellfish face uncertain future in high CO_2 world: influence of acidification on oyster larvae calcification and growth in estuaries. *PLoS Biology* 4: e5661.

Millero, F.J. 1986. The pH of estuarine waters. *Limnology and Oceanography* 31: 839–847.

Najjar, R.G., C.R. Pyke, M.B. Adams, D. Breitburg, C. Hershner, M. Kemp, R. Howarth, M.R. Mulholland, M. Paolisso, D. Secor, K. Sellner, D. Wardrop, and R. Wood. 2010. Potential climate-change impacts on the Chesapeake Bay. *Estuarine Coastal and Shelf Science* 86: 1–20.

Newell, R.I.E., G.S. Alspach Jr., V.S. Kennedy, and D. Jacobs. 2000. Mortality of newly metamorphosed eastern oysters (*Crassostrea virginica*) in mesohaline Chesapeake Bay. *Marine Biology* 136: 665–676.

Newell, R.I.E., V.S. Kennedy, and K.S. Shaw. 2007. Comparative vulnerability to predators, and induced defense responses, of eastern oysters *Crassostrea virginica* and non-native *Crassostrea ariakensis* oysters in Chesapeake Bay. *Marine Biology* 152: 449–460.

O'Donnell, M., L. Hammond, and G. Hofmann. 2009. Predicted impact of ocean acidification on a marine invertebrate: elevated CO2 alters response to thermal stress in sea urchin larvae. *Marine Biology* 156: 439–446.

Parker, L.M., P.M. Ross, and W.A. O'Connor. 2009. The effect of ocean acidification and temperature on the fertilization and embryonic development of the Sydney rock oyster *Saccostrea glomerata* (Gould 1850). *Global Change Biology* 15: 2123–2136.

Paynter, K.T. 1999. Managing around oyster disease in Maryland and Maryland oyster roundtable strategies. In *Oyster reef habitat restoration: a synopsis and synthesis of approaches*, ed. M.W. Luckenbach, R. Mann, and J.A. Wesson, 317–328. Gloucester Point: Virgina Institute of Marine Science Press.

Pörtner, H.O. 2008. Ecosystem effects of ocean acidification in times of ocean warming: a physiologist's view. *Marine Ecology-Progress Series* 373: 203–217.

Pörtner, H.O., M. Langenbuch, and A. Reipschlager. 2004. Biological impact of elevated ocean CO2 concentrations: lessons from animal physiology and earth history. *Journal of Oceanography* 60: 705–718.

Powell, E.N., and J.M. Klinck. 2007. Is oyster shell a sustainable estuarine resource? *Journal of Shellfish Research* 26: 181–194.

Powell, E.N., J.N. Kraeuter, and K.A. Ashton-Alcox. 2006. How long does oyster shell last on an oyster reef? *Estuarine Coastal and Shelf Science* 69: 531–542.

Ringwood, A.H., and C.J. Keppler. 2002. Water quality variation and clam growth: is pH really a non-issue in estuaries? *Estuaries* 25: 901–907.

Rothschild, B.J., J.S. Ault, P. Goulletquer, and M. Heral. 1994. Decline of the Chesapeake Bay oyster population—a century of habitat destruction and overfishing. *Marine Ecology-Progress Series* 111: 29–39.

Salisbury, J., M.A. Green, C. Hunt, and J. Campbell. 2008. Coastal acidifcation by rivers: a threat to shellfish? *EOS, Transactions, American Geophysical Union* 89: 513–514.

Schulte, D.M., R.P. Burke, and R.N. Lipcius. 2009. Unprecedented restoration of a native oyster metapopulation. *Science* 325: 1124–1128.

Smith, S.V., and G.S. Key. 1975. Carbon dioxide and metabolism in marine environments. *Limnology and Oceanography* 20: 493–495.

Smith, G.F., K.N. Greenhawk, D.G. Bruce, E.B. Roach, and S.J. Jordan. 2001. A digital presentation of the Maryland oyster habitat and associated bottom types in the Chesapeake Bay (1974-1983). *Journal of Shellfish Research* 20: 197–206.

Smith, G.F., D.G. Bruce, E.B. Roach, A. Hansen, R.I.E. Newell, and A.M. McManus. 2005. Assessment of recent habitat conditions of eastern oyster *Crassostrea virginica* bars in mesohaline Chesapeake Bay. *North American Journal of Fisheries Management* 25: 1569–1590.

Soetaert, K., A.F. Hofmann, J.J. Middelburg, F.J.R. Meysman, and J. Greenwood. 2007. The effect of biogeochemical processes on pH. *Marine Chemistry* 105: 30–51.

Srna, R.F. and A. Baggaley. 1976. Rate of excretion of ammonia by hard clam *Mercenaria-Mercenaria* and American oyster *Crassostrea-Virginica. Marine Biology* 36: 251–258.

Talmage, S.C., and C.J. Gobler. 2009. The effects of elevated carbon dioxide concentrations on the metamorphosis, size, and survival of larval hard clams (*Mercenaria mercenaria*), bay scallops (*Argopecten irradians*), and Eastern oysters (*Crassostrea virginica*). *Limnology and Oceanography* 54: 2072–2080.

Ulanowicz, R.E., W.C. Caplins, and E.A. Dunnington. 1980. The forecasting of oyster harvest in Central Chesapeake Bay. *Estuarine and Coastal Marine Science* 11: 101–106.

Wilber, K.M., and A.S.M. Saleuddin. 1983. Shell formation. In *The mollusca: physiology*, ed. K.M. Wilber and A.S.M. Saleuddin, 594. New York: Academic Press, Inc.

Yates, K.K., C. Dufore, N. Smiley, C. Jackson, and R.B. Halley. 2007. Diurnal variation of oxygen and carbonate system parameters in Tampa Bay and Florida Bay. *Marine Chemistry* 104: 110–124.

Journal of Shellfish Research, Vol. 30, No. 3, 659–669, 2011.

OYSTER SHELL DISSOLUTION RATES IN ESTUARINE WATERS: EFFECTS OF pH AND SHELL LEGACY

GEORGE G. WALDBUSSER,[1]* RYAN A. STEENSON[2] AND MARK A. GREEN[3]

[1]*College of Oceanic and Atmospheric Sciences, Oregon State University, 104 COAS Administrative Building, Corvallis, OR 97331;* [2]*South Dakota State University, Brookings, SD 57006;* [3]*Division of Natural Sciences, St. Joseph's College of Maine, 278 Whites Bridge Road, Standish, ME 04084*

ABSTRACT Oyster shell is a crucial component of healthy oyster reefs. Shell planting has been a main component of oyster restoration efforts in many habitats and has been carried out on scales from individual and grassroots efforts to multiagency efforts across entire estuaries. However, the cycling and lifetime of the shell that makes up the bulk of an oyster reef has only recently received attention, and most of the work to date has focused on the role of epi- and endobionts on shell degradation. Here we report findings from a laboratory study in which we manipulated pH in a flow-through control system using water from the mesohaline mouth of the Patuxent River to measure dissolution rates of intact oyster shell. Shells from the Eastern oyster (*Crassostrea virginica* Gmelin 1791) with three different legacies were exposed to 4 levels of pH that encompass a range typical of the mesohaline waters of the Chesapeake Bay (~7.2–7.9 on the NBS scale). Mass loss over a 2-wk period was used to measure dissolution rate on 3 shell legacies: fresh, weathered, and dredged. We found that pH and shell legacy had significant effects on shell dissolution rate, with lower pH increasing dissolution rate. Fresh shell had the highest dissolution rate, followed by weathered then dredged shell. Dissolution rates were significantly different among all 4 pH treatments, except between the lowest (~7.2) and the next lowest (~7.4); however, shells lost mass even under noncorrosive conditions (~7.9). We discuss the implications of our findings to ongoing efforts to understand shell budgets and cycling in oyster reef habitat, the interaction of biological and geochemical agents of shell degradation, and the complexity associated with shell carbonate cycling in the unique milieu of the oyster reef.

KEY WORDS: oyster shell, estuarine acidification, pH, oyster reef, biogeochemistry, calcium carbonate

INTRODUCTION

The living oysters that make up the veneer of an intact oyster reef are supported by a framework of dead oyster shell (DeAlteris 1988, Hargis & Haven 1999), although the physical and chemical composition of interior oyster reefs have not been well quantified. Oyster restoration efforts have often focused on replanting dead oyster shell to help provide an initial framework on which oysters recruit. The role of this shell in supporting oyster growth has long been recognized as noted by the naturalist Pliny the Elder in his first-century work, *Natural History*. More recently, the importance of topographic relief in shell plantings has been shown, with taller shell plantings having greater success (Schulte et al. 2009). Although the importance of the shell habitat for recruitment of juveniles is well documented, the cycling of this shell material and lifetime on oyster reefs has only recently received treatment (Powell et al. 2006, Beck et al. 2009). Oyster shell is a dynamic resource on oyster reefs subject to a number of degradation processes that ultimately control the rate of loss from the reef. The integrated effect of physical, biological, and chemical processes degrading shell material over the time it spends in the outer reef layer determines how much of this material ultimately contributes to the reef framework versus being recycled to the water column.

A peculiar requirement of oyster reef health and growth is oyster mortality adding to the shell pool of the reef (Mann & Powell 2007, Southworth et al. 2010); in other words, for a reef to remain static with various degradation processes and effective sea level rise, it must have a healthy population of oysters with a mortality rate at least balancing the rate of shell loss (Powell & Klinck 2007). Recent work in the Delaware River estuary has found that oyster shells on reefs have typical half-lives of years to a decade (Powell et al. 2006). Powell and Klinck (2007) argue that these loss rates should set the target population dynamics needed for self-sustaining populations. Although there are few estimates of shell lifetime from oyster reefs, the seemingly rapid rates from the Delaware suggest that oyster populations require a careful balance of recruitment, growth, and mortality to be self-sustaining (Mann & Powell 2007). Given the evidence that oyster recruitment is less consistent interannually and related to changes in environmental conditions such as salinity (Kimmel & Newell 2007), the processes affecting the lifetime of shells on oyster reefs have potentially important consequences for ongoing restoration efforts. In general, active breakdown of calcium carbonate hard parts is thought to occur in the taphonomically active zone (TAZ) (Davies et al. 1989), which occurs within the bioturbated and oxic zones of sediment seafloor. The important role of biont attack on shell persistence has been previously noted (Carver et al. (2010) and references therein), with shell-burrowing organisms such as polychaetes and sponges rapidly deteriorating oyster shell while it remains in the TAZ. Burial in anoxic conditions generally results in increased preservation (Hu et al. 2011) as a result of the exclusion of shell-boring organisms and ultimately more favorable geochemical conditions for calcium carbonate preservation (Morse 2005, Morse et al. 2007).

Although shell-boring organisms are often considered to be the primary agent of oyster shell degradation (Carver et al. 2010), the thermodynamics of surrounding estuarine waters sets the stage for stability of these biogenic minerals prior to burial. In addition, the pitting associated with fouling organisms changes the surface topography of shell material at small spatial scales (millimeters to centimeters), creating additional surface area

*Corresponding author. E-mail: waldbuss@coas.oregonstate.edu.
DOI: 10.2983/035.030.0308

where thermodynamic dissolution is thereby accelerated (Morse et al. 2007). The low salinity and high rates of production and respiration in many estuaries create conditions that may be transiently corrosive to the calcium carbonate of oyster shell. The calcium carbonate reef framework deposited by oysters in estuarine environments is therefore notable, given the less favorable thermodynamics compared with other reef-building organisms such as corals. Furthermore, oysters are prolific filter feeders, capturing organic matter from the water column and depositing it to the benthos, including their own reef framework. The deposited organic matter stimulates high rates of microbial metabolism contributing respired CO_2, increasing corrosiveness, and ultimately increasing dissolution of shell material until burial below the TAZ. Once buried below the oxic zone, however, this organic matter may, in fact, help preserve shell (Hu et al. 2011).

Adult and juvenile bivalves have been shown to deposit calcium carbonate under corrosive conditions, albeit at a reduced rate (Gazeau et al. 2007, Waldbusser et al. 2010, Waldbusser et al. 2011). After the organism dies, however, no new shell may be deposited, and the stability of the shell mineral is controlled by the corrosiveness of the surrounding conditions. Estuarine carbonate chemistry is variable with respect to many processes that alter the stability of calcium carbonate minerals, driven by production and respiration cycles (Abril et al. 2003, Borges & Gypens 2010, Feely et al. 2010, Waldbusser et al. 2011), freshwater input (Salisbury et al. 2008), and global changes in atmospheric CO_2 (Miller et al. 2009). All have the potential to alter the lifetime of the calcium carbonate oyster reef building blocks in these thermodynamically unstable habitats, and all are susceptible to local and global human impacts.

The importance of calcium carbonate cycling to the global carbon cycle has precipitated significant research efforts on understanding the dissolution/preservation dynamics of the mineral phase (see reviews in Morse (2005) and Morse et al. (2007)) and the calcium carbonate counterpump in the world's oceans (Antia et al. 2001, Zondervan et al. 2001). However, within estuarine habitats, much less is known regarding the cycling of calcium carbonate and feedbacks with calcifiers. A recent analysis by Lebrato et al. (2010) found that echinoderm calcification on continental shelves is a small but significant component of the global carbon cycle. The short-term controls on and dissolution rates of these multicellular biogenic minerals have not been explored with much depth, particularly in relation to population dynamics and resource management timescales. However, taphonomic studies of longer term degradation rates (e.g., Kidwell 2005) and more recent experimental evidence from continental shelf environments provide some insight (Hu et al. 2011, Powell et al. 2011b).

One challenge to understanding these dynamics is that many estuarine calcifiers are multicell organisms producing shells that are heterogenous in nature with high preservation potential (of adult shells) even in relatively corrosive nearshore sediments. The calcium carbonate reaching the seafloor in the open ocean is often from single-cell organisms or small aggregates, whereas temperate estuarine calcifiers are frequently metazoans with large shells (and relatively small surface area (sensu Walter and Morse (1984)) already living on or within the sediment. Bivalve shells vary in mineral composition, proportion of organic matter, microstructure of mineral grains, and the outer organic sheath that protects the mineral component of the shell. These shell characteristics all affect the rate at which shells dissolve resulting from thermodynamic conditions: corrosiveness of surrounding media. However, beyond a first-order thermodynamic argument, others have argued that factors such as biological interactions, bioenergetics of shell formation, and environmental variables ultimately determine the patterns and fate of shell material (Kidwell 2005, Hautmann 2006, Powell et al. 2011b). Shells undergo diagenetic transformation during burial, and that transformation alters surface chemistry and ultimately the dissolvability of the shell mineral. Formation of modified minerals called "micrite" resulting from dissolution and reprecipitation in marine sediments (Kobluk & Risk 1977, Longman 1980) results in elemental changes to the shell surface (Palma et al. 2008) as well as structural changes to the mineral crystallography. Ultimately, these early stages of transformation are the beginning of the fossilization of shell material. It is clear, therefore, that the legacy of the shell would likely impact the solubility under corrosive conditions, and these differences in legacy provide a snapshot into a time-variable component of shell dissolution on and within oyster reefs.

A combination of biological, geochemical, and sedimentary agents will ultimately affect the dissolution rates of intact shells in estuarine environments. To explore the role of estuarine geochemistry and shell characteristics on dissolution rates, we used a flow-through experimental CO_2 system with feedback control to control pH and to measure dissolution rates of intact oyster shell over a 2-wk period. Specifically, our study addresses two primary questions: What is the response of the intact shell dissolution rate to a range of common estuarine pH values? What effect does shell legacy have on shell dissolution rates across these pH values? Furthermore, we explored the implications and mechanisms of dissolution rate response to these factors: pH and shell legacy.

METHODS

Shell Collection

Oyster shells of the species *Crassostrea virginica* were collected from three different sources resulting in three different shell legacies: fresh, dredged, and weathered. Fresh shells were obtained from a local oyster house in Solomons, MD, all meat was removed after shucking, and shells were placed into experimental conditions within 24 h of shucking. Dredged shells were collected from a marina in Stevensville, MD (Langenfelder Marine Inc.), which retained the shells from the State of Maryland dredged shell/oyster reef replenishment program. These dredged shells are sifted from dredge spoils around the Maryland portion of Chesapeake Bay and relocated to oyster restoration areas. Limited aging estimates of the dredged shell from the state of Maryland indicate the oldest shells are approximately 3,000 y BP, with many in the several-hundred-year range (C. Judy, MD-DNR, pers. comm. July, 25, 2011). Shells were collected haphazardly from a large shell pile at the marina, focusing on intact shells of a medium size relative to the rest of the shells in the pile. Weathered shells were originally collected from a local oyster house in Solomons, MD, shucked, and placed in a sandy, beachfront area for approximately 2 y. No differentiation was made between left and right valves of the shells for any of the legacies. The visual differences of the three shell types were obvious (Fig. 1). Fresh shells were generally smooth, and the

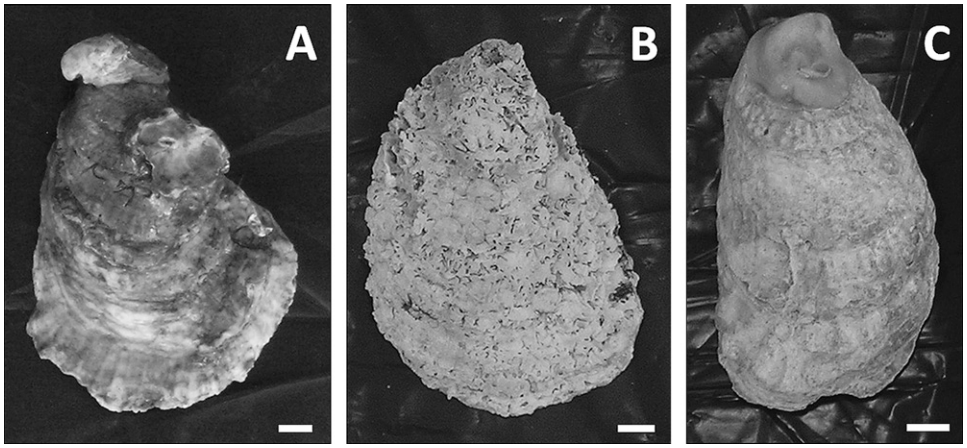


Figure 1. (A–C) Representative images of the 3 shell legacies used in the experiment: fresh (A), weathered (B), and dredged (C). White bars in each picture are 1 cm in length for scale.

outer shell appeared brown and generally had an intact periostracum. Dredged shells were whitish gray with some light secondary coloration of blue gray on a generally smooth surface. Weathered shells were white with extensive pitting on the order of a couple of millimeters in size on the shell exterior but not the interior. Some evidence of polychaete burrowing was found as black blisters on shell interiors of weathered and fresh shell.

pH Control System

A flow-through, feedback-control CO_2 manipulation system was used to control pH in the experimental system. The control system used was a Neptune Aquacontroller 3 (Neptune Systems, San Jose, CA) interfaced with a PC to record pH and temperature values every 2 min throughout the course of the experiment. Four epoxy filled, double-junction pH electrodes were used with an internal Ag/AgCl reference electrode, one within each treatment tank. Probes were calibrated with standard NBS buffers, rinsed and cleaned daily with deionized water, with the calibration checked daily against a standard 7.01 NBS buffer, and recalibrated at a minimum of once per week. During late July and early August 2009, incoming water from the seawater system of the Chesapeake Biological Laboratory was manipulated to obtain treatment levels (Table 1). The incoming water was drawn from the Patuxent River, a mesohaline tributary of the Chesapeake Bay. Throughout the course of the entire experiment salinity varied between 13–14 psu from daily measurements (with no measureable diurnal variability), and temperature was recorded every 2 min with a mean and SD of 27.28 ± 0.48°C, and a maximum and minimum of 29.50°C and 23.80°C, respectively. One hundred percent CO_2 was added in proportions to maintain an average pH (NBS scale) for 3 target treatments of 7.7 (high), 7.4 (mid), and 7.2 (low). Our actual values were 7.67, 7.38, and 7.17, each with an SD of 0.04 pH units throughout the entire course of the experiments, with values recorded every 2 min. These pH values were chosen to represent a range of conditions typical of the mesohaline region of the Chesapeake Bay. A control was also used in which incoming water was bubbled with ambient air to a pH set point of 7.90 with an SD of 0.04 during the experimental period. Bubbling was initiated when values dropped below the set point and was stopped when the set point was reached.

TABLE 1.

Average conditions (± 1 SD) in experimental aquaria during the 2-wk measurement period.

	Temperature	Salinity	Alkalinity (mmol/L)	pH
Inlet	26.50 (0.62)	13.2 (0.4)	1.598 (0.014)	n/a
Control	27.28 (0.48)	13.2 (0.4)	1.604 (0.013)	7.90 (0.04)
High	27.28 (0.48)	13.2 (0.4)	1.613 (0.011)	7.67 (0.04)
Mid	27.28 (0.48)	13.2 (0.4)	1.686 (0.040)	7.38 (0.04)
Low	27.28 (0.48)	13.2 (0.4)	1.704 (0.053)	7.17 (0.04)

Values are pH units on the NBS scale.
n/a, not applicable.

The experimental tank setup was designed to dampen diurnal variation in pH, allow for relatively constant carbonate chemistry, and provide constant flow rates to the experimental aquaria. Incoming water was plumbed to an open 20-L head tank in which river water entered the system, and flowed out from a standpipe to maintain a constant water level and thus head pressure of water fed to experimental aquaria. The water from the head tank was split to the 3 pH treatments and 1 control. Each treatment aquarium consisted of two connected 20-L containers, one for mixing and one for shells. A sheet of bubble wrap was placed on the surface water in both containers to minimize atmospheric exchange. Water and CO_2 were added to the mixing tank that was connected to the experimental tank by a short pipe. Water was cycled back to the mixing tank with a small submersible pump at a rate of roughly 54.4 L/h. The water in the experimental tank spilled over through an outlet, and the flow rate of water through each experimental aquarium (5.4 L/h) was controlled by a valve between the header tank and experimental tanks, and was checked daily and adjusted as necessary. At the volume of the experimental aquaria and target flow rates, the water had a residence time of roughly 8 h in experimental aquaria. We found the ratio of mixing flow to outlet flow (~10:1) resulted in the most stable controller response, and it limited cycling of pH in the experimental system. In addition, each experimental tank was situated on top of a small stir plate, and a magnetic stir bar was used to ensure complete mixing in the experimental tank where shells were suspended in a rack above the bottom of the tank

with several centimeters of space among the total of 15 shells per aquarium.

Shell Weight and Surface Area

The linear rate of decrease measured on individual shell mass was used to estimate dissolution rates for each shell. Five shells per shell legacy and pH treatment were placed in experimental conditions for 10 days prior to the experimental 2-wk mass loss period. Although we collected mass loss data during this initial 10-day period, we used this time to refine handling procedures of shells during weighing, and to allow shells to develop an initial biofilm. After the initial 10-day acclimation, mass loss estimates were based on a linear fit over a 2-wk period in which total mass of individual shells changed by as much as 2–3 g. The linear regression fit of mass loss over time for the experimental mass loss period was typically $R^2 > 0.90$; thus, using the slopes of this relationship were valid for these short-term estimates of shell dissolution. Shells were weighed every 2–3 days by removing shells from treatment aquaria, blotting them dry, and allowing them to air dry for 15 min before measuring mass. This procedure was used because it was found to be reproducible and consistent in obtaining a wet weight of the shell mass without leading to complete drying of shells. Final dry weights of shells were obtained by drying for 24 h at 60°C, and were used to estimate the percent water content of shells.

The interior and exterior surfaces of all shells were photographed just prior to their placement into the experimental aquaria from a planar view on a black background to enhance contrast between shell and background. Shell interior and exterior surface areas were measured by using standard image analysis techniques (ImageJ 1.43u, National Institute of Health, Washington DC), and the measurement difference between interior and exterior surface area estimates was roughly 3%. Briefly, each image of 10 shells was imported into Image J, the color image was converted to a binary image, and the "analyze particles" command was used to compute the total surface area. Estimates of surface area were checked by examining the traces of shells relative to the original images. In some cases, because of the lack of contrast between shell edges and the background, shell edges had to be traced manually and the surface area estimates rerun on touched-up images.

Alkalinity

Alkalinity measurements of incoming and outflow waters for each pH treatment were measured at roughly 2 to 4-day intervals to ensure flow rates were sufficient to prevent alkalinity buildup resulting from shell dissolution. Alkalinity samples were taken from the water entering the experimental system and at the 4 drains from the experimental tanks. These measurements also permitted calculation of the saturation state of calcite at specific time points during the experimental period. Alkalinity was measured by a 2-point end point titration following Edmond (1970), and handled following best practices for open cell titration (Dickson et al. 2007). Samples were analyzed within 20 min of collection, and were kept in sealed syringes until the open cell titration was carried out. Alkalinity was used to calculate the calcite saturation state of the average conditions within the experiments using CO2SYS (van Heuven et al. 2011) with salinity-dependent dissociation constants from Millero et al. (2006).

Electron Microprobe

Elemental analysis of one representative shell from each legacy type in the control pH treatment was selected to determine whether shells from the different shell legacies had been significantly altered. Electron microprobe analysis of calcium, strontium, barium, manganese, iron, magnesium, aluminum, and silica on the interior and exterior shell surfaces, as well as a cross-section of the shell, was conducted. For each shell legacy, the left valve was used to eliminate differential effects between valves. Five replicate locations were analyzed on epoxy-embedded shell samples. Electron microprobe analyses of shells were performed at Oregon State University using a CAMECA SX-100 (CAMECA, Gennevilliers Cedex, France) instrument equipped with five wavelength dispersive spectrometers and high-intensity dispersive crystals for high-sensitivity trace element analysis. The shells were analyzed using a 15-keV accelerating voltage, a 50-nA sample current, and a 10-μm beam diameter. Counting times ranged from 10 to 60 sec, depending on the element and desired detection limit. Data reduction was performed online using a stoichiometric PAP correction model (Pouchou & Pichoir 1984). Barium, manganese, and aluminum were below detection limits in all samples (FEI, Hillsboro, OR).

Scanning Electron Microscopy

Visual inspection of shell surfaces for diagenetic transformation was carried out by electron microscopy. Samples were taken from the same shells as the electron microprobe analyses by chipping a section of the outer shell surface. The shell samples were rinsed with deionized water and dried before sputtering with a Pb/Au layer. Samples were analyzed at the Oregon State University Electron Microscopy Laboratory under an FEI Quanta 600F scanning electron microscope.

Experimental Design and Statistical Analyses

A 2-way full factorial experimental design was used to examine the effects of shell legacy and pH (and their interaction) on dissolution rates of Eastern oyster shell. Five replicate shells of each shell legacy were randomly assigned to 1 of 3 pH treatments. Within each experimental aquarium, 15 shells total (5 of each legacy) were contained during the course of mass loss measurements. Percent mass loss per day was computed as the dependent variable by calculating the regression of mass over time for each shell (in grams per day), then dividing by the initial mass to determine a percent mass corrected dissolution rate for each shell in the experiment. The initial 2-way ANOVA found that the interaction effect of legacy and pH treatment was not significant, and it was therefore dropped from the analysis. Assumptions of ANOVA were checked by examining residuals visually against each independent variable, as well as by using Shapiro-Wilk's test of normality and Hartley's f-max test of heteroscedacity. Residuals were normally distributed for each treatment level within each factor (legacy and pH treatment). Assumption of homoscedascity was met for treatment levels within pH; however, variances were found to be significantly different among the three legacy treatments. To address this violation of assumptions, separate variance estimates were made for each shell legacy to be used in the treatment comparisons, and denominator degrees of freedom were computed using Satterthwaite's method for the subsequent ANOVA with variance groupings by shell legacy. All analyses were run in SAS v9.1.3 (SAS, Cary, NC).

RESULTS

Experimental System Performance

The controller system maintained pH within experimental aquaria for the duration of the experiment, except for three failures that resulted in missing data (Fig. 2). Data were logged every 2 min from the 4 pH probes, and the SD of pH throughout the course of the experiment was roughly 0.04 pH units (Table 1). However, during three periods, the mid and low pH treatment controller circuit froze and no data were logged, as evident by the gaps in Figure 2. On examining the mass data for these periods of controller failure, there are no measurable deviations in the rate of mass loss relative to the overall trends. We recognize the potential pitfalls of the experimental system, but note that this appeared to have no significant consequence on the response variable during these controller blackouts.

Shell Dissolution

During the 2-wk period in which we measured mass loss of shells, we found significant overall effects of shell legacy ($F_{2,35} = 42.94$, $P < 0.0001$) and a positive effect of decreasing pH treatment ($F_{3,28.6} = 50.90$, $P < 0.0001$) on shell dissolution (Fig. 3). The pH values we used in these experiments are not atypical of conditions in this mesohaline section of the Chesapeake Bay, albeit only in a transient sense. All the pairwise comparisons were significantly different between shell legacies, and only the difference between the low and mid pH treatments was not significant (Table 2). Two of the five replicates in the fresh mid-pH group had dissolution rates higher than what would be predicted from the overall trends, thus driving the mean value up (Fig. 4). Closer examination of these shells (and several others) revealed visual evidence of polychaete burrows (black blisters on internal shell surfaces) and sponges (pitting on exterior shell surfaces) in the fresh shells, which corresponded to the higher dissolution rates. In general, linear model fits to the individual shell mass loss over time explained more than 90% of the variance in change in mass over time. The dredged/control-pH shells typically had the worst fits to a linear model because of the very small changes in mass during the experimental period. However, with decreasing pH and corresponding increases in dissolution rates, the linear fits increased, with the dredged low-pH shells all having an R^2 value of more than 0.95. We should also note that the patterns in shell dissolution rates are robust when standardized to planar surface area, rather than mass. Therefore, we will retain the units in per mass because they are in some ways easier to understand and to enable comparisons with estimates of shell half-lives.

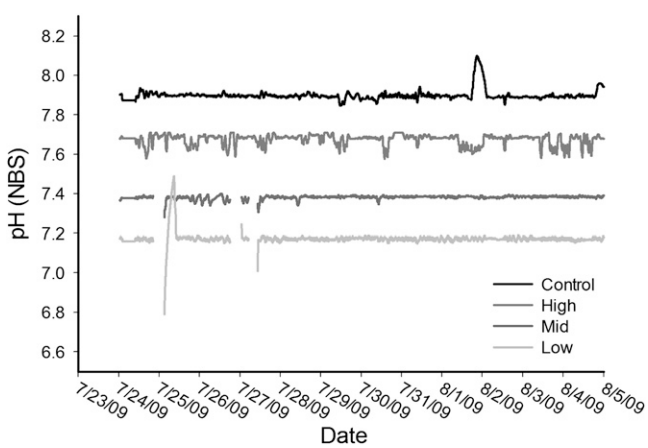


Figure 2. One hour centered mean pH values from each experimental tank.

The average percent water weights by shell legacy (±1 SD) at the end of the experiment were fresh, 3.47 ± 1.38; dredged, 1.90 ± 1.71; and weathered, 5.06 ± 3.63. A simple 1-way ANOVA found a significant effect of shell legacy on percent water weight ($F_{2,32.1} = 11.60$, $P = 0.0002$). Data were log transformed to meet assumption of normality, and variance was grouped by legacy because of unequal variance across the shell type. Significant differences, with Tukey-Kramer adjustment, were found between dredged and fresh shells ($t_{33.6} = 3.76$, $P = 0.0019$) and between dredged and weathered shells ($t_{35.5} = 4.49$, $P = 0.0002$), but not between fresh and weathered shells ($t_{28.6} = 1.93$, $P = 0.1457$). Note that the degrees of freedom vary among comparisons as a result of the Satterwaithe degrees of freedom estimation method used when grouping variance components.

Alkalinity Measures

Alkalinity values were used to determine saturation state for calcite (in conjunction with pH) and to ensure that flow rates were sufficient to prevent buildup of alkalinity in experimental aquaria. Water samples were taken from the inlet water supply and the outlet of the four experimental aquaria. The average inlet water alkalinity was 1.60 ± 0.01 mmol/L. An increase in alkalinity over time was found in all treatments; however, our estimated error in the alkalinity measurements is roughly 0.015 mmol/L, and therefore is greater than the difference we found in the control, and equal to that in the high pH treatment. The differences between inlet and outlet waters for each pH treatment were control, below detection limit; high, 0.015 mmol/L; mid, 0.087 mmol/L; and low, 0.105 mmol/L. The increase of alkalinity in the low-pH treatment equates to a change in saturation state for calcite of 0.02, which highlights the minimal container effect of our system at the chosen flow rates. It should be noted here that remineralization of organic matter and release of nutrients generally decreases alkalinity, so these delta alkalinity values may be underestimating the true carbonate dissolution rate. Remineralization of organic components of the shell would contribute to the mass loss without an increase in alkalinity (and potentially a decrease depending on nutrient release rate). However, the organic components of oyster shell are generally less than 10% by weight. The different shell legacies were mixed in each pH treatment, thus the differences in alkalinity resulting from the dissolution of calcium carbonate are integrated across shell type. The average percentage (by weight) of shell material in each pH treatment was dredged, 25%; weathered, 33%; and fresh, 42%.

Shell Elemental Analyses

Shell elemental composition varied among the representative shell of each shell legacy (Fig. 5). Values presented in Figure 5 are from the inner shell surface of left (or bottom) valves only. Overall recoveries on analyses were generally near 100% on the weathered and fresh shells (indicating we captured nearly all the primary elements), but were as low as 80% on the dredged shell, implicating other possible elements for which we did not probe. The dredged shell had higher strontium concentrations on the inner shell surfaces and measurable concentrations of iron and

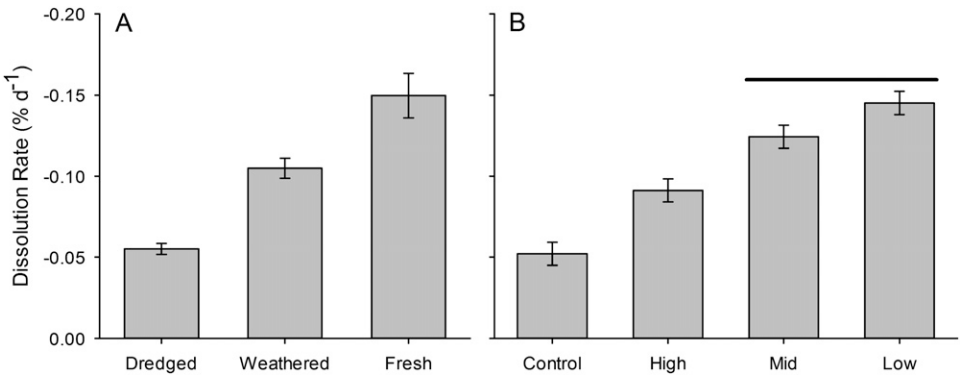


Figure 3. (A, B) Estimates of least square means for legacy (A) and pH treatment (B) effects. Error bars are SE estimates. Note that the different error estimates for the shell legacy means are the result of the individual variance estimates of these treatment effects.

silica, indicating early fossilization, whereas the fresh and weathered shell had lower strontium concentrations, and iron and silica concentrations below detection limits. A lower magnesium concentration was found on the weathered shell compared with other shell legacy types, indicating the preferential loss of the more soluble, higher magnesium calcite in the weathered shells (Morse et al. 2007, Burdige et al. 2010), subject to rainwater and terrestrial weathering processes. These limited data provide geochemical evidence of possible surface control on dissolution, and corroborate the electron microscopy observations below.

Visual Examination of Shell Surfaces

Examination of shell surfaces using electron microscopy highlights further differences in surface texture that likely have additional impacts on dissolution rates of different oyster shell. Figure 6 highlights three representative images of exterior shell surfaces of the same left valves used for electron microprobe analysis. The periostracum is visible on the fresh shell sample (Fig. 6A), with periostracal creases (white arrow) overlaid on the prismatic shell layer (black arrow). Breaks in shell growth are also visible, where edges of prisms are seen with growth occurring in the general lower left to upper right direction. The surface of the weathered shell (Fig. 6B) contains many small fragments and crystals occurring in various directions, indicating significant modification of the shell surface through the weathering process. The dredged shell surface (Fig. 6C) has larger crystal formations, and some of the original prisms are also visible. These shells were buried in organic-rich sediments for significant periods of time; as a result, diagenetic cementing occurs (dissolution and reprecipitation) on the surfaces exposed to the corrosive sediments. It is again important to note that these images are from representative shells, and provide insight (not conclusive evidence) into the possible role of microstructure and surface processes in controlling dissolution rates of intact shells of differing legacies.

TABLE 2.

Results from pairwise comparisons among treatments within each factor of the 2-way ANOVA.

Pairwise Comparisons	*df*	t Value	Adjusted *P* value
Shell legacy			
Dredged vs. fresh	21.1	6.69	<0.0001
Dredged vs. weathered	28.1	7.11	<0.0001
Fresh vs. weathered	26.2	2.97	0.0144
pH treatment			
Control vs. high	28.6	4.86	0.0002
Control vs. mid	28.6	8.96	<0.0001
Control vs. low	28.6	11.55	<0.0001
High vs. mid	28.6	4.10	0.0017
High vs. low	28.6	6.69	<0.0001
Mid vs. low	28.6	2.59	0.0677

DISCUSSION

Our laboratory-based measurements of intact oyster shell dissolution highlight the potential role of environmental changes in carbonate chemistry on this important and limiting estuarine resource. Across a typical range of pH values for this mesohaline region of the Chesapeake Bay, the rate of shell dissolution changed significantly (Figs. 3 and 4). In addition, the legacy of the shell had equally important consequences on the dissolution rate, altering the dissolution rate by about a factor of 10 from the slowest dissolving dredged shells to the more rapidly dissolving fresh shells (Figs. 3 and 4). The short timescale (weeks) of this

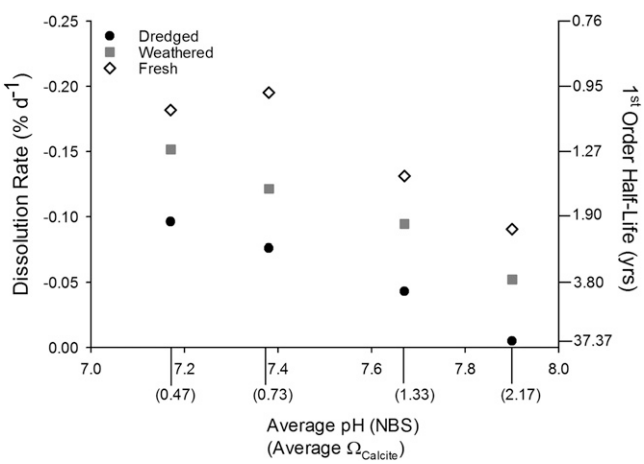


Figure 4. Average dissolution rates of individual treatments related to pH. The saturation state for calcite is presented below the pH values for reference. On the right y-axis are the first-order half-life values computed from the rate constants measured during the experiments. It is important to note the measurements are instantaneous rate constants for a short window of time, roughly 2 wk (see text).

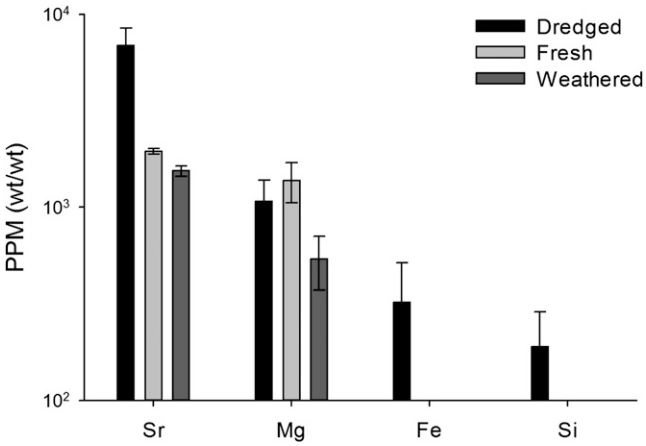


Figure 5. Trace element composition of interior shell surfaces in parts per million (PPM). Iron and silica were less than detection limits on the dredged shell cross-section analysis of the interior shell, and strontium in this region was also similar to that of the fresh and weathered shells.

experiment provides a snapshot of dissolution rates in response to changes in estuarine carbonate chemistry, but excludes other factors that are also responsible for shell degradation, such as shell-boring organisms. Although we noted evidence of shell-boring organisms, none were present on or in the shells during the experiment. Our measured rates in these short-term experiments are, however, within the range of other previous measurements of shell degradation. In an early study of shell dissolution Hecht (1933) showed that, immediately after death, mollusc shells may lose 25% of their mass in a matter of weeks. Driscoll (1970) found mass loss rates of up to 16% per year of shells in Buzzard's Bay, MA, attributed largely to boring organisms. Our measurements of dissolution (excluding bionts) range from roughly 2 to 70% per year, depending on shell type and pH (Fig. 4). This extrapolation to per year for any of our pH values has obvious limitations given the multiple scales of variability in estuarine carbonate dynamics. The most appropriate application of these rate loss values would be to apply to variable pH records of sufficient temporal resolution, such as hours, and integrate rate loss over longer periods of time, thus providing an estimate of geochemical shell loss to estuarine pH. However, if pH values for a given timeframe are normally distributed, using the average pH would provide an adequate estimation. Our experiments were conducted at a narrow range of temperature and salinity—factors important to carbonate thermodynamics. Our experiments do indicate that oyster shell lifetime, within the more surficial and open parts of a reef, may be susceptible to changing estuarine carbonate chemistry, and is supported by recent work in shelf environments on shell breakdown (Hu et al. 2011, Powell et al. 2011a).

Shell Half-Life and Agents of Destruction

Shell dissolution rates may be used to compute shell half-lives to evaluate the role of pH and carbonate chemistry in maintaining/degrading oyster reefs. Recent estimates of Eastern oyster shell half-lives on reefs by Powell et al. (2006) indicate that in the Delaware Bay estuary shell half-lives range from just older than 1 to ~20 y, with the shortest half-lives found in mesohaline waters. Fitting our loss rates to an exponential decay model (as in Powell et al. (2006)), the shortest half-lives we compute are roughly 1 y for fresh shell under mid and low pH, and increase to nearly 40 y in the dredged control pH treatment (Fig. 4). It is important to note half-life increases exponentially with decreasing rate loss, and applied to our experimental data, shell lifetime is therefore most sensitive to changes in the upper pH range. After rate losses drop roughly 0.05 d^{-1} (or roughly 20 y^{-1}), half-lives increase very rapidly, identifying a possible threshold type response of shell to degradation/dissolution rates. The rates of fresh shell decay in our experimental range are well above this threshold, and extrapolating linearly to higher pH values finds that this 0.05 d^{-1} rate would be obtained at a pH of 8.25 for the fresh shells in our experiment. The estimates and extrapolations from our experiments only account for geochemical degradation and exclude effects from shell-boring organisms (e.g., Zuschin et al. 2003, Carver et al. 2010).

In Chesapeake Bay, Pomponi and Meritt (1990) found that 90% of oyster shells were infested with a boring sponge, and these values were not unusual compared with other estuarine habitats (Rosell et al. 1999). Several of the fresh shells in our experiments had visual indicators of biont attack prior to the experiment, with visible black blisters on interior shell surfaces from polychaetes and bore holes from sponges (although no organisms were found in or on shells). Some of the highest dissolution rates we measured on individual shells were in the mid-pH treatment, with evidence of endobiont attack, and evidence of biont attack generally resulted in higher dissolution rates within pH treatment groups of fresh shells.

In the Delaware estuary, however, Powell et al. (2006) noted that the shortest half-lives were found in mesohaline waters, where boring sponges generally decrease in abundance with decreasing salinity (Hopkins 1962). Mesohaline estuarine environments are, however, often the region of chlorophyll maxima (such as in the Delaware River (Mannino & Harvey 1999)), and thus higher

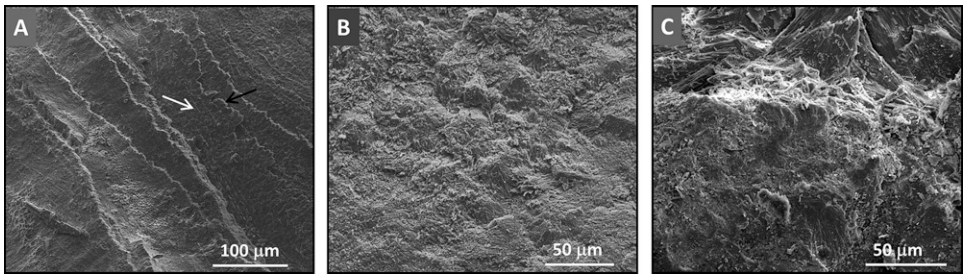


Figure 6. Representative scanning electron micrographs of the 3 shell legacies, outer shell surfaces of the left valves. (A) Fresh shell legacies. The white arrow notes the prisms in the prismatic layer, whereas the black arrow highlights a periostracal crease. (B) Weathered shell legacies. (C) Dredged shell legacies.

rates of production and respiration should result in a greater diurnal range in pH. Coupled with the lower alkalinity buffering in these environments, the overnight respiration signal is likely to create corrosive conditions for calcium carbonate minerals. We are not arguing that biont attack on shell is trivial, rather that biological and geochemical agents likely work in tandem, by creating microzones of respired CO_2 and additional surface area for dissolution. Should continued anthropogenic impacts on estuarine ecosystems result in less thermodynamically favorable estuarine conditions for calcium carbonate (Abril & Frankignoulle 2001, Borges & Gypens 2010, Feely et al. 2010, Waldbusser et al. 2011), shell budgets may be increasingly controlled by geochemical processes.

Time Dependency of Shell Degradation and Shell–Reef Dynamics

The comparison of the fresh shell with the other two shell types provides possible insights into the time dependency of shell degradation rates (noted by Powell et al. (2011a)) and highlights shell cycling dynamics on oyster reefs. Few studies of the physical and geochemical structure of an oyster reef exist, from the outer layer to the interior core (Davies et al. 1989, Hargis & Haven 1999). An important, and peculiar, requirement for an oyster reef to grow is the mortality of oysters and contribution of their shell to the reef framework (Powell et al. 2006, Mann & Powell 2007)—a framework composed of shell, organic deposits, and pore space in poorly constrained proportions. The rates of fresh shell dissolution we measured may be representative of shells during the early postmortality stage. However, as shells age (on a growing reef), they are eventually buried, presumably in an anoxic environment closer to the reef interior, thus excluding biont attack, and in a generally more favorable geochemical environment for preservation of calcium carbonate (Hu et al. 2011). Also during this transition from the near reef surface to reef interior, the exterior of the shell undergoes early diagenetic alteration, altering the surface chemistry of the calcium carbonate mineral and ultimately solubility (Morse & Arvidson 2002).

If the reef interior is in fact a diffusion-limited environment, the shell surface will dissolve and reprecipitate, leading to a less soluble surface for dissolution. Our dredged shell measurements, therefore, may represent a shell after having undergone this early diagenesis and then subsequently having been reexposed to oxic conditions. Thus, the differences in dissolution rate from the fresh to dredged shell may be, to some degree, an estimate of end members in a time-dependent dissolution rate. Furthermore, it is important to note that should the shell be buried within the reef framework, it would be exposed to a very different geochemical milieu. Decreases in reef accretion rates resulting from decreased oyster population growth, or increases in erosion resulting from changed hydrographic conditions could result in exposure of older/deeper more modified shells, perhaps providing a feedback in shell budget as a result of the lower dissolution rates of previously buried shell.

Importance of the Shell Dissolution Surface

Evidence from elemental analyses and electron microscopy of representative shells highlights the importance of surface control on dissolution rates under these moderately corrosive conditions (Morse & Arvidson 2002). Elevated concentrations of silica and iron on the dredged shell surface (Fig. 5) suggest the initial stages of diagenetic transformation (Longman 1980, Palma et al. 2008) and micrite formation (recrystallized calcium carbonate) have altered surface characteristics. Elevated silica and iron values have been measured previously in outer prismatic layers (Carriker et al. 1991) and are similar to our measurements of foliated interior shell surfaces (Fig. 5), but aluminum was below detection limits, providing us with confidence that we captured foliated microsctructure. Our electron microscopic images further support that the dredged shells were in an early stage of diagenetic transformation rather than sampling different microstructures, based on the larger crystal shapes seen in Figure 6C. Larger crystal sizes (and thus less surface area for dissolution) can be generated by dissolution and reprecipitation without altering chemical composition of the mineralogy (Morse & Casey 1988), and/or result in new mineral formation (Rude & Aller 1991). The geochemical and visual evidence of shell surface alteration are suggestive of both processes and, coupled with our dissolution rate measures, highlight the disconnect between bulk mineral properties and dynamics of mineral dissolution (Morse & Arvidson 2002), particularly of intact shells in estuarine environments.

Our study shows that even in supersaturated conditions, with respect to calcite, shells decreased in mass. The remineralization of organic shell components is one likely explanation for this response (Glover & Kidwell 1993), especially given the fact that the fresh shells had the highest dissolution rates (and the most organic material). However, organic components of oyster shell are typically a small percentage. The presence of a surface for microbes to colonize, and the secondary effects of metabolic CO_2 production (Emerson & Bender 1981) and microzone dissolution (Berner 1969) on shell surfaces are likely also at play.

The rates of shell dissolution we measured here are more than an order of magnitude lower than modeled calcite dissolution estimates in the open ocean seafloor (Hales & Emerson 1997). Much of the intact shell mass is not available to dissolve because of the low surface area-to-volume ratio of an oyster shell relative to a calcite grain (Walter & Morse 1984). Although others have examined the differences in calcium carbonate dissolution among biogenic and authigenic minerals, and the differences in available surface area (Cubillas et al. 2005), this work has been done on crushed shell. For example, assuming a perfect spherical calcite grain of 0.2 mm in diameter, one can calculate the mass per surface area as roughly 1.4×10^{-3} g/cm^2, whereas the mass per surface area we measured for shells was 0.854 g/cm^2. Our planer surface area measurements do not account for curvature of shells and microtexture, but do highlight the importance of this basic geometric comparison in regulating shell dissolution. Clearly, there are many dynamics modulating the dissolution of intact oyster shell. Our measurements simply set the stage for determining the magnitude of response to changes in estuarine pH.

Oyster Reefs and Estuarine Calcium Carbonate Dynamics

Oyster reefs are significant biogenic carbonate masses in thermodynamically variable and often unstable estuarine environments. To date, most interest in carbonate dissolution has focused on the seafloor of continental shelves and the open ocean (Honjo & Erez 1978, Cai et al. 2006, Hu et al. 2011) because of the important role of these habitats in global carbon cycling. Significant carbonate dissolution has been found in nearshore marine sediments (Green & Aller 1998, Green & Aller 2001) and estuarine turbidity maximum zones (Abril et al. 2003). The role of keystone species such as seagrass, algae, or corals on carbonate cycling in

coastal environments has also been highlighted (Gattuso et al. 1996, Bensoussan & Gattuso 2007, Burdige et al. 2008), but these studies have generally focused on carbonate-rich tropical environments. Postlarval juvenile and adult oysters can precipitate calcium carbonate in thermodynamically unfavorable conditions (Gazeau et al. 2007, Waldbusser et al. 2011) and, coupled with a high rate of organic matter deposition, this shell material may be readily preserved if buried quickly enough. In an insightful treatment, Kidwell and Jablonski (1983) noted the significant of sediment "shelliness" and positive ecological feedbacks of shell to changing benthic community composition (termed "taphonomic feedback"). If the responses of oyster shell dissolution to changing pH we measured (Figs. 3 and 4) were reasonable estimates of *in situ* conditions, then changes to estuarine geochemistry may be an important factor for oyster restoration.

Estuarine carbonate chemistry is dynamic, however, with many processes occurring across multiple temporal and spatial scales, including production/respiration cycles (Abril et al. 2003, Borges & Gypens 2010), hydrologic and watershed changes (Salisbury et al. 2008, Najjar et al. 2010, Aufdenkampe et al. 2011), and eutrophication (Gypens et al. 2009, Feely et al. 2010, Waldbusser et al. 2011). The importance of shell to the estuarine ecosystem (Gutierrez et al. 2003, Powell et al. 2006, Kelly et al. 2011, Green et al. accepted), the dynamic biogeochemistry in these environments, and feedbacks of shell budgets with population dynamics suggest (Powell et al. 2006, Mann & Powell 2007) this is an important and challenging area of future research.

Managing Shell Resources

One interesting note with regard to the dredged shell is that it had been used extensively by the state of Maryland to restore previously harvested oyster reefs from 1960 until 2006. Approximately 196 million bushels of dredged oyster shell were replaced in Chesapeake Bay during this 46-y period from the program's inception to termination (MD DNR). This is likely the largest coordinated shell planting/reef restoration effort to date, and also perhaps the largest alkalinity buffering experiment conducted. From a kinetic perspective, the use of dredged shell may have helped provide additional benefits from its lower rate of dissolution under all pH conditions (Figs. 3 and 4). The slower dissolution rate may have been especially beneficial during the the early time frame of shell planting when carbonate conditions may have been less favorable for calcium carbonate preservation (Aufdenkampe et al. 2011). This potentially lower loss rate resulting from dredged shell meant that the population size needed to provide shell through mortality could have been smaller, helping to balance the shell budget (Powell et al. 2006). However, it is unknown whether these potential benefits also translate into a lesser degree of biont attack, and how the overall shell budgets were altered by the planting of dredged shell. At the control pH value, the dredged shell had a half-life 10 times higher than the fresh shell or, a loss rate equivalent to fresh shell at a pH of roughly 0.2 pH units lower. Although the shell planting program has been discontinued, in part because of the lack of accessible shell, and it may be difficult to reconstruct previous shell budgets, the likely significant positive effect this effort should not be overlooked (Southworth et al. 2010).

A recent analysis has suggested that oysters are "functionally" extinct because of their precipitous decline in numbers relative to former populations (Beck et al. 2009). Thus the ecosystem functions (e.g., filtering, habitat) that oysters provided in the past have been lost. With ongoing restoration efforts aimed at restoring shells to former oyster grounds in many regions, understanding the controls on the lifetime of shells and how they relate to anticipated changes to estuaries from local and global anthropogenic impacts seems crucial. Conversely, changing the balance of shell material in estuaries may also have significant geochemical implications (Waldbusser et al. in prep). Shell planting may be a worthwhile mitigation strategy for some bivalves (Green et al. 2009); however, without the characterization of oyster population dynamics (Harding et al. 2010, Southworth et al. 2010) needed to provide renewed shell, these activities may be less effective than anticipated. The susceptibility of oyster shell and other bivalve shells in shell beds to dissolution extends beyond simple first-order thermodynamics arguments (Kidwell 2005); however, these first-order processes are important to constrain rate losses and to provide a starting point for understanding how shell resources in estuaries will respond to future anthropogenic impacts, including harvest and changing biogeochemistry. Our study provides limited, but important, insight into the possible future of oyster shell cycling. Relatively small increases in acidity may require shell replacement rates higher than can be achieved by natural populations and, vice versa, relatively small decreases in acidity could help provide natural oyster populations with an advantage. The geochemical control on dissolution of oyster shell needs to be evaluated in the context of other biological and sedimentary processes that ultimately control the lifetime of oyster shell on the reef.

ACKNOWLEDGMENTS

We acknowledge the Research Experience for Undergraduates (REU) program supported by Maryland Sea Grant and the National Science Foundation for supporting R. A. S. to carry out this research. This work was also supported by NSF-OCE#0622999 to M. A. G. and G. G. W. We thank Langenfelder Marine Inc. for allowing us to pick shells from their dredged shell pile, Frank J. Tepley at Oregon State University for carrying out the electron microprobe analyses, Liu Yi at Oregon State University for assistance with electron microscopy, and Bud Millsaps at the Chesapeake Biological Laboratory for his assistance with the seawater system. G. G. W. thanks Chris Judy and Eric Weissberger at the Maryland Department of Natural Resources for information on the Maryland shell planting program, Janet Nye for data on biological oxygen demand of Patuxent River waters, and Sarah E. Kolesar and Eric Powell for comments on a previous version of this manuscript.

LITERATURE CITED

Abril, G. & M. Frankignoulle. 2001. Nitrogen–alkalinity interactions in the highly polluted Scheldt basin (Belgium). *Water Res*. 35:844–850.

Abril, G., H. Etcheber, B. Delille, M. Frankignoulle & A. V. Borges. 2003. Carbonate dissolution in the turbid and eutrophic Loire estuary. *Mar. Ecol. Prog. Ser*. 259:129–138.

Antia, A. N., W. Koeve, G. Fischer, T. Blanz, D. Schulz-Bull, J. Scholten, S. Neuer, K. Kremling, J. Kuss, R. Peinert, D. Hebbeln, U. Bathmann, M. Conte, U. Fehner & B. Zeitzschel. 2001. Basin-wide particulate carbon flux in the Atlantic Ocean: regional export patterns and potential for atmospheric CO_2 sequestration. *Global Biogeochem. Cycles* 15:845–862.

Aufdenkampe, A. K., E. Mayorga, P. A. Raymond, J. M. Melack, S. C. Doney, S. R. Alin, R. E. Aalto & K. Yoo. 2011. Riverine coupling of biogeochemical cycles between land, oceans, and atmosphere. *Front. Ecol. Environ.* 9:53–60.

Beck, M. W., R. D. Brumbaugh, L. Airoldi, A. Carranza, L. D. Coen, C. Crawford, O. Defeo, G. J. Edgar, B. Hancock, M. Kay, H. Lenihan, M. W. Luckenbach, C. L. Toropova & G. Zhang. 2009. Shellfish reefs at risk: a global analysis of problems and solutions. Arlington, VA: The Nature Conservancy. 52 pp.

Bensoussan, N. & J.- P. Gattuso. 2007. Community primary production and calcification in a NW Mediterranean ecosystem dominated by calcareous macroalgae. *Mar. Ecol. Prog. Ser.* 334:37–45.

Berner, R. A. 1969. Chemical changes affecting dissolved calcium during bacterial decomposition of fish and clams in sea water. *Mar. Geol.* 7:253.

Borges, A. V. & N. Gypens. 2010. Carbonate chemistry in the coastal zone responds more strongly to eutrophication than to ocean acidification. *Limnol. Oceanogr.* 55:346–353.

Burdige, D. J., X. Hu & R. C. Zimmerman. 2010. The widespread occurrence of coupled carbonate dissolution/reprecipitation in surface sediments on the Bahamas Bank. *Am. J. Sci.* 310: 492–521.

Burdige, D. J., R. C. Zimmerman & X. Hu. 2008. Rates of carbonate dissolution in permeable sediments estimated from pore-water profiles: the role of sea grasses. *Limnol. Oceanogr.* 53:549–565.

Cai, W. J., M. H. Dai & Y. C. Wang. 2006. Air–sea exchange of carbon dioxide in ocean margins: a province-based synthesis. *Geophys. Res. Lett.* 33. DOI: 10.1029/2006GL026219.

Carriker, M. R., C. P. Swann, R. S. Prezant & C. L. Counts. 1991. Chemical elements in the aragonitic and calcitic microstructural groups of shell of the oyster *Crassostrea virginica:* a proton probe study. *Mar. Biol.* 109:287–297.

Carver, C. E., I. Theriault & A. L. Mallet. 2010. Infection of cultured Eastern oysters *Crassostrea virginica* by the boring sponge *Cliona celata*, with emphasis on sponge life history and mitigation strategies. *J. Shellfish Res.* 29:905–915.

Cubillas, P., S. Kohler, M. Prieto, C. Chairat & E. H. Oelkers. 2005. Experimental determination of the dissolution rates of calcite, aragonite, and bivalves. *Chem. Geol.* 216:59–77.

Davies, D. J., E. N. Powell & R. J. Stanton. 1989. Relative rates of shell dissolution and net sediment accumulation: a commentary: can shell beds form by the gradual accumulation of biogenic debris on the seafloor. *Lethaia* 22:207–212.

DeAlteris, J. T. 1988. The geomorphic development of wreck shoal, a subtidal oyster reef of the James River, Virginia. *Estuaries* 11:240–249.

Dickson, A. G., C. L. Sabine & J. R. Christian, editors. 2007. Guide to best practices for ocean CO2 measurements. *PICES Spec. Publ.* 3: 191 pp.

Driscoll, E. G. 1970. Selective bivalve shell destruction in marine environments: a field study. *J. Sediment. Petrol.* 40:898.

Edmond, J. M. 1970. High precision determination of titration alkalinity and total carbon dioxide content of sea water by potentiometric titration. *Deep-Sea Res.* 17:737.

Emerson, S. & M. Bender. 1981. Carbon fluxes at the sediment–water interface of the deep sea: calcium carbonate preservation. *J. Mar. Res.* 39:139–162.

Feely, R. A., S. R. Alin, J. Newton, C. L. Sabine, M. Warner, A. Devol, C. Krembs & C. Maloy. 2010. The combined effects of ocean acidification, mixing, and respiration on pH and carbonate saturation in an urbanized estuary. *Estuar. Coast. Shelf Sci.* 88:442–449.

Gattuso, J. P., M. Pichon, B. Delesalle, C. Canon & M. Frankignoulle. 1996. Carbon fluxes in coral reefs. 1. Lagrangian measurement of community metabolism and resulting air–sea CO2 disequilibrium. *Mar. Ecol. Prog. Ser.* 145:109–121.

Gazeau, F., C. Quiblier, J. M. Jansen, J. P. Gattuso, J. J. Middelburg & C. H. R. Heip. 2007. Impact of elevated CO2 on shellfish calcification. *Geophys. Res. Lett.* 34. DOI: 10.1029/2006GL028554.

Glover, C. P. & S. M. Kidwell. 1993. Influence of organic matrix on the postmortem destruction of molluscan shells. *J. Geol.* 101:729–747.

Green, M. A. & R. C. Aller. 1998. Seasonal patterns of carbonate diagenesis in nearshore terrigenous muds: relation to spring phytoplankton bloom and temperature. *J. Mar. Res.* 56:1097–1123.

Green, M. A. & R. C. Aller. 2001. Early diagenesis of calcium carbonate in Long Island Sound sediments: benthic fluxes of Ca2+ and minor elements during seasonal periods of net dissolution. *J. Mar. Res.* 59:769–794.

Green, M. A., G. G. Waldbusser, L. Hubazc, E. Cathcart & J. Hall. Carbonate mineral saturation state as the recruitment cue for settling bivalves in marine muds. *Estuaries Coasts.*

Green, M. A., G. G. Waldbusser, S. L. Reilly, K. Emerson & S. O'Donnell. 2009. Death by dissolution: sediment saturation state as a mortality factor for juvenile bivalves. *Limnol. Oceanogr.* 54:1037–1047.

Gutierrez, J. L., C. G. Jones, D. L. Strayer & O. O. Iribarne. 2003. Mollusks as ecosystem engineers: the role of shell production in aquatic habitats. *Oikos* 101:79–90.

Gypens, N., A. V. Borges & C. Lancelot. 2009. Effect of eutrophication on air–sea CO(2) fluxes in the coastal southern North Sea: a model study of the past 50 years. *Glob. Change Biol.* 15:1040–1056.

Hales, B. & S. Emerson. 1997. Evidence in support of first-order dissolution kinetics of calcite in seawater. *Earth Planet. Sci. Lett.* 148:317–327.

Harding, J. M., R. Mann, M. J. Southworth & J. A. Wesson. 2010. Management of the Piankatank River, Virginia, in support of oyster (*Crassostrea virginica,* Gmelin 1791) fishery repletion. *J. Shellfish Res.* 29:867–888.

Hargis, W. J. & D. S. Haven. 1999. Chesapeake oyster reefs: Their importance, destruction, and guidelines for restoring them. In: M. W. Luckenbach, R. Mann & J. A. Wesson, eds. Oyster reef habitat restoration: A synopsis and synthesis of approaches. Gloucester, VA: Virginia Institute of Marine Science Press. 372 pp.

Hautmann, M. 2006. Shell mineralogical trends in epifaunal Mesozoic bivalves and their relationship to seawater chemistry and atmospheric carbon dioxide concentration. *Facies* 52:417–433.

Hecht, F. 1933. Der Verbleib der organische Substanz der Tiere bei meerischer Einbettung. *Senckenb. Biol.* 15:165–219.

Honjo, S. & J. Erez. 1978. Dissolution rates of calcium carbonate in deep ocean: *in situ* experiment in North Atlantic Ocean. *Earth Planet. Sci. Lett.* 40:287–300.

Hopkins, S. H. 1962. Distribution of species *Cliona* (boring sponge) on the eastern shore of Virginia in relation to salinity. *Chesap. Sci.* 2:121–124.

Hu, X., W. J. Cai, Y. Wang, X. Guo & S. Luo. 2011. Geochemical environments of continental shelf: upper slope sediments in the northern Gulf of Mexico. *Palaeogeogr. Palaeoclimatol. Palaeoecol.*

Kelly, R. P., M. M. Foley, W. S. Fisher, R. A. Feely, B. S. Halpern, G. G. Waldbusser & M. R. Caldwell. 2011. Mitigating local causes of ocean acidification with existing laws. *Science* 332:1036–1037.

Kidwell, S. M. 2005. Shell composition has no net impact on large-scale evolutionary patterns in mollusks. *Science* 307:914–917.

Kidwell, S. M. & D. Jablonski. 1983. Taphonomic feedback: ecological consequences of shell accumulation. In: M. J. S. Tevesz & P. L. McCall, editors. Biotic interactions in recent and fossil benthic communities. New York: Plenum Press. pp. 195–248.

Kimmel, D. G. & R. I. E. Newell. 2007. The influence of climate variation on Eastern oyster (*Crassostrea virginica*) juvenile abundance in Chesapeake Bay. *Limnol. Oceanogr.* 52:959–965.

Kobluk, D. R. & M. J. Risk. 1977. Calcification of exposed filaments of endolithic algae, micrite envelope formation and sediment production. *J. Sediment. Petrol.* 47:517–528.

Lebrato, M., D. Iglesias-Rodriguez, R. A. Feely, D. Greeley, D. O. B. Jones, N. Suarez-Bosche, R. S. Lampitt, J. E. Cartes, D. R. H. Green & B. Alker. 2010. Global contribution of echinoderms to the marine carbon cycle: CaCO(3) budget and benthic compartments. *Ecol. Monogr.* 80:441–467.

Longman, M. W. 1980. Carbonate diagenetic textures from near-surface diagenetic environments. *AAPG Bull. Am. Assoc. Petrol. Geologists* 64:461–487.

Mann, R. & E. N. Powell. 2007. Why oyster restoration goals in the Chesapeake Bay are not and probably cannot be achieved. *J. Shellfish Res.* 26:905–917.

Mannino, A. & H. R. Harvey. 1999. Lipid composition in particulate and dissolved organic matter in the Delaware Estuary: sources and diagenetic patterns. *Geochim. Cosmochim. Acta* 63:2219–2235.

Miller, A. W., A. C. Reynolds, C. Sorbino & G. F. Riedel. 2009. Shellfish face uncertain future in high CO2 world: influence of acidification on oyster larvae calcification and growth in estuaries. *PLoS Biol.* 4:e5661.

Millero, F. J., T. B. Graham, F. Huang, H. Bustos-Serrano & D. Pierrot. 2006. Dissociation constants of carbonic acid in seawater as a function of salinity and temperature. *Mar. Chem.* 100:80–94.

Morse, J. W. 2005. Formation and diagenesis of carbonate sediments. In: F. T. Mackenzie, editor. Sediments, diagenesis, and sedimentary rocks. Oxford: Elsevier-Pergamon. pp. 67–86.

Morse, J. W. & R. S. Arvidson. 2002. The dissolution kinetics of major sedimentary carbonate minerals. *Earth Sci. Rev.* 58:51–84.

Morse, J. W., R. S. Arvidson & A. Luttge. 2007. Calcium carbonate formation and dissolution. *Chem. Rev.* 107:342–381.

Morse, J. W. & W. H. Casey. 1988. Ostwald processes and mineral paragenesis in sediments. *Am. J. Sci.* 288:537–560.

Najjar, R. G., C. R. Pyke, M. B. Adams, D. Breitburg, C. Hershner, M. Kemp, R. Howarth, M. R. Mulholland, M. Paolisso, D. Secor, K. Sellner, D. Wardrop & R. Wood. 2010. Potential climate-change impacts on the Chesapeake Bay. *Estuar. Coast. Shelf Sci.* 86:1–20.

Palma, R. M., G. S. Bressan & D. A. Kietzmann. 2008. Diagenesis of a bioclastic oyster deposit from the Lower Cretaceous (Chachao Formation), Neuquen Basin, Mendoza Province, Argentina. *Carbonates Evaporites* 23:39–49.

Pomponi, S. A. & D. W. Meritt. 1990. Distribution and life history of the boring sponge *Cliona truitti* in the upper Chesapeake Bay. In: K. Rutzler, editor. New perspectives in sponge biology. Washington, DC: Smithsonian Institution Press. pp. 384–390.

Pouchou, J. L. & F. Pichoir. 1984. A new model for quantitative X-ray microanalysis. 1. Application to the analysis of homogeneous samples. *Recherche Aerospatiale* 3:167–192.

Powell, E. N., C. E. Brett, K. M. Parsons-Hubbard, W. R. Callender, G. M. Staff, S. E. Walker, A. Raymond & K. A. Ashton-Alcox. 2011a. The relationship of bionts and taphonomic processes in molluscan taphofacies formation on the continental shelf and slope: eight-year trends: Gulf of Mexico and Bahamas. *Facies* 57:15–37.

Powell, E. N., J. N. Kraeuter & K. A. Ashton-Alcox. 2006. How long does oyster shell last on an oyster reef? *Estuar. Coast. Shelf Sci.* 69:531–542.

Powell, E. N. & J. M. Klinck. 2007. Is oyster shell a sustainable estuarine resource? *J. Shellfish Res.* 26:181–194.

Powell, E. N., G. M. Staff, W. R. Callender, K. A. Ashton-Alcox, C. E. Brett, K. M. Parsons-Hubbard, S. E. Walker & A. Raymond. 2011b. Taphonomic degradation of molluscan remains during thirteen years on the continental shelf and slope of the northwestern Gulf of Mexico. *Palaeogeogr. Palaeoclimatol. Palaeoecol.* [in press].

Rosell, D., M. J. Uriz & D. Martin. 1999. Infestation by excavating sponges on the oyster (*Ostrea edulis*) populations of the Blanes littoral zone (north-western Mediterranean Sea). *J. Mar. Biol. Assoc. UK* 79:409–413.

Rude, P. D. & R. C. Aller. 1991. Fluorine mobility during early diagenesis of carbonate sediments: an indicator of mineral transformations. *Geochim. Cosmochim. Acta* 55:2491–2509.

Salisbury, J., M. A. Green, C. Hunt & J. Campbell. 2008. Coastal acidification by rivers: a threat to shellfish? *Eos Trans. AGU* 89:513–514.

Schulte, D. M., R. P. Burke & R. N. Lipcius. 2009. Unprecedented restoration of a native oyster metapopulation. *Science* 325: 1124–1128.

Southworth, M., J. M. Harding, J. A. Wesson & R. Mann. 2010. Oyster (*Crassostrea virginica,* Gmelin 1791) population dynamics on public reefs in the Great Wicomico River, Virginia, USA. *J. Shellfish Res.* 29:271–290.

van Heuven, S., D. Pierrot, J. W. B. Rae, E. Lewis & D. W. R. Wallace. 2011. MATLAB Program Developed for CO_2 System Calculations. ORNL/CDIAC-105b. Carbon Dioxide Information Analysis Center, Oak Ridge National Laboratory, U.S. Department of Energy, Oak Ridge, Tennessee. DOI: 10.3334/CDIAC/otg.CO2SYS_MATLAB_v1.1.

Waldbusser, G. G., H. Bergschneider & M. A. Green. 2010. Size-dependent pH effect on calcification in post-larval hard clam *Mercenaria* spp. *Mar. Ecol. Prog. Ser.* 417:171–182.

Waldbusser, G. G., E. P. Voigt, H. Bergschneider, M. A. Green & R. I. E. Newell. 2011. Biocalcification in the Eastern oyster (*Crassostrea virginica*) in relation to long-term trends in Chesapeake Bay pH. *Estuaries Coasts* 34:221–231.

Walter, L. M. & J. W. Morse. 1984. Reactive surface-area of skeletal carbonates during dissolution: effect of grain-size. *J. Sediment. Petrol.* 54:1081–1090.

Zondervan, I., R. E. Zeebe, B. Rost & U. Riebesell. 2001. Decreasing marine biogenic calcification: a negative feedback on rising atmospheric pCO(2). *Global Biogeochem. Cycles* 15:507–516.

Zuschin, M., M. Stachowitsch & R. J. Stanton. 2003. Patterns and processes of shell fragmentation in modern and ancient marine environments. *Earth Sci. Rev.* 63:33–82.

Appendix 2

Additional Comments in Response to the Shenandoah River

Jeff Kelble <jeff@potomacriverkeeper.org>

RE: Algae

Jeff Kelble <jeff@shenandoahriverkeeper.org> Thu, Sep 23, 2010 at 1:35 PM
To: "Marshall, Harold" <hmarshal@odu.edu>

I understand. For the present, I was most intersted in identifying the presence of blue greens in the Shenandoah and my lack of ability to find them in the Rappahannock.

We aren't hanging anything on this one sample, except that it confirms the presence of cyanobacteria in a high energy ridge/valley river. There are several different actions that we're initiating. The first is mostly an effort to persuade Virginia to consider additional criteria such as nutrification, fish health problems and algae blooms as evidence of impairment due to loss of use or diminished use of the river.

Then the next job is getting Virginia to finally promulgate nutrient standards for moving rivers.

I don't know how to thank you enough for doing this in your "spare time".

Jeff
On Thu, Sep 23, 2010 at 12:51 PM, Marshall, Harold <hmarshal@odu.edu> wrote:

> Hi Jeff,
>
> To answer your questions:
>
> There are seasonal changes that are common in the algal composition within these rivers. The bluegreen algae are also more frequently found during the warmer months of the year, and often are bloom producers during summer and early fall in Virginia habitats. These blooms may be brief or extended, composed of a few dominant species or a variety. Two important determiners are temperature and nutrient availability. In the 2 samples you sent the species of bluegreens would be more typical of higher nutrient levels, but remember all you have is a snapshot of one day of the year. We don't know if this sample is characteristic for the whole river, this one location, or for any particular time period (month, or all the time). The comparison you have may represent the basis of inquiry to get nutrient data. I do not know what is necessary regarding the nutrient impairment status, but I am sure extended nutrient sampling and/or historical data would be required to support your position.
>
> Sincerely,
>
> Harold G. Marshall, Ph.D.
>
> Professor, Department of Biological Sciences
>
> Old Dominion University
>
> Norfolk, Virginia 23529-0266
>
> hmarshal@odu.edu 1-757-683-4204
>
> FAX 1-757-683-5283

From: Jeff Kelble [mailto:jeff@shenandoahriverkeeper.org]
Sent: Wednesday, September 22, 2010 6:27 PM
To: Marshall, Harold
Subject: Re: Algae

Hi Harold,
Thank you for working up those samples so thoroughly. The Rappahannock sample I believe was taken from September 6th instead of May 6th, we can probably blame my handwriting for that error.

I left you a message on your voicemail and I'd like to run a thing or two by you. We've been trying to "encourage" Virginia to list the Shenandoah River for the nutrient impairment that I think everyone recognizes it has. Unless you let me know otherwise I plan to include your blue green algae findings in our correspondence/comments to the state in the most recent 303D listings public comment period.

That brings me to the question: does the presence of the three cyanobacteria's in the Shenandoah seem to indicate a nutrification issue? We don't see it in the Rappahannock which has considerably less nutrient pollution.

Thank you for your thoughts,

Jeff

On Wed, Sep 22, 2010 at 2:29 PM, Marshall, Harold <hmarshal@odu.edu> wrote:

Jeff,

The samples you sent me represented a small spring bloom of algae occurring in May, plus what would be considered a late summer bloom from August. I have provided you general identifications, e.g. many of the diatoms would require more intense time (SEM) and preparation, some of the filamentous taxa require reproductive bodies which were not available.

1.Main stem Shenandoah 8/27/10. Bloom by several cyanobacteria filamentous taxa; *Oscillatoria limosa, Oscillatoria princeps*, and *Lyngbya* sp.; plus numerous diatoms were present and included representatives from the following genera *Pinnularia, Achnanthes, Tabellaria, Navicula, Gomphonema*, and *Gyrosigma*.

2.Rappahannock River 5/6/10. The most common species was a filamentous chlorophyte *Spirogyra* sp.; other chlorophytes included a variety of life stages of a variety of *Scenedesmus/Desmodesmus* spp., including *Desmodesmus quadricauda, D. armatus, and Scenedesmus bijuga*, and*, Ankistrodesmus falcatus*, and the desmid *Cosmarium* sp. Also present were the diatoms *Navicula* sp., *Synedra* sp., and *Tabellaria fenestrata.*

Sincerely,

Harold G. Marshall, Ph.D.

Professor, Department of Biological Sciences

Old Dominion University

Norfolk, Virginia 23529-0266

hmarshal@odu.edu 1-757-683-4204

FAX 1-757-683-5283

--
Jeff Kelble
Shenandoah Riverkeeper
P.O. Box 405
Boyce, VA 22620
Phone: 540-837-1479
Cell: 540-533-6465
Email: jeff@shenandoahriverkeeper.org
Website: www.shenandoahriverkeeper.org
Recognized as "one of the best small nonprofits" by Catalogue for Philanthropy

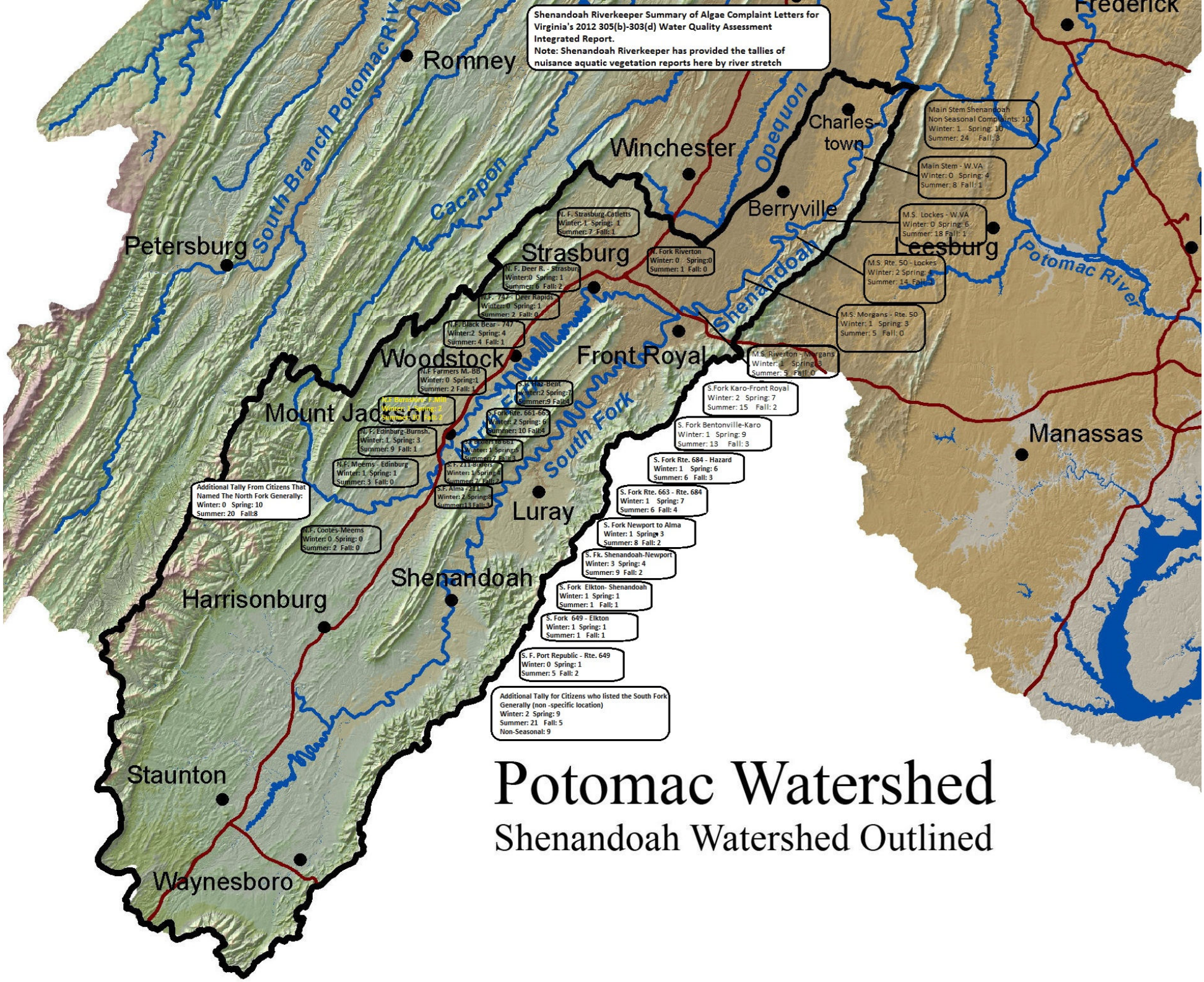
United Way # 9335 * CFC # 87828 *

Shenandoah Riverkeeper uses citizen action and enforcement to protect and restore water quality in the Shenandoah River Valley for people, fish and aquatic life

--
Jeff Kelble
Shenandoah Riverkeeper
P.O. Box 405
Boyce, VA 22620
Phone: 540-837-1479
Cell: 540-533-6465
Email: jeff@shenandoahriverkeeper.org
Website: www.shenandoahriverkeeper.org
Recognized as "one of the best small nonprofits" by Catalogue for Philanthropy

United Way # 9335 * CFC # 87828 *

Shenandoah Riverkeeper uses citizen action and enforcement to protect and restore water quality in the Shenandoah River Valley for people, fish and aquatic life


Shenandoah Riverkeeper Summary of Algae Complaint Letters for Virginia's 2012 305(b)-303(d) Water Quality Assessment Integrated Report.
Note: Shenandoah Riverkeeper has provided the tallies of nuisance aquatic vegetation reports here by river stretch
Romney
Frederick
South Branch Potomac River
Cacapon
Opequon
Winchester
Charles-town
Berryville
Leesburg
Potomac River
Shenandoah
Petersburg
Strasburg
Woodstock
Front Royal
Mount Jackson
South Fork
Luray
Shenandoah
Harrisonburg
Staunton
Waynesboro
Manassas
Main Stem Shenandoah Non Seasonal Complaints: 10 Winter: 1 Spring: 10 Summer: 24 Fall: 3
Main Stem - W.VA Winter: 0 Spring: 4 Summer: 8 Fall: 1
M.S. Lockes - W.VA Winter: 0 Spring: 6 Summer: 18 Fall: 1
M.S. Rte. 50 - Lockes Winter: 2 Spring: 4 Summer: 14 Fall: 3
M.S. Morgans - Rte. 50 Winter: 1 Spring: 3 Summer: 5 Fall: 0
M.S. Riverton - Morgans Winter: 1 Spring: 3 Summer: 5 Fall: 0
N. F. Strasburg-Cotletts Winter: 1 Spring: 1 Summer: 7 Fall: 1
N. Fork Riverton Winter: 0 Spring: 0 Summer: 1 Fall: 0
N. F. Deer R. - Strasburg Winter: 0 Spring: 1 Summer: 6 Fall: 2
N.F. 747 - Deer Rapids Winter: 0 Spring: 1 Summer: 2 Fall: 0
N.F. Black Bear - 747 Winter: 2 Spring: 4 Summer: 4 Fall: 1
N.F Farmers M.-BB Winter: 0 Spring: 1 Summer: 2 Fall: 1
N.F. Burnsnors F.Mill Winter: 1 Spring: 2 Summer: 1 Fall: 2
N. F. Edinburg-Burnsh. Winter: 1 Spring: 3 Summer: 9 Fall: 1
N.F. Meems - Edinburg Winter: 1 Spring: 1 Summer: 3 Fall: 0
N.F. Cootes-Meems Winter: 0 Spring: 0 Summer: 2 Fall: 0
Additional Tally From Citizens That Named The North Fork Generally: Winter: 0 Spring: 10 Summer: 20 Fall: 8
S.F. Haz-Bent Winter: 2 Spring: 7 Summer: 9 Fall: 4
S. Fork Rte. 661-662 Winter: 2 Spring: 6 Summer: 10 Fall: 4
S.F. Elkton to 661 Winter: 1 Spring: 6 Summer: 7 Fall: 3
S.F. 211-Briers Winter: 1 Spring: 4 Summer: 7 Fall: 2
S.F. Alma - 211 Winter: 2 Spring: 8 Summer: 13 Fall: 3
S.Fork Karo-Front Royal Winter: 2 Spring: 7 Summer: 15 Fall: 2
S. Fork Bentonville-Karo Winter: 1 Spring: 9 Summer: 13 Fall: 3
S. Fork Rte. 684 - Hazard Winter: 1 Spring: 6 Summer: 6 Fall: 3
S. Fork Rte. 663 - Rte. 684 Winter: 1 Spring: 7 Summer: 6 Fall: 4
S. Fork Newport to Alma Winter: 1 Spring: 3 Summer: 8 Fall: 2
S. Fk. Shenandoah-Newport Winter: 3 Spring: 4 Summer: 9 Fall: 2
S. Fork Elkton- Shenandoah Winter: 1 Spring: 1 Summer: 1 Fall: 1
S. Fork 649 - Elkton Winter: 1 Spring: 1 Summer: 1 Fall: 1
S. F. Port Republic - Rte. 649 Winter: 0 Spring: 1 Summer: 5 Fall: 2
Additional Tally for Citizens who listed the South Fork Generally (non -specific location) Winter: 2 Spring: 9 Summer: 21 Fall: 5 Non-Seasonal: 9
Potomac Watershed
Shenandoah Watershed Outlined

ANTHONY MORRIS
EVERGREEN
P. O. BOX 560
MADISON, VA 22727-0560

E-mail ANTHONYMORRIS@NEXET.NET

April 2, 2012

Mr. John M. Kennedy
DEQ Office of Water Monitoring and Assement
P.O. Box 1105
Richmond, Va. 23218-1105

Re: Draft 2012 305(b)/303(d)
Water Quality Assessment Integrated Report

Dear Mr. Kennedy, I have fished all my life, worked on streams and Rivers, life long member of Trout Unlimited including several committees.

Today, I am writing about the Shenandoah River, and the decline of the enjoyment. I fish near Elkton Va., both up and down river (north & south, and prefer to wade, as well as small craft. Recently, it is quite slippery with the Filamentous algae. "rock snot", as it is called, causes danger, and takes away the joy of fly fishing, or spin fishing. There is an odor as the warm days approach and it is plain to see, that some farms, chicken and others have damaging amounts of excrement washing into the River. I could write a volume of problems, but that would be boring! Let me close by saying, when you factor in, smell, algae, the concern for sickness, (I no longer would bring a pet) the River decline removes a way for families to spend time together. Please consider the Shenandoah as if it were a part of your life. Thank you, Anthony Morris

4 April 2012

ATTN: John M. Kennedy
DEQ Office of Water Monitoring and Assessment
P.O. Box 1105
Richmond, Virginia 23218-1105
RE: Draft 2012 305(b)/303(d) Water Quality Assessment Integrated Report

Dear Mr. Kennedy,

I am writing to ask that the Virginia Department of Environmental Quality include the North Fork, the South Fork, and the Main Stem of the Shenandoah River as impaired waters in the 2012 305(b)3/303(d) Water Quality Assessment Integrated Report. The entire Shenandoah watershed has been experiencing severe algae problems for several years.

When I started fishing the North Fork and Big Stoney Creek in the mid-1970s, the Shenandoah and Stoney were crystal-clear watersheds. I used to take my Sons fishing out there with me, and took our Boy Scout Troop out there quite a number of times. But then I was assigned overseas for several years, and when I came back, Stoney and the North Fork were mere shadows of themselves by the mid- to late 1980s. Many fish had sores on them and there definitely weren't the numbers of earlier years.

I still fish the North Fork, South Fork and Main Stem of the Shenandoah. However, for much of the year, large sections of each river experiences a series of algae blooms that seriously diminish water conditions and my use and enjoyment of the rivers. In each of the past 6 years during late winter there have been algae blooms. Over the course of the year I have seen this on all three rivers near Edinburgh, Shenandoah, and below the Front Royal Dam. This bloom turns the river a dark murky green well into summer. When the algae blooms I often stop wet wading, fearing an infection. In fact, a couple of years back a couple of members of my fishing club scratched themselves in some of the rivers in the Potomac watershed, not sure if it was the Shenandoah system, but they almost died. When I do fish, I find the fishing is poor.

By mid-May or June "rock snot" takes over the river bottoms, and the native grasses, which are trying to emerge, turn brown and lose their leaves and remain bare the rest of the year. There is also a floating bubbly type of algae that comes at this time. Clumps rise up and float on the surface.

The Shenandoah watershed is a mess, and I urge you to ask that DEQ include the three sections of the Shenandoah River on the 305B/303D list, determine why we are having these algae problems, and to develop and implement a plan to clean up the Shenandoah. I don't know if you are a fisherman, but when I first started fishing it, the 'Doah was pristine. Now we've had several major fish kills and the algae problems. The river used to attract fishermen from all over Virginia and all along the East Coast. Old friends of mine who live in New Hampshire used to come down every year to fly fish for smallmouth bass. They haven't come down in several years. Heck, why would anyone drive 500 miles to get skunked by the fish and the algae.

I am the activity chairman of the Potomac River Smallmouth Club, with over 100 members. If you would ever like to hear our comments on the state of the Shenandoah, we would be proud to host you. We meet most months on the last Wednesday of the month from 1930 to 2100 at the Vienna Fire House. Please check out our Website at www.prsc.org.

Respectfully,

William J. "Bill" Amshey, Jr.
3007 Rayjohn Lane
Oak Hill, Virginia 20171
703-716-0838

Members of the Virginia Department of Environmental Quality:

I am writing this letter in support of listing the Shenandoah River as a Endangered Waterway. During the past 25 years, my children and I have used the Shenandoah for swimming, kayaking, and fishing. They are grown now, and are off to live in other parts of Virginia. They would be sad to see the condition of the Shenandoah as it is now. Through the years I have seen a steady decline in the quality of the water.

Gone are the days when the Shenandoah was a clear river full of wonderful smallmouth bass of huge size.

Because of the string of years with fish kills, I have limited my use of the Shenandoah during the past five or six summers. Something is killing those fish. Whatever it is couldn't be good for people either.

Last summer, however, I decided to enjoy the river again. I fished the river at least ten ten days. I found it in worse shape than ever. There was a summer-long algae bloom on the Main Stem. The water was an olive-drab green rendering the water nearly opaque. Gone were the lush grass beds that sheltered smaller fish and enriched the river's water with oxygen. Fishing was poor, and I certainly didn't want to stand in that water to fish, let alone swim in it.

Others seem to have found problems with the river, too. There were several days on the Shenandoah last summer when I saw not a single other angler on the river other those with me. The few anglers I encountered reported catching few if any smallmouth bass on the Main Stem, and those caught were of small size.

One day I fished the Main Stem with a friend, an experienced angler, who had never fished the Shenandoah. He was struck by the awful condition of the water. He's not fished the Main Stem since, but continues to tell me of wonderful fishing in less degraded waters elsewhere. Why would he want to spend his fishing money here?

It is obvious to anyone who has spent time on the Shenandoah that the river is in serious trouble. With the help of the DEQ, the Shenandoah can come back to health. Recognizing the serious nature of the problem is a necessary first step.

I hope one day in the future I will be able to enjoy the Main Stem with my grandchildren and show them a river about which we call all be proud.

Sincerely,
Bill Prokopchak
1714 Lewis St.
Winchester, VA 22601

April 18, 2012

April 16, 2012

John M. Kennedy
DEQ Office of Water Monitoring and Assessment
P.O. Box 1105
Richmond, VA 23218-1105

Re: Adding Shenandoah River to 2012 305(b)/303(d)Water Quality Assessment Integrated Report

Dear Mr. Kennedy:

I am writing to request that Virginia' Department of Environmental Quality add the North Fork, South Fork and Main Stem of the Shenandoah River as impaired waters on the 2012 305(b)3/303(d) Water Quality Assessment Integrated Report because of the severe algae problems the river experiences every year.

I have fished all sections of the Shenandoah River for many years and, since my retirement as a civil engineer in 2003, I have kept a log of each fishing trip. I have made 121 catch-and-release fishing trips on various Shenandoah River locations since 2003. The majority of the trips were on the Main Stem between Route 50 and Locke's landing. The fishing consisted of wading and canoeing, with smallmouth bass the most desired quarry. I was usually accompanied by my fishing buddies, my sons or my grandson on these river expeditions.

In general, I have observed increased weed growth over the years, often to the point where it is not possible to fish some locations by the middle of summer. There is no question that this excessive growth is due to over-fertilization by nitrogen and phosphorous. Based on more than 30 years' experience in the study and design of wastewater treatment facilities, I know more must be done to up upgrade these facilities to meet the requirements of the Chesapeake Bay cleanup initiative. In addition, the non-point sources of storm water and agricultural waste must be controlled to reduce this problem growth in the Shenandoah River.

Algae growth was a major problem on the Main Stem last year and the murkiness of the water was aesthetically very unpleasant. It severely diminished the fishing until it cleared up in early fall. This algae pollution was very evident when one looked on Google Maps at the confluence of the Potomac and Shenandoah Rivers at Harpers Ferry. The dirty Shenandoah water was in stark contrast with the clear Potomac flow.

In addition, the slimy film that accumulates on the river bottom fouls up fishing line and lures and is particularly a problem for wade fishing. The growth is slippery and as I move around, I have occasionally fallen even when using a stout wading staff and studded fishing boots with felt soles.

The Main Stem smallmouth bass population has declined as reflected in my 9 years of fishing logs. Fish do not thrive in polluted water and the long-term consequences are not good for this section of the Shenandoah watershed. Most important, the water from the Shenandoah River merges with the Potomac River and eventually becomes the source of drinking water for Loudoun County, most of Fairfax County, and the Washington D.C. metropolitan area. The pollution of the Shenandoah requires additional treatment and accompanying expense so that the drinking water in these areas will comply with the Safe Drinking Water Act.

As our nation's population expands, the use and enjoyment of outdoor recreation is more and more important for the spiritual rejuvenation and physical wellbeing of our people. The proximity of the Shenandoah River to major Virginia population centers makes it imperative that we preserve its beauty and wildness. Please consider placing these waters on the impaired list under the 305(b)/303(d) Water Quality Assessment to determine the various sources causing the weed growth and algae problems, and to develop, implement and finance a plan to solve them.

Thank you for your consideration of this request.

Sincerely,

Robert E. Forbes
2316 Concert Court
Vienna, VA 22181
703 281-5283
Bforbes32@verizon.net

Jeff Kelble <jeff@potomacriverkeeper.org>

Form submission from: Take Action!- Help the Shenandoah Riverkeeper Get Rid of Algae

Potomac Riverkeeper <sarah@potomacriverkeeper.org> Mon, Apr 23, 2012 at 2:29 AM

To: jeff@potomacriverkeeper.org

Submitted on Monday, April 23, 2012 - 2:29am
Submitted by anonymous user: [166.248.32.86]
Submitted values are:

Your Letter:
John M. Kennedy
DEQ Office of Water Monitoring and Assessment
P.O. Box 1105 Richmond, Virginia 23218-1105

Dear Mr. Kennedy,

I'm writing in concern of the algae blooms consistently occurring in the North Fork, South Fork, and Main Stem of the Shenandoah River, and request the Virginia Department of Environmental Quality (DEQ) lists these waters on thee 2012 305(b)3/303(d) Water Quality Assessment Integrated Report.

I rely on several properties of friends to access the main stem Shenandoah for fishing, and often use public access ramps for canoeing with friends as well. Over the past several years I have found my fishing experiences continuing to decrease along the main stem directly because of algae blooms. I have noticed extensive algae blooms occurring in March and April to the point of not even fishing from frustration of cleaning a green fishing lure, then during May I have been catching less fish than I have recorded in past years. I feel my low fish numbers are in direct relation to the death of these extensive algae blooms decreasing the levels of oxygen in the river when decomposing.

I have also noticed there is less aquatic grass establishing itself in the river along my fishing spots after algae blooms. This is likely an additional response to the less dissolved oxygen from decomposing algae, and has also diminished by experiences and use of the river because there is no cover for fish.

As new years come I find myself using the river lesser than previous years, more recently even safety issues arising with the concern for loved ones. The river use to be a great place to spend time with my girlfriend to fish, and introduce the outdoors to my nephew. During the summer the river bed becomes extensively covered with an algae film on the rocks bank to bank. Both my girlfriend and nephew have had problems keeping upright when wading within the river with the rocks so slippery.

My experiences catching fish on the Shenandoah are not near what they were when I were a kid, and these algae blooms are an issue directly affecting enjoyable leisure. Still I hope one day my nephew gets the same enjoyable opportunities to fish the Shenandoah like I had in the past. To restore what the Shenandoah could be I request the DEQ includes all three sections of the river on the 305(b)/303(d) impaired waters list.

Thank you for considering by suggestion and best regards,

Cory M. Miller
280 Buckhorn Road
Middletown, VA 22645
Email: cmiller082@su.edu
Phone: 540-333-7086

Name: Cory M. Miller
Email: cmiller082@su.edu

The results of this submission may be viewed at:
http://www.potomacriverkeeper.org/node/428/submission/830

Jeff Kelble <jeff@potomacriverkeeper.org>

RE; Draft 2012 305(b)/303(d)Water Quality Assessment Integrated Report

bishbox@comcast.net <bishbox@comcast.net> Wed, Apr 11, 2012 at 9:44 PM
To: jeff@shenandoahriverkeeper.org

To John M. Kennedy
DEQ Office of Water Monitoring and Assessment
P.O .Box 1105
Richmond Va.23218-1105
RE; Draft 2012 305(b)/303(d)Water Quality Assessment Integrated Report

Dear Mr. Kennedy,
I have enjoyed owning property on the South Fork of the Shenandoah River for thirty years now and in two weeks I'll be sitting down at a closing table to purchase the land lot next to mine. I am fifty-one years old and hope to retire there someday as I think its one of the most serene and relaxing places in the state of Virginia.

Some years ago I do remember reading that the river had made the list of the COUNTRYS most endangered rivers and these last few years my friends and family has experienced the decline for ourselves.

The fishing has dropped of dramatically, the turtles are gone and birds seem fewer.
There is an algae that seems to be covering the bottom most of the year, I don't think the science community ever solved the "fish-kill" problem, but the bad news just keeps piling on.

This stubborn and horrible algae will hurt tourism, sport, wildlife and all of us who make Virginia our home, as a result I would like to ask that DEQ include all three sections of the Shenandoah River on 305B/303D LIST, determine why we are having these algae problems, and to develop and implement a plan to bring 'The Daughter of the Stars" back to her great self. I have six Grandkids who are going want to go fishing at Grandpa's country house!
Thank You for considering these concerns,

Mr. Craig Bishop
1000 Canoe River Dr.
Rileyville Va.22650

DRS. DONNA AND ZAHRL SCHOENY

Dear Jeff,

We are long time friends of the Browne's at 456 Misty Meadow Lane in Bentonville on the Shenendoah River. Since they have been property owners there we have spent many days and weeks on the river. When we first started going there, we fished and ate the fish we caught. We also were avid kayakers, keeping our kayak at their house. We were very excited when they also purchased the cabin on Misty Meadow Lane and spent almost every weekend or more there for years, getting to know the neighbors and feeling a part of the valley community. We have watched the decline of the river and are very saddened by the amount of algae, flotsam, and diseased fish that continues to worsen every year. Allowing one of the most beautiful rivers in this country (and we have travelled all 50 states} to decline at this rate is a travesty and embarrassment to the state of Virginia. We would implore the state to recognize that the Shenendoah should be on Virginia's Dirty Water's List. Our children and grandchildren love to come to the river to swim, fish, and float. One even worked for a summer for an Outfitter and lived with me at the cabin. We are concerned if it is still safe for them to continue enjoying the recreation available at this once idyllic spot. Please let us know if there is anything we can do to encourage the clean up our favorite river

Donna and Zahrl Schoeny

300 Lake Forest Lane

Charlottesville, Virginia 22901

April 10, 2012

John M. Kennedy
DEQ Office of Water Monitoring and Assessment
P.O. Box 1105
Richmond, Virginia 23218-1105
RE: Draft 2012 305(b)/303(d) Water Quality Assessment Integrated Report

Dear Mr. Kennedy,

I am writing to request that Virginia's Department of Environmental Quality add the North Fork, South Fork and Main Stem of the Shenandoah as impaired waters on thee 2012 305(b)3/303(d) Water Quality Assessment Integrated Report because of the severe algae problems the river experiences every year.

I fish the South Fork of the Shenandoah extensively and I also use the river to kayak and take my family members fishing. However, for much of the year, large sections of the river seem to experience a series of noxious algae blooms that seriously diminish my use and enjoyment of the rivers.

Over the course of the past five years I've fished the South Fork from Luray to Front Royal. The places I like to wade and fish are mile 13, 16 and up stream from Andy Guest Park. The past two years I've fished these locations less due to the fact that I not only find algae as mentioned above but I find fish that have abnormal growths on them. I typically fish these areas from May through September; however, I've begun seeking out other locations due to the poor conditions of the water. I've been in my kayak when I've had to paddle though thick pockets of algae.

This cycle of algae diminishes both my use and my enjoyment of the Shenandoah River system. As a result I would like to ask that DEQ include the South Fork and the other sections of the Shenandoah River on 305B/303D list, determine why we are having these algae problems, and to develop and implement a plan to make them go away.

Thank you for considering my comments,

Elwyn "Chip" Comstock
6822 Wemberly Way
Mclean, Va 22101
Email: comburns@aol.com
Phone: 703-356-9124
Potomac River Smallmouth Bass Club Librarian

April 17, 2012

John M. Kennedy

DEQ Office of Water Monitoring and Assessment

P.O. Box 1105

Richmond, Virginia 23218-1105

RE: Draft 2012 305(b)/303(d) Water Quality Assessment Integrated Report

Dear Mr. Kennedy,

By now, you know the story.

I am writing to request that Virginia's Department of Environmental Quality add the North Fork, South Fork and Main Stem of the Shenandoah as impaired waters on thee 2012 305(b)3/303(d) Water Quality Assessment Integrated Report because of the severe algae problems the river experiences every year.

I fish the Shenandoah often, from Front Royal to Harper's ferry (which is where the confluence with the Potomac is). But it often looks like a pond (noxious algae blooms in the summer) and that seriously diminish my use and enjoyment of the river. Last year, the nasty, oppressive algae bloom spilled over into the Upper Potomac. That's where I fish too! So now, that impairs that fishery as well. And it stinks! No way would I want to get out of my kayak into that stuff….

These rivers deserve better!!

You must take some action to preserve our use of this river. It cannot die. It's that simple. Algae is a killer of life in the water but you already know that.

Thank you for considering my comments,

Mr. Ernesto J Rojas

21317 Comus Ct

Ashburn VA 20147-4864

Email: ernierojas@verizon.net

Phone: 703-729-0128

April 12, 2012
John M. Kennedy
DEQ Office of Water Monitoring and Assessment
P.O. Box 1105
Richmond, Virginia 23218-1105
RE: Draft 2012 305(b)/303(d) Water Quality Assessment Integrated Report

Dear Mr. Kennedy,
I am writing to request that Virginia's Department of Environmental Quality add the North Fork, South Fork and Main Stem of the Shenandoah as impaired waters on the 2012 305(b)3/303(d) Water Quality Assessment Integrated Report because of the severe algae problems the river experiences every year.

Since my retirement in 2004, my principle recreational activity from April through October is fishing. I wade fish on all three sections of the Shenandoah about 40 times a year. A review of my fishing log for last year showed that last year I recorded the lowest average daily catch rates on the Shenandoah since I retired. I attribute this reduction to the condition of the river. In a normal year, the river water is fairly stained from the Spring rains until about the latter part of June. It then becomes clear enough to see the bottom when wading for the rest of the fishing season. Last year, the main stem and the South Fork stayed cloudy due to algae blooms until sometime in September. This condition reduced the number of fish I was catching and made the wading more dangerous. As a result, I basically stopped fishing on these two sections of the river until the water cleared and a normal fish catch rate resumed in September.

In June through September, I was forced to do most of my fishing on the North Fork. While the area in this section of the river where I fished didn't suffer the algae bloom that the other two sections did, it was loaded with rock snot. On just about every cast, I would have to clean the rock snot off my bait (even if the bait was rigged for weedless fishing) before making the next cast. I also had to be much more careful when wading to avoid slipping on or stumbling over coated rocks. As a result, my fishing experience was much less enjoyable and productive.

This cycle of algae diminishes both my use and my enjoyment of the Shenandoah River system. As a result I would like to ask that DEQ include the three sections of the Shenandoah River on 305B/303D list, determine why we are having these algae problems, and to develop and implement a plan to make them go away.
Thank you for considering my comments,

George Moran
4132 Point Hollow Ln
Fairfax, VA 22033
Email: morangm@yahoo.com
Phone: 703-818-2146

C
Dr. John R. Cook
Dr
3254 Holly Hill Dr
Falls Church, VA 22042

3/29/12

John M Kennedy
DEQ Office of Water Monitoring & Assessment
P.O. Box 1105
Richmond, Va 23218 - 1105
RE: Draft 2012 305(b)/303(d) Water Quality Assessment
Integrated Report

Dear Mr. Kennedy,

I am writing to express my concern for the water quality of the Shenandoah River. The algae bloom on the Shenandoah made it almost impossible to fish from mid-summer on. It also affected the Potomac particularly the south bank. For the past 10 years I have averaged 40 days fishing mostly on Virginia series and the Potomac, I find the floating algae and the filamentous algae (snot grass) a serious detriment to my fishing

For the past 25 years I have been the record keeper for the Potomac River Smallmouth Club catch and release fishing contest, after the floods of 96 the fishing improved rapidly. The results of 03, 04 + 05 were our best and they have been in decline since, I believe the decline reflects declining river quality.

The purpose of this letter is to ask that the North Fork, South Fork + Main Stem Shenandoah be added to the list of impaired waters on the 2012 305(b)/303(d) Water Quality Assessment Integrated Report by the Va Dept of Environmental Quality.

I don't know if you are a fisherman or not but if the fishing is good that means that things are right in the river and that makes a lot of people happy!

Good fishing,

Jack

Patricia & John R. Cook
3254 Holly Hill Dr.
Falls Church, VA 22042-3660

4/2/12

John M Kennedy
DEQ office of Water Monitoring and assessment
PO Box 1105
Richmond, Va 23218-1105
RE Draft 2012 305(b) 303(d) Water Quality assessment
Integrated Report

Dear Mr Kennedy,

I am writing an addendum to the letter I sent to you thru Jeff Kelble, the Shenandoah River Keeper. He requested that I be specific as to the timing of the algae bloom. I'm not exactly sure but I think it started late spring, May/June. It continued well thru the fall. As I said I didn't fish the Shenandoah much after early summer because of the algae. However I did fish the Potomac below Harper's Ferry and it had a problem particularly the South bank from the algae coming down from the Shenandoah.

Thank you for your consideration of matter.

Sincerely

Jack

Jeff Little
2703 Rowe Road
New Windsor, MD 21776

410-635-3857 Home
443-244-2592 Cell

yakfish@verizon.net

April 9, 2012

John M. Kennedy
DEQ Office of Water Monitoring and Assessment
P.O. Box 1105
Richmond, Virginia 23218-1105
RE: Draft 2012 305(b)/303(d) Water Quality Assessment Integrated Report

Dear John M Kennedy,

The last time I visited the Shenandoah River to fish was last September near Pugh's Run on the North Fork. I took my two sons to wade and fish where I have fond memories of catching feisty smallmouth bass. We spent about three hours wading downstream, catching a few small bass, a far cry from my memories of this previously impressive fishery.

The slippery filamentous algae that permeated the river bottom made wading treacherous for my sons. The "snot grass" coated their water sandals and when the decided to jump in further, the rest of their clothes. It also made for frustrating fishing, as each cast yielded a clump of algae that prevented our catching many fish. This was not the case several years ago. My tales of successful fishing were met by skepticism by two young anglers who would rather not return. Certainly the algae, symptomatic of the water quality issues that affect the fishery has diminished how often I have visited the river.

I would request that the Virginia Department of Environmental Quality include the North Fork, South Fork and Mainstem Shenandoah on its impaired waters 303D/305B list. Feel free to contact me if you have questions. Thank you for your time.

Sincerely yours,

Jeff Little

Jeff Kelble <jeff@potomacriverkeeper.org>

Form submission from: Take Action!- Help the Shenandoah Riverkeeper Get Rid of Algae

Potomac Riverkeeper <sarah@potomacriverkeeper.org> Tue, Apr 17, 2012 at 1:56 PM
To: jeff@potomacriverkeeper.org

Submitted on Tuesday, April 17, 2012 - 1:56pm
Submitted by anonymous user: [76.21.150.216]
Submitted values are:

Your Letter:
Dear Mr. Kennedy,
I and many of my friends USED to travel to Virginia to fish the Shenandoa River. We did so for many years because the water used to be clean, and the fishing was great. That stopped over 5 years ago. I'm not sure what's happened, but I assume it's increased pollution entering the river, including pesticides, road salts, and lawn fertilizers. So, for the last 5 years, we've avoided your state, and none of our out of state money is being spent there either, for hotels, gas, restuarants, etc. I wish you could support cleaning up the river by including the three sections of the Shenandoah River on 305B/303D list, and identifying what's causing the terrible algae growths. Sincerely.
Name: John Cabala
Email: jrockjrock@comcast.net

The results of this submission may be viewed at:
http://www.potomacriverkeeper.org/node/428/submission/824

April 17, 2012
John M. Kennedy
DEQ Office of Water Monitoring and Assessment
P.O. Box 1105
Richmond, Virginia 23218-1105
RE: Draft 2012 305(b)/303(d) Water Quality Assessment Integrated Report

Dear Mr. Kennedy,

I am writing to request that Virginia's Department of Environmental Quality add the North Fork, South Fork and Main Stem of the Shenandoah as impaired waters on thee 2012 305(b)3/303(d) Water Quality Assessment Integrated Report because of the severe algae problems the river experiences every year.

I live in Northern Virginia and am an avid angler. I fish most rivers and lakes in the state. In the past I have had some great fishing experiences on the Shenandoah River. I have fished mostly the North Fork and Main Stem sections by myself and with my family. Over the last few years I have avoided this river due to heavy algae blooms and filamentous algae (rock snot) that seem to cycle yearly. The bad smell of the algae blooms, lack of fish and slime have made any river outing unpleasant. This is not to mention the fish kills and lesions on fish I have seen.

This cycle of algae diminishes both my use and my enjoyment of the Shenandoah River system. As a result I would like to ask that DEQ include the three sections of the Shenandoah River on 305B/303D list, determine why we are having these algae problems, and to develop and implement a plan to make them go away.

Thank you for considering my comments,

John R. Durbin
21841 Maywood Terrace
Sterling, VA 20164
E-mail: john_d_20164@yahoo.com
Phone: 703-406-9096

Shenandoah River

I am writing a letter requesting that the North fork, South fork, and main stem of the Shenadoah river are added to the impaired waters list.

I like to go to the Shenadoah river with my mommy and my daddy. Some things I do in the river are fishing, swimming, and canoeing. My enjoyment has been diminished because of big, slimy, mushy, icky clumps of algae floating around, causing me to try to avoid those areas, therefore lessening my enjoyment by limiting the areas that I can go in.

Sincerely,

Kaitlyn Potts, age 8

Mark J. Frondorf
4114 N. 21st Street
Arlington, VA 22207
Phone: 571-969-0746

13 April 2012

John M. Kennedy
DEQ Office of Water Monitoring and Assessment
P.O. Box 1105
Richmond, VA 23218-1105

RE: Draft 2012 305(b)/303(d) Water Quality Assessment Integrated Report

Dear Mr. Kennedy,

The purpose of this letter is to request that Virginia's Department of Environmental Quality add the North Fork, South Fork and the Main Stem of the Shenandoah as impaired waters on the 2012 305(b)/303(d) Water Quality Assessment Integrated Report.

I have lived and fished in Virginia since 1984. My favorite river is the Shenandoah and I fish multiple stretches of the river every year. Over the course of the last five years I have noticed a real and persistent problem with algae blooms on the Shenandoah River. I have encountered these blooms on the North Folk, the South Fork as well as the Main Stem of the Shenandoah River. As a result of the algae blooms, I no longer fish the Shenandoah from July – September. The river smells, it turns a putrid green, the fishing drops off dramatically, as well as fouling your line on almost every cast.

This past summer, I donated my time as a fishing guide to the Shenandoah River Rodeo that took place in Front Royal, VA. I was embarrassed to take major contributors out on the supposed crown jewel of Virginia rivers as rock snot coated every rock and eddy pocket on the river. If not addressed, this algae will ultimately result in the loss of jobs and businesses as anglers and tourists take their hard-earned dollars elsewhere to enjoy the outdoors.

I understand you are in a difficult position with a tight budget and stretched staff. I don't envy your duties and responsibilities. But these responsibilities require you to add the Shenandoah River to the impaired waters on the Water Quality Assessment Report.

Respectfully,

Mark J Frondorf

Mark J. Frondorf

Jeff Kelble <jeff@potomacriverkeeper.org>

Thanks for your help with the Shenandoah algae issue

Frondorf, Matthew <matthew.frondorf@cinbell.com> Thu, Apr 19, 2012 at 2:30 PM
To: "jeff@shenandoahriverkeeper.org" <jeff@shenandoahriverkeeper.org>

April 19, 2012

John M. Kennedy
DEQ Office of Water Monitoring and Assessment
P.O. Box 1105
Richmond, Virginia 23218-1105

Dear Mr. Kennedy,

I am writing to request that Virginia's Department of Environmental Quality add the North Fork, South Fork and Main Stem of the Shenandoah as impaired waters on thee 2012 305(b)3/303(d) Water Quality Assessment Integrated Report because of the severe algae problems the river experiences every year.

I fish the North Fork, South Fork and Main Stem of the Shenandoah occasionally and I also use all three rivers to take my family canoeing and camping. However, for much of the year, large sections of each river seems to experience a series of noxious algae blooms that seriously diminish my use and enjoyment of the rivers. Driving from Cincinnati Ohio to the Commonwealth takes time and money and I am afraid this will cease if the present conditions continue. My family spends money on gas, lodging, food, souvenirs, licenses, and camping gear totally several hundred dollars per trip – we will be forced to find another destination for our leisure time that is algae free. I am certain you and your family would make the same decision.

In each of the past six years during late winter a planktonic algae blooms, usually after a high water event, but not always. Over the course of the year I use the entire river system and have seen this on all three rivers from Port Republic down to Front Royal, from Broadway to Front Royal and from Front Royal to the Confluence with the Potomac River. This bloom turns the river a dark murky green color, like green paint, from late winter until about July. When the algae blooms I often choose not to swim or fish and I don't like to take my family swimming because they don't like the off-color murk water. When I do fish I find the fishing is poor and I don't enjoy the experience as much. Whenever the river is this murky color, it's disturbing to fish and the fish are usually lethargic and often they don't feed at all. Activity in the river drops to near zero. For the past two years I simply could not fish the lower south fork or the entire Main Stem from July through August because of the plankton green water.

Beginning in mid-May or early June each year as the river clears the planktons seem to die back. But then a filamentous algae (rock snot) takes hold covers the river bottom and the native grasses which are trying to emerge. The native grasses turn brown and lose their leaves and remain bare the rest of the year. Additionally, a floating bubbly type of algae (I'm told its Cynabacteria or Blue Green Algae) comes at this time. Clumps rise up to and float on the water surface and then accumulates on anything sticking out of the river and in back eddies. This algae smells like sewage or rotting broccoli. When these algae are blooming I literally do not want to be anywhere near the river and I often choose to go somewhere else.

When I have no other place to go then it just takes all the enjoyment out of my day. The fishing is frustrating because you cannot fish without fouling your line on the algae, the fish won't bite lures or bait with algae sticking to it. I find being around the river very unpleasant due to the odors and annoyance. I found this problem in the following areas last year from July through August; Broadway, Lupton, Deer Rapids, Edinburg, Woodstock, Strasburg, Front Royal, Goods Mill, Luray Dam, 211, Alma, Port Republic, Warren Dam, Shenandoah, Route 50, Route7 among other places.

Finally, in the fall each year the native grasses die back and a bright green filamentous algae again covers the entire bottom of the river from Broadway to Strasburg. All three of these algae make it nearly impossible to fish and again diminishes and sometimes eliminates my ability to enjoy the river.

This cycle of algae diminishes both my use and my enjoyment of the Shenandoah River system. As a result I would like to ask that DEQ include the three sections of the Shenandoah River on 305B/303D list, determine why we are having these algae problems, and to develop and implement a plan to make them go away.
Thank you for considering my comments,

Mr. Matt Frondorf

2715 Eugenie Lane

Cincinnati OH 45211

513-251-0700

Email: matthew.frondorf@cinbell.com

Matt Frondorf

Cincinnati OH

The information transmitted is intended only for the person or entity to which it is addressed and may contain confidential and/or privileged material. Any review, retransmission, dissemination or other use of, or taking of any action in reliance upon, this information by persons or entities other than the intended recipient is prohibited. If you receive this in error, please contact the sender and destroy any copies of this document.

Mr. John Kennedy
Virginia Department of Environmental Quality
Office of Water Monitoring and Assessment
P.O. Box 1105
Richmond, VA 23218-1105

April 9, 2012

Subject: Draft 2012 305(b)/303(d) Water Quality Assessment Integrated Report

Dear Mr. Kennedy:

I am writing to request that the Virginia Department of Environmental Quality add the North Fork Shenandoah River, South Fork Shenandoah River, and main stem Shenandoah River to the 305(b)/303(d) list of Virginia's impaired waters.

I am an angler and naturalist who buys annual out-of-state Virginia fishing licenses each year. I have floated and fished the Shenandoah River in late spring and early summer, normally near Front Royal and Luray and downstream near the Shenandoah's confluence with the Potomac, for over 30 years. Over the past 5 years or so, the increased predictability of blooms of planktonic and filamentous algae throughout the Shenandoah River system has caused me to avoid the Shenandoah and either go further afield in Virginia or stick to Maryland waters. Excess algae makes river use unpleasant for me by making rocks excessively slippery, fouling lines and lures, and causing the river to look polluted and "dead." The now-routine occurrence of spring and early summer fish kills in the Shenandoah, which are apparently tied to algae blooms and nutrient enrichment, further inhibits my desire to travel to the Shenandoah.

The occurrence of algae blooms and fish kills on the Shenandoah, and using my valuable recreation time to travel to the Shenandoah only to have these environmental insults impinge on my recreation, significantly diminishes my use and enjoyment of that waterway.

The Shenandoah River system's worsening water quality now routinely makes the local and national news and is therefore common knowledge to fishermen and other river users throughout the mid-Atlantic.

The degradation of the Shenandoah's waters needs to be addressed by the Virginia DEQ as quickly as possible by adding the North Fork Shenandoah River, South Fork Shenandoah River, and main stem Shenandoah River to Virginia's 305(b)/303(d) list of impaired waters so that appropriate studies can be conducted and solutions can be found.

Thank you for your attention to this important issue.

B. Peter Yarrington
1809 Crystal Lane
Silver Spring, MD 20906
301-871-3120

Jeff Kelble <jeff@potomacriverkeeper.org>

Form submission from: Take Action!- Help the Shenandoah Riverkeeper Get Rid of Algae

Potomac Riverkeeper <sarah@potomacriverkeeper.org> Fri, Apr 13, 2012 at 6:06 PM
To: jeff@potomacriverkeeper.org

Submitted on Friday, April 13, 2012 - 6:06pm
Submitted by anonymous user: [107.4.147.5]
Submitted values are:

Your Letter:
April 13, 2012

John M. Kennedy DEQ Office of Water Monitoring and Assessment P.O. Box 1105 Richmond, Virginia 23218-1105

RE: Draft 2012 305(b)/303(d) Water Quality Assessment Integrated Report

Dear Mr. Kennedy,

I am writing to request that Virginia's Department of Environmental Quality add the North Fork, South Fork and Main Stem of the Shenandoah as impaired waters on thee 2012 305(b)3/303(d) Water Quality Assessment Integrated Report because of the severe algae problems the river experiences every year.
I am an avid fly fishing angler and have worked with the VDGIF and Jeff Kelble closely on various projects involving the health of the Shenandoah River. When I first moved the Harrisonburg, VA from Philadelphia and instantly noticed the quality difference in the water of the Shenandoah compared to the Delaware River where I grew up fishing.

The noxious algae blooms I have observed and come in contact with worry my deeply. As a graduate of EMU with a B.A in Environmental Sustainability I know the fact that pollution run off is harming our beautiful river. I have come to love the Shenandoah River even with all its problems and would love it even more if it was cleaner.

I spend the better part of a year working as an intern with The Shenandoah Riverkeeper trying to pin point the worst polluters of the fresh water systems in Rockingham Co. After working with the Riverkeeper I was more aware of all the problems we have with the river. Also, as a fanatic of small mouth bass fishing on a fly rod, it was hard to not blame the depletion of their species on the health of the river.

The algae diminishes both my use and my enjoyment of the Shenandoah River system. As a result I would like to ask that DEQ include the three sections of the Shenandoah River on 305B/303D list, determine why we are having these algae problems, and to develop and implement a plan to make them go away.

Thank you for considering my comments,

Sam Berenstain
531 E. Wolfe Street
Harrisonburg, VA 22801

Name: Sam Berenstain
Email: samberenstain05@gmail.com

The results of this submission may be viewed at:
http://www.potomacriverkeeper.org/node/428/submission/821

March 27, 2012

John M. Kennedy DEQ Office of Water Monitoring and Assessment P.O. Box 1105 Richmond, Virginia 23218-1105

RE: Draft 2012 305(b)/303(d) Water Quality Assessment Integrated Report

Dear Mr. Kennedy,

I am writing to request that Virginia's Department of Environmental Quality add the North Fork, South Fork and Main Stem of the Shenandoah as impaired waters on thee 2012 305(b)3/303(d) Water Quality Assessment Integrated Report because of the severe algae problems the river experiences every year.

I fish the South Fork and Main Stem of the Shenandoah extensively and in the past, I have used all three rivers to take my family canoeing and camping. However, for much of the year, large sections of the river appears to experience a series of noxious algae blooms that seriously diminish my use and enjoyment of the rivers.

In each of the past six years I have observed a late winter planktonic algae blooms. Over the course of the year I use the river system and have seen this on all three rivers from Lurey down to Front Royal on the South Fork, and from Route 50 to the Confluence with the Potomac River in Harpers Ferry. This bloom turns the river a dark murky green color, like green paint, from late winter until about July. When the algae blooms I often choose not to swim or fish and I don't take my family swimming because they don't like the off-color murk water. In fact, during the times the river gets real bad, I choose to go elsewhere to take my family. This represents an economic loss to the area, because I tend to spend money on gasoline, dinners, the occasional hotel, and do some of my home shopping in the boutique stores in the area.

By mid-May or June each year as the river clears the planktons seem to die back. But then a filamentous algae (rock snot) takes hold covers the river bottom and the native grasses which are trying to emerge. The native grasses turn brown and lose their leaves and remain bare the rest of the year. Additionally, a floating bubbly type of algae (I'm told its Cynabacteria or Blue Green Algae) comes at this time. This algae rises up and floats on the water surface, and then accumulate on anything sticking out of the river and in back eddies. This algae smells like sewage or rotting broccoli. When the algae blooms I literally do not want to be anywhere near the river and I often choose to go somewhere else. The fishing is frustrating because you cannot fish without fouling your line on the algae, the fish won't bite lures or bait with algae sticking to it. I find being around the river very unpleasant due to the odors and annoyance. I found this problem in the following areas last year from July through August; Front Royal, Luray, Alma, Port Republic, Bentonville, Millville, and from Route 50 down to Route7, then to Confluence with the Potomac River.

This cycle of algae diminishes both my use and my enjoyment of the Shenandoah River system but also the economy of the area. As a result I would like to ask that DEQ include the three sections of the Shenandoah River on 305B/303D list, determine why we are having these algae

problems, and to develop and implement a plan to make them go away. Thank you for considering my comments,

George “HookUp” Thurston
21079 Ethan Court
Sterling, VA 20164
Email: rd400d@hotmail.com
Phone: (202) 267-3545

April 21, 2012

John M. Kennedy
DEQ Office of Water Monitoring and Assessment
P.O. Box 1105
Richmond, Virginia 23218-1105
RE: Draft 2012 305(b)/303(d) Water Quality Assessment Integrated Report

Dear Mr. Kennedy,
I am writing to request that Virginia's Department of Environmental Quality add the North Fork, South Fork and Main Stem of the Shenandoah as impaired waters on the 2012 305(b)3/303(d) Water Quality Assessment Integrated Report because of the severe algae problems the river experiences every year.

I own property adjacent to the South Fork of the Shenandoah River in the Shenandoah/Shipwreck area about 1 mile downstream from the dam in Shenandoah. My family and I have enjoyed the fact that we live in such a beautiful area and have been able to enjoy the numerous opportunities the river has to offer. There has been a significant change in the river in the past years and the time we spend there has been far less enjoyable. In fact, we spend less and less time there and have been traveling to other areas to enjoy similar activities on other waters.

The main issue is an algae that has been dominating the river waters in recent years. When we first moved to the area, the river was prolific with numerous fish species of great size and the river bottom was dominated by native grasses and aquatic invertebrates. In addition, the river valley was well populated by a plethora of migratory and resident bird species and ducks. What we have noticed is a significant decline in the number, size and variety of all species mentioned above. After a significant fish kill several years ago, the smallmouth bass never recovered. There are still smaller fish around, but as they grow, sores develop and I find dead fish about 12-14 inches in length along the shore later in the summer. Rarely do I ever find them bigger than that. The numbers of minnows, darters, chubs, bluegill and suckers have also declined significantly. The native grasses that would form huge underwater forests in the shallows have all but disappeared. These areas were very productive feeding grounds for the fish as well as excellent retreats for breeding fish and a nursery for the juveniles to feed and hide. The area would also support numerous birds such as kingfishers, ducks of all kinds, tanangers, orioles, green herons, etc., that thrived on the grasses, fish or invertebrates produced in these vital areas. These birds are rarely seen here anymore and the diversity and enjoyment of seeing and studying them is all but gone.

What we have noticed is that late in the winter, very early spring, a green, slimy algae will form on the rocks and the water will turn a greenish color. It is variable based on the water flow in the river and tends to occur after huge rains when the water levels starts to fall. It will last for months and will be replaced later in the late spring and early summer with long green filaments of what has been called "rock snot" by my neighbors. The native grasses try to take hold but they are soon overwhelmed by this algae which sticks to the blades and smothers it out. Even in faster flowing waters, this algae is very sticky it will weigh the grass down and eventually it dies out. What is left behind is a slimy rock bed that is all but devoid of life except for the small black snails come to graze on the algae is unbelievable numbers. When you wade the shores and start flipping rocks to see what is left, there is not much at all. A few hearty inverts, but no crayfish, very few if any helgramites or large stonefly larvae, and just a small sample of tiny minnows. I have also noticed a decline in the number of frogs and snakes that frequent the shores, and as for

the beavers, we have not seen one in three years. It's no wonder why the diversity of life has moved or died off.

By late summer, when the water levels are low, we really don't even spend time at our section of the river. There is a funky green foam that occurs in the shallow, still waters and in the eddies. It has a rancid smell at times and to wade or swim is out of the question for my family. Even to paddle, it is not very enjoyable and when friends or family come to visit, they are shocked to see the water quality. We have been going to other waters far further upstream in the national forest or across the mountain into West Virginia to enjoy the experience we once had right here at home.

There is little doubt in my mind that the cause for this is the inorganic and organic chemical surplus that gets added to the water each year. The amount of nitrogen and phosphorous added to our beautiful watershed must be excessive but little if any monitoring is done to control it. There is also a huge trend in "no till' farming in our immediate area that is based on tremendous amounts of "round-up" or other herbicides being sprayed and ending up in our river. I do not have the facts to prove this which is why I am requesting that the DEQ include the three sections of the Shenandoah River on 305B/303D list to determine why we are having these algae problems and diversity loss and come up with a plan to protect this section of the river.

Sincerely,

Thomas A. Bahleda, M.D.
449 Shipyard Rd.
Shenandoah, VA 22849

Jeff Kelble <jeff@potomacriverkeeper.org>

my letter

icepeep@aol.com <icepeep@aol.com> Thu, Apr 5, 2012 at 9:30 AM
To: jeff@shenandoahriverkeeper.org

April 5, 2012
John M. Kennedy
DEQ Office of Water Monitoring and Assessment
P.O. Box 1105
Richmond, Virginia 23218-1105
RE: Draft 2012 305(b)/303(d) Water Quality Assessment Integrated Report

Dear Mr. Kennedy,
I am writing to request that Virginia's Department of Environmental Quality add the North Fork, South Fork and Main Stem of the Shenandoah as impaired waters on thee 2012 305(b)3/303(d) Water Quality Assessment Integrated Report because of the severe algae problems the river experiences every year.

I fish the North Fork, South Fork and Main Stem of the Shenandoah extensively and I also use all three rivers for canoeing, nature watching and nature photography. However, for much of the year, large sections of each river seems to experience a series of noxious algae blooms that completely destroy my use and enjoyment of the rivers.

In each of the past six years during late winter a planktonic algae blooms, usually after a high water event, but not always. Over the course of the year I use the entire river system and have seen this on all three rivers from Port Republic down to Front Royal, from Broadway to Front Royal and from Front Royal to the Confluence with the Potomac River. This bloom turns the river a dark murky green color, like green paint, from late winter until about July. When the algae blooms I often choose not to fish because fishing is smelly and slimy. When I do fish I find the fishing is poor and I don't enjoy the experience as much. Whenever the river is this murky color, it's disturbing to fish and the fish are usually lethargic and often they don't feed at all. Activity in the river drops to near zero. For the past two years I simply could not fish the lower south fork or the entire MainStem from July through August because of the plankton green water.

By mid May or June each year as the river clears the planktons seem to die back. But then a filamentous algae (rock snot) takes hold covers the river bottom and the native grasses which are trying to emerge. The native grasses turn brown and lose their leaves and remain bare the rest of the year. Additionally, a floating bubbly type of algae comes at this time. Clumps rise up to and float on the water surface and then accumulates on anything sticking out of the river and in back eddies. This algae smells like sewage. When these algaes are blooming I literally do not want to be anywhere near the river and I often choose to go somewhere else. When I have no other place to go then it just takes all the enjoyment out of my day. The fishing is frustrating because you cannot fish without fouling your line on the algae, the fish won't bite lures or bait with algae sticking to it. I find being around the river very unpleasant due to the odors and annoyance.This happens throughout the river system.

Finally, in the fall each year the native grasses die back and a bright green filamentous algae again covers the entire bottom of the river from Broadway to Strasburg. All three of these algaes make it nearly impossible to fish and again diminishes and sometimes eliminates my ability to enjoy the river.

This cycle of algae diminishes both my use and my enjoyment of the Shenandoah River system. As a result I would like to ask that DEQ include the three sections of the Shenandoah River on 305B/303D list, determine why we are having these algae problems, and to develop and implement a plan to make them go away.
Thank you for considering my comments,

Robert Abrams
6504 Byrnes Drive
McLean, Virginia 22101-5225

703 734 1238
icepeep@aol.com

BOYD W. POST
12158 Cathedral Drive
Lake Ridge, VA 22192

April 6, 2012

John M. Kennedy
DEQ Office of Water Monitoring and Assessment
P. O. Box 1105
Richmond, VA 23218-1105

RE: Draft 2012 305(b)/303(d) Water Quality Assessment Integrated Report

Dear Mr. Kennedy,

This is a request that the Virginia Department of Environmental Quality to add all of the Shenandoah River (North Fork, South Fork, and Main Stem) as impaired waters on the 2012 305(b)/303(d) Water Quality Assessment Integrated Report because severe algae problems have occurred over the past several years.

I frequently canoe and fish the above mentioned streams. Very often I find the river so greatly affected by nasty algal blooms that my enjoyment of being on the river is severely impacted and diminished. It is really disgusting to experience retrieving a lure covered with rock snot and have to clean it off. The thought of what that is doing to the fishery is frankly depressing. At other times I experience floating clumps of apparently rotting algae that smell like sewage. This is even more disgusting than the rock snot from the bottom rocks.

I believe the State of Virginia has the responsibility to correct these problems so we citizens can again enjoy this very wonderful resource without the vexing algae problems. Therefore I am asking that DEQ include these mentioned parts of the Shenandoah River in the 305(b)/303(d) list so that a solution to these algae problems can be achieved – the sooner, the better!

Thank you for your consideration of my request.

Sincerely,

Boyd W. Post

Dr. Boyd W. Post
Email: boydpost28@comcast.net
Telephone: 571 408-4227

April 5, 2012

John M. Kennedy
DEQ Office of Water Monitoring and Assessment
P.O. Box 1105
Richmond, Virginia 23218-1105
RE: Draft 2012 305(b)/303(d) Water Quality Assessment Integrated Report

Dear Mr. Kennedy,
I am writing to request that Virginia's Department of Environmental Quality add the North Fork, South Fork and Main Stem of the Shenandoah as impaired waters on thee 2012 305(b)3/303(d) Water Quality Assessment Integrated Report because of the severe algae problems the river experiences every year.

I fish the North Fork, and Main Stem of the Shenandoah extensively during the summer months for more than 40 years. Recently, however, for much of the summer, particularly the warmer months, large sections of the river experience a series of noxious algae blooms that seriously diminish my use and enjoyment of the rivers.

In each of the past six years during late winter a planktonic algae blooms, usually after a high water event, but not always. This bloom turns the river a dark murky green color, like green paint, from late winter until about July. When the algae blooms I often choose not to fish as I don't like the off-color murky water. When I do fish I find the fishing is poor and I don't enjoy the experience as much. Whenever the river is this murky color, it's disturbing to fish and the fish are usually lethargic and often they don't feed at all. Activity in the river drops to near zero. For the past two years I simply could not fish the lower south fork or the entire Main Stem from July through August because of the plankton green water.

By mid May or June each year as the river clears the planktons seem to die back. But then a filamentous algae (rock snot) takes hold covers the river bottom and the native grasses which are trying to emerge. The native grasses turn brown and lose their leaves and remain bare the rest of the year. Additionally, a floating bubbly type of algae (I'm told its Cynabacteria or Blue Green Algae) comes at this time. Clumps rise up to and float on the water surface and then accumulates on anything sticking out of the river and in back eddies. This algae smells like sewage or rotting broccoli. When these algaes are blooming I literally do not want to be anywhere near the river and I choose to go somewhere else. I have found that if you do fish; the fishing is frustrating because you cannot fish without fouling your line on the algae, the fish won't bite lures or bait with algae sticking to it. I find being around the river very unpleasant due to the odors and annoyance. I found this problem in the following areas last year from July through August;, Deer Rapids, Edinburg, Woodstock, Strasburg, Front Royal, Goods Mill, Shenandoah, Route 50, Route7 among other places.

Finally, in the fall each year the native grasses die back and a bright green filamentous algae again covers the entire bottom of the river from Broadway to Strasburg. All three of these algaes make it nearly impossible to fish and again diminishes and many times eliminates my ability to enjoy the river.

This cycle of algae diminishes both my use and my enjoyment of the Shenandoah River system. As a result I would like to ask that DEQ include the three sections of the Shenandoah River on 305B/303D list, determine why we are having these algae problems, and to develop and implement a plan to make them go away.
Thank you for considering my comments,

Carl Onesty 703 978-4684 conesty@aol.com
8529 Pappas Way
Annandale, VA 22003

Shenandoah River Algae Complaint

April 12, 2012
John M. Kennedy
DEQ Office of Water Monitoring and Assessment
P.O. Box 1105
Richmond, Virginia 23218-1105
RE: Draft 2012 305(b)/303(d) Water Quality Assessment Integrated Report

Dear Mr. Kennedy,
I am writing to PLEADING Virginia's Department of Environmental Quality add the North Fork, South Fork and Main Stem of the Shenandoah as impaired waters on thee 2012 305(b)3/303(d) Water Quality Assessment Integrated Report because of the severe algae problems the river experiences every year.

I am the owner and one of the head fishing guides for Mossy Creek Fly Fishing in Harrisonburg, Virginia. My twin brother Brian and I opened our shop and guide service in April of 2003. The NUMBER 1 reason for opening our shop in this location was its proximity to the South Fork of the Shenandoah River. We have been fishing the Shenandoah River for 20 years and guiding on it for 12. We are on the river 12 months a year and almost daily in the spring and summer. We have 6 boats and run more float fishing trips on this river than any other fishing guide service. We do target the James River and New River for smallmouth bass and musky, however we consider the Shenandoah River our home water. Unfortunately claiming the Shenandoah our home water is becoming more and more embarrassing each year as we see constant algae blooms, fish kills, disease, foul smelling water, and more. Some of our guests will not return to fish the Shenandoah or our area again as a result of what they see on the water.

I am amazed at what I see each day and each season I am on the Shenandoah River. I actually grew up in Richmond Virginia about 1 mile from the James River. I was used to seeing crystal clear water through the spring, summer, and fall when of course the river wasn't flooding. Despite water temp and flow the James always looked clean and clear. That also holds true for the middle and upper James River where we now guide and fish. The James, New, Rappahannock, Potomac, and many of their large tributary streams run clean and clear most of the year. I can say the same for possibly 1major South Fork Shenandoah tributary. The upper South River near Waynesboro seems fairly free of algae most of the year. The North River and Middle River, both major tributaries to the South Fork of the Shenandoah, run green most of the year. I have actually never witnessed a day in my lifetime when the Middle River near Verona, VA has ever not been green. I guess the first time I really started to understand what was wrong with the river was back in 2005 when the first major fish kill occurred. I took pictures that spring, March, April, and May. I noticed that the water actually looked like it belonged in a theme park like King's Dominion rather than in a natural environment. The water was actually a leafy green color and you couldn't see more than about 2 feet into it on a good day. Not only was the color horrendous but the river had a very strong odor of ammonia. So strong some days it would make my eyes and the eyes of my fishing clients water most of the day! Other issues occurred later in the summer time when clumps of slimy algae would detach from the river bottom and rise up to the surface. You can't even cast a line without getting your gear covered in this slimy, liquidy, brown gunk. That same gunk covers much of the river bottom nowadays. This slimy brownish algae is something I have never seen before until the past few years. Now, I remember 20 years ago seeing the river a "Margarita Green", a color term coined by all the local fishermen and fishing guides, on occasion but not all year. This past season from 2011-2012 our guides were on the Shenandoah multiple times each week throughout the year. Even in December, January, and February, when most rivers are devoid of plant life and algae, the Shenandoah River had no more than 2 feet of visibility ALL WINTER! What is even more frustrating is that the Main Stem Shenandoah from Front Royal North to Harper's Ferry was so discolored all The upper James River, virtually the same size as the South Fork of the Shenandoah was running crystal clear as was the New River, Rappahannock, Potomac, Staunton, and

more. I have photos of the river from EVERY month over the past 10 years I have been in business and it is difficult to find 1 photo where the Shenandoah River actually looks clean or clear.

Our busy season on the Shenandoah River is usually from May into September. You can expect to see one of our boats on the South Fork or Main Stem Shenandoah at a minimum of 5 days a week through this time period. We see the same algae problems on the river from Port Republic all the way into West Virginia! Clients are always asking us, 'did it rain recently?' 'Why is the river so dirty?' . Again, the embarrassment sets in as we explain the nutrient loading into the river and the algae problems that exist year round! Clients ask us constantly if the water is safe to swim in or even touch. I recommend they do not swim or even touch the water. I have my clients enter the boat at a boat ramp and exit at a boat ramp. At no time during the day do I allow them out of the boat. If they ask to swim I quote what DEQ officials told me at the first Fish Kill Task Force meeting….."Do not enter the Shenandoah River with open cuts or sores and NEVER fully submerge your head in the Shenandoah River". I used this same quote in a discussion with the General Assembly in Richmond back in 2006 concerning the state of the Shenandoah River. So does this algae problem make me less likely to use the Shenandoah River for recreation? Yes. Unfortunately my business depends on our guide services for me to make a living and support my family. So rather than use our home water, 15 miles down the road from our shop, we are fishing more and more on the James River, over 2 hours away. This 4 hour round trip haul costs us about $80 in fuel per day rather than the $15 it costs us to fish locally. However our customers are much happier and feel safer fishing a river that doesn't look as sick as the Shenandoah.

The current state of the Shenandoah River has diminished my enjoyment of the river. My shop staff and guides love this river and we spend time with VDGIF and DEQ volunteering our efforts to help figure out these problems and issues. However watching fish die as a result of unhealthy water and watching anglers become frustrated as they constantly battle with algae on their lines or catching and handling diseased or dying fish is getting too much to handle on a daily basis. I have been to the doctor twice and my brother once in the past 3 years with bacterial infections we were told were derived from a water source. The most likely culprit was the Shenandoah River as that was the only body of water we were on throughout the incubation time of our ailments. These infections are obviously not a result of algae in the river, but a direct result of an unhealthy waterway. Back in the 90's articles were written about how the Shenandoah River was one of the top rivers to fish for smallmouth bass in the world! I based my business model around this river system and its history of being such a quality fishery. How did we go from a world recognized fishery to a waterway that is unhealthy to even enter? It not only has diminished my enjoyment of the river but has diminished my business and livelihood.

I am now asking that DEQ include the three sections of the Shenandoah River on 305B/303D list, determine why we are having these algae problems, and develop and implement a plan to make them go away. I currently do not see major issues on our other large river systems like the James, New, or Rappahannock. This is a Shenandoah River problem. This River is the life of the Shenandoah Valley and it is slowly dying before our eyes. Action should have been taken years ago but we are begging that something be done NOW! Thank you for considering my comments,

Colby Trow
Owner Mossy Creek Fly Fishing
Harrisonburg, VA 22801
Email: store@mossycreekflyfishing.com
Phone: 540-434-2444

Some Planktonic Algae & Floating Scum Examples all Taken Before/During 2005 Fish Kills:

Jeff Kelble <jeff@potomacriverkeeper.org>

shenandoah river algae

Dylan Cooper <deerslayer88@embarqmail.com> Sun, Apr 15, 2012 at 7:12 PM
To: jeff@shenandoahriverkeeper.org

Dear Mr. Kelble,

I am voicing to you my dear concern about the health of the Shenandoah River, particularly about the problem of algae.

I am a lifelong user of the Shenandoah River, mostly in the South Fork because I live in Luray, VA but I've covered almost all parts of the Shenandoah River system. During the spring and summer, I fish it, canoe it, and swim or wade in it, and I also hunt it in the fall and winter. For years I have noticed the increasing problem of algae in the Shenandoah and I would like something to be done about it.

My biggest problem is that with a fishing lure that goes anywhere near the bottom, and sometimes on top of the water as well, I must take green and/or brown algae off of the lure after almost every single cast. This not only becomes annoying, but it slows up fishing and decreases my chances of catching fish. No fish will think a lure covered in algae is something to eat. The algae gets tangled in my line and ends up fouling up my fishing reels or rod guides.

The algae also stains the water a dark green color. With water that is not clear, it's impossible to fish by sight.

I also like to catch live bait in the river to use for fishing. Brown-snot-covered, slippery rocks are not the best habitat for the macroinvertbrate I am looking for, nor is it any good for footing.

Overall the algae has diminished my enjoyment of fishing my favorite fishing water and summer paradise in very many ways.

Other problems I notice while boating is that the algae gets stuck on the anchor and then it gets carried into the boat to dry out and stink up my whole boating trip. Also, anything sticking out of the water has algae on it that has floated to the surface during certain times of the year and that stuff really stinks up my river trip.

Sometimes I will get out of the boat to wade or swim (when I slip off the brown-snot-covered, slippery rocks) and the algae gets stuck on my shoes or clothes. This algae can stay on my shoes even after I try to pick it off, especially if the shoes have Velcro or mesh on them. Then once my shoes dry, they stink for days! And don't try to sit down on the bottom of the river unless you want to ruin your bottoms with brown stains from algae.

I also experience problems with the algae when I waterfowl hunt on the river in fall or late winter. I wear chest waders to go out in the icy cold water and the brown-snot-covered, slippery rocks are once again dangerous to walk on. I've been in many situations where I barely kept my footing on the algae-covered bottom to prevent myself from falling in the hypothermia-inducing water. And now because of the algae being spread to different river systems (that's a whole different problem), felt bottomed shoes and waders are not allowed. So there goes any extra chance of holding one's footing.

I have a Labrador retriever that absolutely loves the water. He'll go in the river all year long to swim and drink. Many times, he'll stink afterwards from getting algae in his fur. I always have to give him a bath after taking him to the river. Sometimes he'll also throw up from drinking the river water.

As you can tell, I have many different problems with the algae in the Shenandoah River. The algae severely diminishes both my use and my enjoyment of the Shenandoah River system. As a result, I would like to ask that DEQ include the three sections of the Shenandoah River on 305B/303D list, determine why we are having these algae problems, and to develop and implement a plan to clean up our river and to make the algae go away!

Thank you very much for allowing my input and for sticking up for our river,

Dylan Cooper

Virginia Tech Biological Systems Engineering Major

306 Winkler Rd

Luray, VA 22835

Email: deerslayer88@embarqmail.com

Phone: 540-742-3273

April 12, 2012

John M. Kennedy
DEQ Office of Water Monitoring and Assessment
P.O. Box 1105
Richmond, Virginia 23218-1105

Dear Mr. Kennedy:

I have lead the Northern Virginia Chapter of Trout Unlimited (a TU chapter with over 800 members) on trips to local streams for over 10 years. Until the last two years, I had always taken groups to the N. Fork of the Shenandoah during the summer to fish for smallmouth bass. The current high nutrient content of the river creates oxygen depleting algae blooms, and causes fish kills and sickens bass. Consequently I have been cancelling trips pending improvement of the condition of the river.

I ask that VA DEQ include the North Fork, South Fork and/or Main Stem on its impaired waters 303D/305B list. Thank you.

Sincerely,
George Paine
Secretary
N. Virginia Chapter
Trout Unlimited

John M. Kennedy
DEQ Office of Water Monitoring and Assessment
P.O. Box 1105
Richmond, Virginia 23218-1105
RE: Draft 2012 305(b)/303(d) Water Quality Assessment Integrated Report

Hello,

I run a local non-profit that removes physical pollution from the river (tires,cars, culverts,stoves, etc,) and I am also a life long fisherman and kayaker of the Shenandoah River. I use the Shenandoah many times a week pretty much year round. I have observed a mass problem with the content of algae growth and contamination over the course of time and I have also noticed that it gets increasingly worst as the year goes on then reappears thicker the next year. I spend most of my time on the North fork of the Shenandoah from New Market to Front Royal, but I have also noticed this issue on the South Fork as well. This seems to start in about May and seems to worsten untill the river is unbearable to use around July. I don't know how much time to spend on the river, but I spend quite a bit with many people and this is a universal problem that seems to be the tip of everyones discussion when it comes to outdoors recreation at these times in the year. The algae is so thick in spots that it becomes a solid mass at the top of the water and one could hardly get a canoe to glide across it. I assure you that most of the public that uses the Shenandoah for whatever they may recognizes this as an issue if not only an eye sore but also a safety issue due to lack of visibility (and trust me there are plenty of sharp man made things of the bottom of the river to cut your self on) and you should do the same and include this on your impaired waters 303D/305B list. Recognize the problem and please do something about it. This stuff isn't going to get rid of itself.

Beau Morgan
President Earth Korps Inc.
93 Short Mountain Road.
Edinburg VA 22824
540-335-8144
earthkorps@yahoo.com
www.earthkorps.org

1423 N. GREENBRIER ST. ARLINGTON, VA 22205 ▪ 703-534-4543 ▪ JAYEICHE@HOTMAIL.COM

April 18, 2012

John M. Kennedy
DEQ Office of Water Monitoring and Assessment
P.O. Box 1105
Richmond, Virginia 23218-1105

Dear Mr. Kennedy,

RE: Draft 2012 305(b)/303(d) Water Quality Assessment Integrated Report

I am writing to request that Virginia's Department of Environmental Quality add the North Fork, South Fork and Main Stem of the Shenandoah as impaired waters on the 2012 305(b)3/303(d) Water Quality Assessment Integrated Report because of severe algae problems the river experiences every year.

I own over 500 ft of riverfront property on the North Fork of the Shenandoah near Maurertown, Virginia. I frequently fish and float the river upstream and downstream of my property, but also have paddled most of the North Fork from Broadway to Front Royal, the South Forth from above Luray to Front Royal, and the Main stem Shenandoah River from Front Royal into West Virginia. I have fished most of the mid Atlantic rivers for the past 20 years, and unfortunately have seen a marked increase in noxious algae blooms and fish kills during that time period.

Specifically, in each of the past six years during late winter there are algae blooms which typically occur after high water events most likely caused excessive nutrient runoff. This bloom will turn parts of the river a dark murky green color with strands of gook the consistency of paint until about mid-summer. When the algae blooms, I generally don't swim or fish, nor do I recommend our guests and friends do the same as I have health concerns with the off-color murky water. When I do fish, I find the fishing is poor and take precautions upon returning home such as cleaning any scrapes or wounds with hydrogen peroxide and using antibiotic ointment. We also discourage pets and small children from entering the water at all.

By mid-summer each year, the river clears as the planktons seem to die back. But then a filamentous algae (rock snot) completely takes over and covers the river bottom which kills the native submerged aquatic vegetation trying to emerge. The native grasses turn brown, lose their leaves, and remain bare the rest of the year. Additionally, a floating bubbly type of algae (probably Cynabacteria or Blue Green Algae) comes at this time. Clumps of it often cover the water surface and accumulate on anything sticking out of the river, as well as in back eddies. This algae smells like sewage. When these algaes are blooming I literally do not want to be anywhere near the river. The fishing is frustrating because you cannot cast without fouling your line on the algae, the fish won't bite, and I find being around the river very unpleasant due to the odors and annoyance.

This cycle of algae diminishes both my use and my enjoyment of the Shenandoah River system. We caution our guests regarding the use of the river during the summer months, and feel it is responsible in

part for a decrease in tourism in the Shenandoah Valley, and has negatively impacted property values. As a result I would like to ask that DEQ include all three sections of the Shenandoah River on 305B/303D list, determine why we are having these algae problems, and to develop and implement a plan to improve water conditions and restore the river to a world class smallmouth bass fishery and national treasure.

Thank you for considering my comments, and please feel free to contact should you have additional questions.

Very Respectfully,

Jay Eiche

Jeff Kelble <jeff@potomacriverkeeper.org>

Letter writing

James Nashed <jamesnashed@hotmail.com> Tue, Apr 17, 2012 at 5:00 PM
To: Jeff Kelbe <jeff@shenandoahriverkeeper.org>

John M. Kennedy
DEQ Office of Water Monitering and Assessment
PO Box 405
Richmond, VA 22620

Dear Mr. Kennedy,

I am writing this letter as a concerned citizan of the state of Virginia and a person who enjoys the recreational use of the Shenandoah river. I use the river extensively throughout the spring, summer, and fall. mostly for fishing but also picnics and wading. I have been extremely alarmed at the amount of algae blooms over the past few years on differnet sections of the river. I have seen them extensively on the North Fork and South Fork, but also along the main stem where I fish a great deal.

Unfortunately this has impacted my ability to fish and certainly wade and swim safely in parts of the river. I find this especially bad in the summer on the North Fork south of Strasburg which I tend to avoid fully. But lately the parts of the South Fork from Luray northward and the main stem from Front Royal to the route 7 bridge also seem greatly affected. The algae and slime sometimes makes fishing a near impossibility due to the poor visibility and inability to keep lines and flies clear of algae. Myself and my family also are concerned enough to not swim or wade where large blooms are present due to health concerns. I have 2 small children who we also prohibit from swimming when blooms are present. This is a tragedy. The river and the surrounding land are some of the most beautiful in the state. I end up traveling to West Virginia or Maryland in search of fishable waters and recreation even with such an amazing river as the Shenandoah is so close to home.

The kinds of algae seem to be many. The floating algae seen in calmer waters in the late summer has a strong odor and colors the entire area a pudrid yellow-green color. The rocks which are already slick in the summer become impossible to walk on even with rubber and metal studded wading boots because on an immense amount of green "rock snot" that encompasses everything. Fishing under these circumstances is both impractical and unenjoyable. Of course no one in my family would swim or wade with this and instead we save our swimming for the beach trips in outer banks of North Carolina or the lakes and streams of Western Maryland. My enjoyment and use of the river the past few summers has been dimished significantly to be sure.

I love the Shenandoah river and wish the algae could be controlled. I urge the VA DEQ to include the North Fork, the South Fork, and the Main stem of the Shenandoah on the list of impared waters 303D/305B.

Sincerely

James Nashed MD
118 Woodfield Lane
Winchester, VA 22602
jamesnashed@hotmail.com

Date: Tue, 17 Apr 2012 11:57:38 -0400
Subject: Re: Letter writing
From: jeff@shenandoahriverkeeper.org
To: jamesnashed@hotmail.com
[Quoted text hidden]

April 15, 2012

John M. Kennedy
DEQ Office of Water Monitoring and Assessment
P.O. Box 1105
Richmond, Virginia 23218-1105

RE: Draft 2012 305(b)/303(d) Water Quality Assessment Integrated Report

Mr. Kennedy
I own a cottage on the banks of the North Fork of the Shenandoah River at 286 Ridgeley Road in Woodstock, Virginia. The reason I have a place on the river is that I am an avid canoeist and fisherman. I regularly wade and fish the half mile of the river in front of my property and float and fish the 5 miles that are up river, from the Pugh's Run intersection with the North Fork at the Artz Road Bridge to my house, between March and October. I use the river for my enjoyment several times a week on average during these periods. Excessive algae in this part of the river is diminishing my use and enjoyment of the river in a significant way and I ask that this area be designated impaired, and that the state take action to reduce the nutrient discharges that are causing this impairment.

For a number of years now, I have noticed excessive and increasing algae growth and accumulation in the river in the summer and early fall. The algae is a major impediment to my enjoyment of the river. In particular, as the algae accumulates, it makes fishing difficult and then impossible. The algae floating on the top interferes with casting and the algae on the bottom and on the grasses, fouls the lure, which renders it ineffective. As a result, I have to give up fishing much of the river during the months of August and September.

In addition, as this algae accumulates, it appears to rot and gives off a nauseating odor…similar to sewage. When this happens I simply cannot enjoy canoeing or fishing this stretch of the river.
A big part of my enjoyment of the river, is having friends and family out for the weekend. When the river is clogged with algae, I end up cancelling family visits and cannot share the river with my friends. I have tried using other parts of the river when this condition exists and find the same thing from my house twenty miles up river and five down river

This algae impairment of the river is significant. I have expected the state and local authorities to remedy it but nothing has happened. I understand that one step in the process is to have this part of the river designated as impaired under 303D and 305B of the Clean Water Act. Please complete the designation as soon as possible.

Sincerely,

John Holmes
286 Ridgeley Road
Woodstock VA 22664

April 10, 2012
John M. Kennedy
DEQ Office of Water Monitoring and Assessment
P.O. Box 1105
Richmond, Virginia 23218-1105
RE: Draft 2012 305(b)/303(d) Water Quality Assessment Integrated Report

Dear Mr. Kennedy,

I am writing to request that Virginia's Department of Environmental Quality add the Main Stem of the Shenandoah as impaired waters on thee 2012 305(b)3/303(d) Water Quality Assessment Integrated Report because of the severe algae problems the river experiences every year.

I own property on Main Stem of the Shenandoah River near Rt. 7. My family and I swim, paddle, float and fish the river mostly in our vicinity (from Rt. 50 to the WV line). During the summer one unfortunately has to check first for the presence of green algae clumps to determine if the river experience will be worth pursuing. These clumps smell terrible and are a strong indicator for my family and me to avoid recreating on or in the river.

The seemingly annual growth of algae is a disappointment and diminishes both my use and my enjoyment of the Shenandoah River. As a result I would like to ask that DEQ include the Shenandoah River on 305B/303D list, determine why we are having these algae problems, and to develop and implement a remediation plan.

Thank you for your consideration,

Jonathan M. Turkel
96 Kent Farm Lane
Bluemont, VA 20135
Email: jmturkel@gmail.com
Phone: (540) 955-0305

April 2, 2012

John M. Kennedy
DEQ Office of Water Monitoring Assessment
P.O. Box 1105
Richmond, Virginia 23218-1105
RE: Draft 2012 305(b)/303(d)Quality Assessment Integrated Report

Dear Mr. Kennedy,

I am writing to you to request the Virginia Department Of Environmental Quality add the North and South Forks as well as the main stem of the Shenandoah River as impaired waters on the 2012 305(b)/303(d) Water Quality Assessment Integrated Report. The reason is the severe algae problems the river experiences every year.

I and friends of mine as well as our families visit the river a number of times a year to enjoy fishing, wading and picnics. We have found that often the algae problems render the river practically unusable not to mention the disgusting odor at certain times. I am aware that this has been approached before in the past and the state has turned a blind eye to it. I find that to be a poor way of treating one of Virginia's great rivers that has been such an important part of the history of the state as well as this part of the country. I fear that we as a people have fallen short of being good stewards of the many wonders of nature that we are blessed with. It is said that the native American Indians often spoke of how the white man would eventually mess up his environment. Indians even today look upon nature as gifts from the creator and as such are to be taken care of. I feel that if the situation is not addressed many people who use and love this beautiful river will find this algae so unpleasant as to be deprived of its use and enjoyment. I love to fish the river but after reeling in your line covered with smelly slime time after time really discourages me from fishing there. You are never sure when these algae blooms are taking place so instead of traveling to the Shenandoah River we would reluctantly choose some other water. Many people enjoy kayaking including myself because it affords you the opportunity to fish as well as do some wading. Of course this type of activity is not possible during the algae blooms. To sum up I find the algae bloom situation renders the Shenandoah River less than an ideal place to enjoy my summer and fall favorite past times.

I would like to ask the DEQ to investigate this situation and if possible find and put into action a plan to remedy this problem. Thank you for considering my remarks.

Lawrence DiJoseph
6715 Moly Drive
Falls Church, Va 22046
Email: dijosephjr@gmail.com
Phone: (703) 241-2393

April 12, 2012

Leslie D. Mitchell
P. O. Box 675
Woodstock, VA 22664

John M. Kennedy
DEQ Office of Water Monitoring and Assessment
P.O. Box 1105
Richmond, Virginia 23218-1105

RE: Draft 2012 305(b)/303(d) Water Quality Assessment Integrated Report

Dear Mr. Kennedy,

I am writing to request that Virginia's Department of Environmental Quality add the North Fork, South Fork and Main Stem of the Shenandoah as impaired waters on thee 2012 305(b)3/303(d) Water Quality Assessment Integrated Report because of the severe algae problems the river experiences every year.

I am a volunteer water monitor for Friends of the North Fork of the Shenandoah River, so I observe the portions of the North Fork near Strasburg on a bimonthly basis. As director of Friends of the North Fork of the Shenandoah River I am also alerted by the public to algae blooms that occur on an annual basis. I paddle the river and also simply enjoy viewing its splendor as well. However, for much of the year, the North Fork seems to be experiencing a series of noxious algae blooms that impair my use and enjoyment of the river.

In recent years, I have noticed or been alerted to numerous and different types of algae blooms and observed thick algae growth on the river's surface and below the surface. The blooms I have observed have been in the summer and early fall of the year. Please see photos below of an algae blooms: 1) June 22, 2010 between about a mile downstream of Deer Rapids Bridge; 2) Same location and date; 3) and 4) Bloom that occurred in the North Fork between Deer Rapids south of Strasburg and the Rt. 55 Bridge across the North Fork, northeast of Strasburg in July of 2011. The algae smells bad, is difficult to paddle through and creates an unpleasant recreational experience in general, especially as it causes one to wonder what it is that is causing these imbalances in the water, allowing this unusual algae growth to occur

The presence of these algae diminishes both my use and my enjoyment of the Shenandoah River system. As a result I would like to ask that DEQ include the three sections of the Shenandoah River on 305B/303D list, determine why we are having these algae problems, and to develop and implement a plan to avoid these blooms in the future.

Thank you for considering my comments,

Ms. Leslie D. Mitchell

P. O. Box 675
Woodstock VA 22664
Email: ldmwtaa@yahoo.com
Phone: 540-459-8457

Photos of Algae:

April 20, 2012

Mr. John M. Kennedy
DEQ Office of Water Monitoring and Assessment
PO Box 1105
Richmond, Virginia 23218-1105

RE: Draft 2012 30(b)/303(d) Water Quality Assessment Integrated Report

Dear Mr. Kennedy:

I am writing to request that the Virginia Department of Environmental Quality add the Shenandoah River system as impaired waters to the 2012 305(b)3/303(d) Water Quality Assessment Integrated Report because of severe and growing algae growth and blooms.

My husband and I have had a riverfront home on the North Fork outside of Woodstock since 1991. Over the years, I have made considerable use of the North Fork for fishing, canoeing and kayaking. I have also collected water samples and benthic data to support the Citizen's Water Quality Monitoring program through the Friends of the North Fork of the Shenandoah River. I have been saddened to observed considerable changes in water quality over the years.

The visible issue is primarily an increase in attached aquatic vegetation. During the dry summer months, algae also increases dramatically to the point that it covers and clogs grass beds, sometimes making it difficult to even kayak or canoe over it. In recent summers, we have consistently observed rooted filamentous brown algae coating rocks on the river bottom. By late summer, large masses of stinky brown slime accumulate on the surface, detracting considerably from our enjoyment of the river. There seems to be little doubt that the health of the North Fork of the Shenandoah River is deteriorating.

I encourage DEQ to include the Shenandoah River system in its 2012 Water Quality Assessment Report.

Sincerely,
Mary Gessner
829 Hickory lane
Woodstock, VA 22664

Jeff Kelble <jeff@potomacriverkeeper.org>

algae

coolmtnman@verizon.net <coolmtnman@verizon.net> Wed, Apr 11, 2012 at 9:07 PM
To: jeff@shenandoahriverkeeper.org

1. I like to use the Shenandoah River for kayaking nearly every weekend. I have kayaked nearly every section of the Shenandoah river in the past 3 or 4 years. I'm also an avid bird watcher and take my binoculars with me on every trip.

2 In areas where ducks congregate which tends to be still water areas, I've noticed algae underwater and floating. It seems to be more prevalent during the very warm months when the ducks are raising their young.

3 I remember one day when I became ill after floating past the sewage treatment plant in Mt Crawford. I'm very healthy and it's extremely rare that I would become ill during the summer months.

4. Becoming ill has diminished my use of the river in this area.

5. I request that the VA DEQ include the North Fork,South Fork and Main Stem on its imparied waters 303D/305B list

6. Orv Lehman 5947 Harpine Hwy Linville Va 22834 540-560-4847

April 14, 2012

John M. Kennedy
DEQ Office of Water Monitoring and Assessment
P.O. Box 1105
Richmond, Virginia 23218-1105
RE: Draft 2012 305(b)/303(d) Water Quality Assessment Integrated Report

Dear Mr. Kennedy,

I am writing to request that Virginia's Department of Environmental Quality add the North Fork, South Fork and Main Stem of the Shenandoah as impaired waters on thee 2012 305(b)3/303(d) Water Quality Assessment Integrated Report because of the severe algae problems the river experiences every year.

I own property on the South Fork of the Shenandoah near Front Royal. My family and I enjoy the river upstream and downstream of my property, most frequently in areas between the Guest Shenandoah River State Park and the Front Royal Luray Avenue boat landings. For each of the past eight years, we have used the river approximately 4 to 8 days per month between May and September to kayak, tube, swim or fish. We also picnic near the river and exercise our dogs along our riverfront.

While we hope to enjoy the peace and serenity of my land, for much of the year the South Fork seems to be experiencing a series of noxious algae blooms. In each of the past six years we have seen the river turn a dark murky green color, like green paint, from late winter or early spring until about mid-summer. These planktonic algae blooms negatively impact our enjoyment and use of the river, because we do not like to swim or fish in the murky water, and do so less frequently when we see them. These blooms seem to follow high water events, but not always.

By mid May or June each year as the river clears the planktons seem to die back. But then a filamentous algae (rock snot) takes hold covers the river bottom and the native grasses which are trying to emerge. The native grasses turn brown and lose their leaves and remain bare the rest of the year.

Additionally, a floating bubbly type of algae (Cynabacteria or Blue Green Algae) comes at this time. Brown-green clumps fill the water surface and accumulate on anything sticking out of the river. This algae smells like sewage or rotting broccoli. When these algaes are blooming we do not want to be anywhere near the river. An experience we had with one of these blooms in late June 2011 left such a negative impression on us that we did not tube or kayak for months afterward.

The cycle of algae diminishes both my use and my enjoyment of the Shenandoah River system. As a result I would like to ask that DEQ include the three sections of the Shenandoah River on 305B/303D list, determine why we are having these algae problems, and to develop and implement a plan to keep the river system clear of them.

Thank you for considering my comments,

Stuart C. Harding
1325 Stoney Bottom Rd.
Front Royal, VA 22630
Email: stuart_c_harding@yahoo.com
Home: 540-636-9721
Cell: 540-683-1801

LTC Terrence E. Cooney, USA (RET)
7514 Rambling Ridge Drive
Fairfax Station, VA 22039

30 March 2012

John M. Kennedy
DEQ Office of Water Monitoring and Assessment
P.O. Box 1105
Richmond, VA 23218-1105

Subject: Draft 2012 305(b)/303(d) Water Quality Assessment Integrated Report

Dear Mr. Kennedy,

My wife and I retired from the military in the Old Dominion over 20 years ago. I used to be a very frequent visitor to the Shenandoah River, both the forks and the main stem. I have enjoyed Andy Guest State Park. We enjoyed romantic stays at the local valley B&Bs, while canoeing the river and picnicking on its banks. I now fish and float elsewhere, and we enjoy B&Bs elsewhere. The Shenandoah, the pride of the valley, has become a *cesspool.* The Shenandoah River featured in numerous "Virginia is for Lovers" ads, and books and movies, synonymous with tales of the Civil War, is now a filthy breeding ground for algae – the green slime.

This is not merely an issue of "green think" or "tree hugging;" the Commonwealth is *seriously* in danger of losing one of its premier natural beauties … and sources of tax revenue. That loss translates to more taxes for me to offset loss of tourism dollars. *Enough.* Please take the appropriate steps to include the three sections of the Shenandoah River on the 305B/303D list. Find out what the algae problem is and fix it.

It is unconscionable that citizens of the Commonwealth should even have to write such a request to our Government. All our native Virginia American President's have known and loved this river. Will our generation be the one to abandon it?

I am Very Respectfully Sir,

Terrence Eugene Cooney

LTC Terrence Eugene Cooney, USA (RET)

Copy: Governor Bob McDonnell, Office of the Governor
Patrick Henry Building, 3rd Floor, 1111 East Broad Street, Richmond, Virginia 23219

Jeff Kelble <jeff@potomacriverkeeper.org>

A letter

Trace Noel <noeltrace@gmail.com> Thu, Apr 19, 2012 at 7:23 PM
To: Jeff Kelble <jeff@shenandoahriverkeeper.org>

April 19, 2012

John M. Kennedy
DEQ Office of Water Monitoring and Assessment
P.O. Box 1105
Richmond, Virginia 23218-1105

RE: Draft 2012 305(b)/303(d) Water Quality Assessment Integrated Report

Dear Sir,

I write to loudly protest the lack of action of your agency and the Commonwealth with regard to the decades of algal growth that continues to plague the South and North Fork as well as the Main Stem of the Shenandoah River. Further I request that this internationally recognized water source be placed on Virginia's impaired waters 303/305B list. To do otherwise is a breach of public trust.

Amongst the many problems the Shenandoah River(s) has is crippling algal blooms. This stuff stinks, ruins camping trips and makes a travesty of the fabled shanty "Oh, Shenandoah". I do not believe even the second or third verses contain the words stink or algae. Might want to 'google' that!

Currently, I personally use the river fishing, boating, wild life/bird photography and canoe camping more than 50 days a year. Recently, the impact of the poor health of the Shenandoah(s) has seriously diminished my river use. I no longer invite friends to enjoy this flowing cesspool. Assuring them that the algae is, in fact, not human waste somewhat diminishes the experience and knocks the wind out of my life long enjoyment of sharing Virginia's great outdoor tradition.

I am a fly rod angler and the algae - in both forms - are ruinous to fishing flies and have reduced my participation on and use of this watershed dramatically. In the area I most often use - Page and Warren Counties, the slack water stench of the floating algal lubs and the spun green algal silk - often measured in meters - foul my boat, PFD, fishing gear, clothing, shoes and a great deal of my angling equipment. I rarely swim in the river other than a quick 'cool off' dunk.

The rivers' condition actually breaks my heart.

As a retired outfitter on the South Fork of the Shenandoah with more than 20 years of daily and first hand experience I can speak directly to the impact that both phases of the algal bloom has on the river.

During the Spring and Summer large clots of algae break loose and head downstream. Resembling tumbling and floating human waste, these algal turds gross out urban guests, exasperate anglers, collect in slow moving water and leave a vomitus stench that diminish the experience by both private landowner and thousands recreational users. One simply has to visit the Guest/Shenandoah River State Park to view the septic floaters, fecal looking river bottom or the 20 yard long ribbons of bright green fibrous strings.

The impact to the watersport recreation industry in the Shenandoah Valley – read economic loss to struggling rural communities - is substantial From float tubers to anglers with tangled lines our operation suffered diminished participation from urban guests who chose other ways to spent discretionary income. The

Shenandoah River is the economic engine that drives valley growth and tourism. Without protection it will continue its long slow decline.

No one will sing songs lovely songs about river. The river is now under your watch.

Respectfully,

Trace Noel

352 Shenandoah Hts. Road
Front Royal, VA 22630
noeltrace@gmail.com

2012 AlgaeLetter.docx
21K

April 10, 2012

John M. Kennedy

DEQ Office of Water Monitoring and Assessment

P.O. Box 1105

Richmond, VA 23218

RE: Draft 2012 305B/303D Water Quality Assessment Integrated Report

Dear Mr. Kennedy,

This letter is meant to show my support for adding the Shenandoah River and her North and South Forks onto the 2012 305B/303D Water Quality Assessment Integrated Report because of the persistent algae problems I see while boating there. I have been an employee of Shenandoah Riverkeeper since October of 2009, and have worked as the agricultural manager. I kayak the river as a part of my work responsibility, but have been a lifetime recreational user of the North Fork, South Fork and Main Stem of the Shenandoah as well as most of the floatable major tributaries such as Cedar Creek, Passage Creek, North River, South River, Middle River etc. Outside of my work duties I continue to use the river on a nearly weekly basis taking my family and friends on float trips.

I regularly kayak and canoe on the Main Stem Shenandoah River and both the North and South Forks with my friends and family. Our family (my brothers and myself) owns 10 kayaks and 3 canoes, which we use for recreation on the rivers in the Shenandoah Valley. My wife grew up along the South Fork in Rockingham County. We usually see algae in one form or another on these waterways. We try to get our children involved with our paddling adventures. But when the algae blooms are at their worst in July and August, my daughters resist my invitations because they don't like the smelly soup that is floating in the Shenandoah. They would prefer we travel farther to the Potomac Highlands or elsewhere for cleaner water, since they like to also swim occasionally, while boating on hot days. We fear our children will choose other activities that don't involve the rivers at all unless conditions improve.

I've seen globs of floating algae on the back eddies and channels on the North Fork Shenandoah River in March and April of this and past years, near Woodstock, New Market and Toms Brook. In May and June of each of the past few years, I've seen filamentous algae on the bottom of the North Fork near Toms Brook and Strasburg, and also on the bottom of the South Fork near Island Ford, Elkton, Shenandoah, Luray, and on the Main Stem Shenandoah River near Morgans Ford Landing, Rt. 50 and Rt. 7 in Clarke County. I've seen the smelly floating algae on the North Fork in July, August, and September near New Market, Mt. Jackson, Edinburg, Woodstock, Strasburg, Toms Brook, and Riverton. This smelly floating algae is also persistent on the South Fork in late summer around Port Republic, Island Ford, Elkton, Shenandoah, Newport, Alma, Luray, and near the Andy Guest/Shenandoah River State Park in Warren County. I have also seen it in large globs on the Main Stem Shenandoah River in late summer in Clarke County and all the way downstream to Harpers Ferry.

I flew over Harpers Ferry with a pilot friend in his small airplane last August and noticed how the Shenandoah River looked like a green pea soup compared to the clear water of the Potomac River at the confluence. I could see the river bottom of the Potomac River, but the bottom of the Shenandoah River was not visible through the murky green water. We could see them mix together downstream of the confluence as the Shenandoah River soup slowly got diluted by the clear Potomac. This is rather disconcerting since we get our drinking water from the North Fork Shenandoah here in Winchester.

I feel this algae plague has diminished my family's enjoyment of the Shenandoah River and her Forks in the Shenandoah Valley. Please include these precious rivers on your 303D/305B impaired waters list, and do everything you can to help return them to clear flowing waterways.

Thank you for reading my opinion.

Sincerely,

Alan Lehman

1314 Vanceright Circle

Winchester, VA 22601

lehman_4@yahoo.com

Jeff Kelble <jeff@potomacriverkeeper.org>

Draft

Andrew Riccobono <ariccobono@yahoo.com> Fri, Apr 13, 2012 at 9:01 AM
Reply-To: Andrew Riccobono <ariccobono@yahoo.com>
To: Jeff Kelble <jeff@shenandoahriverkeeper.org>

Jeff,

[Quoted text hidden]

==========

401 Sherrow Avenue
Falls Church, Virginia 22046

April 12, 2012

John M. Kennedy
DEQ Office of Water Monitoring and Assessment
P.O. Box 1105
Richmond, Virginia 23218-1105
RE: Draft 2012 305(b)/303(d) Water Quality Assessment Integrated Report

Dear Mr. Kennedy:

I am writing to you today on behalf of my family and our concern about the Shenandoah River and it's status on the 305B/303D list. I have been canoeing and fishing on the river since the 1990s and have grown increasingly concerned about our stewardship of the river.

The condition of the river, in my experience, has steadily declined since I first started fishing the South Fork of the Shenandoah. Back then the water was cleaner, the fish were bigger and healthier, and there was rarely any algae at all. In fact, the entire river was so clean that it looked like an aquarium. The conditions started to decline well before the fish kills in 2005.

I still regularly fish the for smallmouth bass and panfish at the Shenandoah River Andy Guest State park near Bentonville and my experiences from spring through summer have become alarmingly predictable. In the spring, when the water is a little higher and a little cooler, the river remains pleasant to fish. By July my flies are covered in green muck after every cast – whether I am fishing on the surface or with a sinking lure. When the algae die off, the decomposing clumps smell pretty nasty. As a result, I will try and fish the spring creeks of the Shenandoah National Park or travel to fish in Pennsylvania or New York where the water remains cooler, cleaner, and more pleasant.

The Shenandoah is an important resource to the Commonwealth. We should do much more to protect it. Please add the Shenandoah River to the 305B/303D list. It is time for us to find out what is causing all this algae and fix our river.

Yours truly,

Andrew Riccobono

Jeff Kelble <jeff@potomacriverkeeper.org>

Shenandoah River

Vernon Mann <vomann@hughes.net> Mon, Apr 9, 2012 at 2:01 PM
Reply-To: vomann@hughes.net
To: Jeff Kelble <jeff@shenandoahriverkeeper.org>
Cc: Michael Hobert <lawyers2@verizon.net>

Dear Mr. Kennedy, I am writing this letter to you to request that Virginia Departmental of Enviormental Quality add the North Fork,South Fork and Main Stream of the Shenandoah River as Impaired Waters on the 2012 305(b)3/303(d) Water Quality Assessment Intergrated Report because of the severe algae and other polution problems.

We live along the Shenandoah River, 3.3 miles south of the Route #7 Bridge and have 247 feet of River Frontage which we keep well groomed. In the summer time we love to sit down on the river front and just watch the river run by. I am 87 yrs old and years ago I used to be an ardent fisherman and did a lot of wade fishing on the Shenandoah all the way from Luray area down to the Rt. 7 bridge. Back is those days the Shenandoah was something to be proud of pure clean spring fed water and if you needed a drink of water you just dipped you hand in and took a drink which I did many times - but not anymore.

I personally don't fish anymore but when my three sons and our grand kids come they like to fish and get into the water but I no longer permit this due to the algae and other polution problems -

In addition to the Algae problem we have another problem which is getting worse by the years - Every morning from about 7am till about 8:30am there are mounds of white foam (my guess is Phosphates) floating down the river. This is apparently controlled up stream somewhere as during the warm weather on week ends the foam does not run on Sat and Sunday but on Monday it's turned loose again - this has been going on for quite a few years 10 or 15 years and is gradually getting worse by the year. I have reported this before but to no avail - just can't figure out why you can't find the source .

W e used to have friends out to fish and swim or float down the river in iner tubes but since the river is in such bad shape most of them have given up .

I have even seen some fish that were caught that had sores on them- Who wants to eat fish like that?

Sure hope you can help clear up this problem on the Shenandoah so that our up coming people can enjoy the river as much as we did. Thanks for Your Help and May God Bless You !! Vernon & Eileen Mann

3295 River Rd.

Bluemont,Va.20135

Phone:540 955-3851

April 13, 2012

John M Kennedy

DEQ Office of Water Monitoring and Assessment

P.O. Box 1105

Richmond Virginia 23218-1105

Dear Mr. Kennedy,

My son and I fish the Shenandoah River several times a year. The Shenandoah is a wonderful gift and treasurer to be appreciated. However, each year for the past several years we have noticed more and more as well as larger and larger algae blooms. The blooms turn the river a dark murky green color, almost like green paint. Where there are blooms there are less fish and when there are fish there are less of them. The algae sticks to our fishing line, clings to the bottom of our boat, smells bad and looks bad. In short, algae blooms and all there ill side effects have diminished our enjoyment of the river and as a result we find ourselves using the Shenandoah less and less every year. We have heard from other fisherman that during July and August last year the blooms were so thick and had such a bad odor that the areas of Woodstock, Strasburg, Front Royal and Goods Mills were so unpleasant due the algae that they were unfishable.

Please include the three sections of the Shenandoah River on the 305B/303D list to help determine why we are having these algae problems and to develop and implement a plan to make them go away.

Thank you in advance for considering my comments and we both look forward to the day when the Shenandoah is restore as one of our nation's great fisheries.

Bill McGrath

6402 Broad Street

Bethesda Maryland 20816

Duncan McGrath

150 Franks Street

Fayetteville West VA 25840

April 6, 2012
John M. Kennedy
DEQ Office of Water Monitoring and Assessment
P.O. Box 1105
Richmond, Virginia 23218-1105
RE: Draft 2012 305(b)/303(d) Water Quality Assessment Integrated Report

Dear Mr. Kennedy,

I am writing to request that Virginia's Department of Environmental Quality add the North Fork, South Fork and Main Stem of the Shenandoah as impaired waters on the 2012 305(b)3/303(d) Water Quality Assessment Integrated Report because of the severe algae problems the river experiences every year.

I fish the North Fork, South Fork and Main Stem of the Shenandoah extensively and I also canoeing and swim on the South Fork. I have been doing this for 40 years. However, recently, for much of the year, large sections of each river experience a series of noxious algae blooms that seriously diminish my use and enjoyment of the rivers.

In each of the past six years during late winter a planktonic algae blooms, usually after a high water event. Over the course of the year I use the river system from Compton Rapids to Front Royal and from Front Royal to the Confluence with the Potomac River. This bloom turns the river a dark murky green color, like green paint, from late winter until about July. When the algae blooms I often choose not to swim or fish and I don't go swimming because they don't like the off-color murky water. When I do fish I find the fishing is poor and I don't enjoy the experience as much. Whenever the river is this murky color, the fish are usually lethargic and often they don't feed at all. For the past two years I simply could not fish the lower south fork or the entire Main Stem from July through August because of the plankton problem.

By mid May or June each year as the river clears the planktons seem to die back. But then a filamentous algae (rock snot) takes hold covers the river bottom and the native grasses which are trying to emerge. The native grasses turn brown and lose their leaves and remain bare the rest of the year. Additionally, a floating bubbly type of algae (I'm told its Cynabacteria or Blue Green Algae) comes at this time. Clumps rise up to and float on the water surface and then accumulate on anything sticking out of the river and in back eddy. This algae smells like sewage or rotting broccoli. When these algaes are blooming, the fishing is frustrating because you cannot fish without fouling your line on the algae, the fish won't bite lures or bait with algae sticking to it. I just cannot use the River due to the odors and annoyance. I found this problem in the following areas last year from July through August: Strasburg, Bentonville, Front Royal, Luray Dam, 211, Shenandoah, Route 50, and Route 7.

Finally, in the fall each year the native grasses die back and a bright green filamentous algae again covers the entire bottom of the river from Broadway to Strasburg. All three of these algaes make it nearly impossible to fish and again diminishes and sometimes eliminates my ability to enjoy the river.

This annual cycle of algae severely diminishes both my use and my enjoyment of the Shenandoah River system. As a result I would like to ask that DEQ include the three sections of the Shenandoah River on 305(b)/303(d) list, determine why we are having these algae problems, and to develop and implement a plan restore the health and beauty of our Shenandoah River system.

Thank you for your consideration,

Bill Millhouser
3325 Prince Charles Ct.
Falls Church, VA 22044
Email: bmillhouser2010@cox.net
Phone: 703-532-2446

Bernard C. Nagelvoort
3043 Parshall Road
Berryville, VA 22611

April 13, 2012

John M. Kennedy
DEQ Office of Water Monitoring and Assessment
P.O. Box 1105
Richmond, Virginia 23218-1105
RE: Draft 2012 305(b)/303(d) Water Quality Assessment Integrated Report

Dear Mr. Kennedy:

Please be aware of the very serious condition of the main stem of the Shenandoah River as indicated by the presence of an excessive brown algae presence for most of last summer seriously reducing visibility in the river even during periods of low flows when it would normally appear to be very clear. This was the most unusual and most serious indication of excess nutrient/pollutants I have seen in the River in the twenty four years I have lived on property bordering the River with 400 feet of river frontage.

As an avid fisherman, I am also discouraged by the dramatic reduction in both small mouth bass and red breast sunfish in my stretch of the river as a result of the fish kills in recent years.

This beautiful River has been subjected to gross mis-use over the past 100 years from industrial and agricultural run-off along with inadequate municipal and industrial wastewater treatment and urban run-off. Its potential for healthy recreation has been substantially diminished over time and the condition last summer is a powerful indication that conditions are not improving.

As I look at the River today with the unusually warm spring we've experienced and modest rainfall, I'm inclined to believe the bad algae conditions are again appearing and will diminish the attractiveness and health of the River yet another summer.

Please accept this letter as an indication of my support for whatever actions DEQ may be able to take to develop and implement plans to reduce the pollution problems causing these serious degraded conditions in the River.

Sincerely,

Bernard C. Nagelvoort

Jeff Kelble <jeff@potomacriverkeeper.org>

Shenandoah Riverkeeper - Help

Harry and Debbie Allan <cedarview_1@hotmail.com> Sat, Apr 14, 2012 at 2:53 PM
To: jeff@shenandoahriverkeeper.org

Jeff – Here is my letter to DEQ. Please feel free to edit as you see fit. Good luck and thanks again. – Harry Allan

---

April 14, 2012

John M. Kennedy

DEQ Office of Water Monitoring and Assessment

P.O. Box 1105

Richmond, VA 23218-1105

Dear Mr. Kennedy,

I fish the South Fork of the Shenandoah River extensively and have been doing so since the mid-1970's. The majority of my fishing time has taken place in the Bentonville area. In these past 35-40 years, I have personally witnessed the degradation of this once beautiful river and its fishery. I am writing to request that Virginia's Department of Environmental Quality add this river, along with the North Fork and mainstem as 'impaired' waters on the 2012 305(B)/303(D) Water Quality Assessment Report. Specifically, severe noxious algae blooms and massive springtime fish kills have seriously diminished my recreational use and enjoyment of the Shenandoah River.

From my extensive fishing experience on this river, I have found that July and August have historically been stellar months of the year for fishing quality. This is no longer the case due to the massive algae blooms early in the season. This problem, in addition to the well-publicized fish kills of the past few years, have forced me to all but abandon the Shenandoah River as a recreational destination.

I respectfully urge the Department of Environmental Quality to include the Shenandoah River on the 305 (B)/303(D) list and begin to take the steps necessary that would return this river to it's legendary status.

Thank you.

Harold Allan

P.O. Box 436

Aldie, VA 20105

703.327.4322

Date: Sat, 7 Apr 2012 14:01:35 -0400
Subject: Re: Shenandoah Riverkeeper - Help
From: jeff@shenandoahriverkeeper.org
To: cedarview_1@hotmail.com
[Quoted text hidden]

Henry J. Staudinger
2218 Riverview Dr
Toms Brook, VA 22660
April 15, 2012

Tel: 540-436-3491
Fax: 540-436-3099
E-Mail hjs@shentel.net

John M. Kennedy
DEQ Office of Water Monitoring and Assessment
P.O. Box 1105
Richmond, VA 23218-1105

Dear Mr. Kennedy:

My wife and I are landowners in Shenandoah County, with almost a mile frontage along the North Fork of the Shenandoah River . The river is an important feature of our property, enabling walks along the river to see wildlife, fishing, wading, canoeing, swimming as well as the view from our home.

Over the years the quality of the water deteriorated, adversely affecting our use and enjoyment as well as diminishing the value of our property. Some years ago we discovered that our cattle operation was contributing to the problem. As a result, we voluntarily put up fences along the river. We also converted a number of acres close to the river from crop land to hay ground and incorporate a number of best management practices to reduce erosion.

We are aware that DEQ monitors and at times has been active in reducing pollution sources. We look forward to DEQ's continued efforts to do. However, more needs to be done. One of the most obvious areas is to identify and address the cause of the algae that has become a more severe problem.

Although the source of the algae is not entirely clear to us, it is becoming an increasing problem. In particular, it discourages and at times makes it impossible to swim or fish. This not only adversely affects our use and enjoyment of the river, but the use and enjoyment of many others. It also diminishes the value of our property. It is not clear what action, if any, we might take to reduce the algae problem. However, it is clear that this is well beyond the ability of individual landowners to address. Please let me know what action DEQ is taking to address this particular problem.

Sincerely,

Henry J. Staudinger

Jeff Kelble <jeff@potomacriverkeeper.org>

North Fork river problems

Jack Chapman <chappyje@gmail.com> Fri, Apr 13, 2012 at 12:45 PM
To: Jeff Kelble <jeff@shenandoahriverkeeper.org>, leslie.watson@fnfsr.org

April 13, 2012
Mr. John M. Kennedy
DEQ Office of Water Monitoring and Assessment
P.O. Box 1105
Richmond, Virginia 23218-11-5

RE: Draft 2012 305(b)/303(d) Water Quality Assessment Integrated Report

Dear Mr. Kennedy,

I am concerned about the quality of the Shenandoah River system and have particular knowledge of the North Fork, near Edinburg. I believe it should be considered "impaired".

We live on Swann Rd., outside of Edinburg. This road parallels the river. I began visiting this area in the mid 1980's due to the quality of the fishing. My wife and I bought a small place on the North Fork in 1999, with the intent to retire there. Which we did in 2007. Prior to retirement we were here most weekends and holidays. We are of modest means, which I state only to emphasize the importance of that purchase. The river and beauty of the area are what drew us here.

I want to outline the problems I've seen, in rough chronological order:

1. The rock-bass (redeye, goggleeye) have all but disapeared. This occurred possibly 15 years ago. They were formerly caught in abundance, sized up to 10" or so. Even though the smallmouth bass were the big draw, the redeye were a prized secondary catch. I talk to a lot of fisherpeople, and they are very aware of this.

2. The fish kills of bass and sunfish are well known. One weekend, I believe early April, the first year of the fishkill, I caught a number of nice bass/sunfish. The next weekend nothing. I caught one sunfish that entire season and quit fishing the area for several years. The sunfish continue to be scarce and small. The bass have had several good years, but are still uniformly small. I did some guiding in the area for several years and quit, partly because of the poor fishing.

3. Currently, the bass fishing is o.k. The fallfish are larger, more abundant and more fun than the bass. Very large carp are moving in to the area.
We have fishtanks we popluate with local critters and often look around and net them. It seems the crayfish & possbily sculpins are much less abundant.

4. We used to have grasses in the river. They grew up as the weather warmed, in the slower sections. I'm not sure if they were all native, but they seemed to house all kinds of aquatic insects and baitfish. These grasses all but disapeared, something like four or five years ago. We have instead, little bright green balls of some kind of algae attached to rocks.
And, the filamentous algae. It is disgusting. It strings out on any available surface, a genuine eight to ten feet. and literally chokes the river. Any significant rainfall causes some algae to detach and we have it floating down the river for days at a time. We hook way more algae that fish during those times.
I frequently wade fish upstream to the bridge (Rt. 698) and we often kayak downstream as far as the first rapids below the deteriorating low water bridge where Swann Road terminates, and paddle back. This algae certainly has diminished our enjoyment of the river. Lifted a paddle with eight feet of algae hanging from it lately? How about backcasting a popping bug with a half pound of gunk attached.

5. We see folks paddling and/or fishing early in the year. The river level us up and the algae has yet to bloom. It's a beautiful trip from Red Banks Rd. to Swann Rd. July through September, people rarely recreate on the river. I frequently go elsewhere.

I feel like we are two of many who live on or near the river and can't enjoy it, or invite friends and family to enjoy it, through large parts of the warm weather. This must be having a significant negative impact both enviromentally and economically.

I would hope these problems will be recognised and addressed by, at least, having this water declared impaired.

Thank You.

Sincerely,

Jack Chapman
P.O. Box 123
393 Swann Rd.
Edinburg, Va. 22824-123
Phone: 703-507-2226
Email: chappyje@gmail.com

Jeff Kelble <jeff@potomacriverkeeper.org>

Letter Writing.,.,. Please help Jeff out.

Jack McAllister <jackmcal@gmail.com> Thu, Apr 19, 2012 at 7:56 AM
To: Jeff Kelble <jeff@shenandoahriverkeeper.org>

Jeff, please add my name to the petition of fishermen that have almost stopped using the South, North and Mainstem of the Shenandoah River due to the algae.....Jack McAllister, Stephenson, Va
[Quoted text hidden]

Trout Unlimited
Northern Virginia Chapter
PO Box 12182
Burke, VA 22009

April 6, 2012

John M. Kennedy
DEQ Office of Water Monitoring and Assessment
PO Box 1105
Richmond, VA 23218-1105

Regarding: Draft 2012 305(b)/303(d) Water Quality Assessment Integrated Report

Dear Mr. Kennedy:

As the President of the Northern Virginia Chapter of Trout Unlimited, I represent approximately 850 dedicated anglers. Please disregard the word "trout" in my organization's name since our members actively pursue smallmouth bass during the summer.

Over the last several years, we have become increasingly concerned about the decline in the quality of fishing on the Shenandoah River. It started with fish kills several years ago and continues with the horrible algae bloom we experienced last year. The impact of the fish kill on recreational angling is obvious, but last year's persistent algae bloom diminished our enjoyment of the river almost as much. The cloudy, murky water made fishing a challenge and diminished our ability to use the river during the summer months. In addition, my members have begun to complain more and more about smell and wonder if contact with the water is dangerous. It seems as if the entire South Fork from Port Republic to Front Royal is equally impacted by this issue.

As a result of the algae, we are less likely to use the river for recreational angling. This will have an adverse economic impact on businesses in the valley as well as on my members as they drive farther to reach other locations in Maryland and Pennsylvania that offer better recreational angling opportunities.

I urge you to take action now and include the North Fork, South Fork and the Main Stem on your impaired waters 303D/305B list.

Sincerely,

Jay Lovering
President, NVTU
703 734-1558
jrlovering@aol.com

April 8, 2012

John M. Kennedy
DEQ Office of Water Monitoring and Assessment
P.O. Box 1105
Richmond, Virginia 23218-1105
RE: Draft 2012 305(b)/303(d) Water Quality Assessment Integrated Report

Dear Mr. Kennedy,

I am writing to you as a concerned Virginia resident, an avid fly fisherman, and an active member of Trout Unlimited and the Friends of the Shenandoah River (FOSR) organizations; as such, I would like to request that the Department of Environmental Quality add the North Fork, South Fork and Main Stem of the Shenandoah as impaired waters on the 2012 305(b)3/303(d) Water Quality Assessment Integrated Report because of the severe algae problems the river experiences every year.

I fish and float the North Fork, South Fork and Main Stem of the Shenandoah often, and as a former monitor for FOSR, I participated for over 5 years in twice-monthly water sampling at various sites on the Main Stem of the river in Clarke County. For much of the year, I have observed that large sections of each river experience a series of noxious algae blooms that seriously diminish my use and enjoyment of these waterways. The blooms turn the river a dark murky green color, like green paint, and make the water far less enjoyable to wade and fish in, as well as far less productive to fish in.

During summer months, there is often a large amount of "rock snot" covering the river bottom, which inhibits growth of the native grasses and also seems to make the fishing worse. There is also a lot of a floating bubbly-type algae on the surface of the water, which has a very foul rotting odor – needless to say, trying to wade-fish the river with that type of algae in abundance is unpleasant at best, and many times will lead me to choose other waters to fish instead.

Based on my fishing and canoeing experiences on other waterways in Virginia, West Virginia, and Maryland, I believe the algae problems are specific to the Shenandoah, which suggests to me that they are likely due to polluted run-off; if that's the case, the causes of the pollution should be identifiable and correctable.

PLEASE ensure that DEQ includes the three sections of the Shenandoah River on the 305B/303D list, so that it can be determined why we are having these algae problems, and so that a plan can be developed and implemented to make the Shenandoah River a pleasant place to fish, canoe/kayak, and swim. I appreciate in advance your consideration of this request.

Sincerely,

Dr. Mark Zimmerman
"Trout in the Classroom" and Communications Coordinator
Winchester Virginia Chapter of Trout Unlimited
309 Huntersridge Rd.
Winchester, VA 22602
E-mail: mbzimmerman@comcast.net
Phone: 540-722-4914

April 14, 2012

John M. Kennedy
DEQ Office of Water Monitoring and Assessment
P.O. Box 1105
Richmond, Virginia 23218-1105
RE: Draft 2012 305(b)/303(d) Water Quality Assessment Integrated Report

Dear Mr. Kennedy,

I am writing to request that Virginia's Department of Environmental Quality add the South Fork and Main Stem of the Shenandoah River as impaired waters on the 2012 305(b)/303(d) Water Quality Assessment Integrated Report due to the severe algae problems that the river is experiencing every year.

Every year, as many others in this wonderful watershed, my thoughts--post winter--turn to the river. In my backyard, so to speak, this refers to the Shenandoah. I have spent the past several seasons kayaking various sections of the south branch and middle segment, from as far south as Port Republic to Island Ford (October, 2011) to the more northern sections of the South Branch, including Foster's Landing to Burners Ford (July 2010), Alma to Rt. 211 (September 2010 river cleanup day), and the middle stem, Myerstown WV, to Millville Mills Landing (Nov 2011).

Sadly, I have witnessed the increasing degradation of water conditions from algae blooms and growth, visible in increasing intensity every year. It chokes out the normal native grasses that usually are seen, gently wafting in waters offering protection for young fish and various aquatic life. Later in the season, some areas become so thickened by growth, that areas paddled in early spring are almost unrecognizable. The areas that are affected by the algae blooms suffer such a lack of oxygen that the fish population suffers. This in turn affects where the birds nest, and the vibrancy of their young. I have seen fish in areas with large sores on their sides, and hear stories of others from fellow paddlers. The connection of it all is becoming harder to miss, even for those visiting on a more occasional basis.

I am writing at the commencement of the season in hopes that what I have witnessed while kayaking these waters can be properly addressed and dealt with in a serious fashion by those in governmental positions. As I have been involved in a small way on occasion to join in organized cleanups on the river, I realize that the effort to eradicate or more effectively control the pollutants that are responsible for the explosion of algae growth requires action well above the efforts of small groups of volunteers. We can, and will, continue to attend to the removal of trash that ends up in the river, but this issue is out of our league.

Please help us with the ecology of this lovely watershed by attending to the protection of this vibrant and important area of Virginia. Many people travel from all over the state, around the country--and various parts of the world-- to enjoy watersports, fishing and camping on the Shenandoah.

Being the daughter of an Air Force Colonel, I have lived all over the country, and halfway around the world. I have been in every state of the Union, except Alaska. I can state without equivocation that the Shenandoah Valley is unsurpassed in her raw, native beauty. That is one main reason why I chose to live here.

The Shenandoah River is a crown jewel of the state, and is threatened to be a yearly eyesore if this is not vigorously addressed. We cannot afford to let this happen; economically from a tourism standpoint, or ethically, as entrusted stewards of these lands. Thank you.

Sincerely,

Kara Rice
37 Wellspring Road
Browntown, VA 22610

Shenandoah Riverkeeper
P.O. Box 405
Boyce, VA 22620
540.837.1479
keeper@shenandoahriverkeeper.org
www.shenandoahriverkeeper.org

March 27, 2012
John M. Kennedy
DEQ Office of Water Monitoring and Assessment
P.O. Box 1105
Richmond, Virginia 23218-1105
RE: Draft 2012 305(b)/303(d) Water Quality Assessment Integrated Report

Dear Mr. Kennedy,
I am writing to request that Virginia's Department of Environmental Quality add the North Fork, South Fork and Main Stem of the Shenandoah as impaired waters on thee 2012 305(b)3/303(d) Water Quality Assessment Integrated Report because of the severe algae problems the river experiences every year.

I fish multiple sections of the South Fork of the Shenandoah River from the Town of Shenandoah to the Town of Luray. The algae and grass problems continue to rise more and more each year. I do not know the reason but I do know that it is frustrating to fish because my fishing lures are constantly getting slime or grass on them. Wading the river during the summer used to be a favorite pastime of mine. I now feel it is unsafe due to the slime on the rocks making footing unstable and I really don't feel safe walking around in the foamy water that is often in the river. I have began heading away from my favorite hometown river in favor of lakes that require me to drive much longer distances.

I also fish in the lower Potomac River several times a year because my family owns a house there. The stuff I see floating in that river and the fact that fishing has really gotten bad there makes me wonder if the algae I am seeing in the Shenandoah River is also having an impact on the Potomac River. If the fishing continues to get worse on the Potomac the house will go up for sell there.

My brother recently got his Captains License and can now begin his own charter guide service but the two areas (South Fork of the Shenandoah and Lower Potomac) are not looking to be a option due to water quality issues. I have also looked into starting a bait and tackle shop in my hometown but now I am having second thoughts on this as well.

I have volunteered my time to help with water cleanup in my hometown. I have been involved in rain garden installations, river bank erosion reduction projects, Save the Bay clean up days, runoff water control, tree plantings, willow spiling projects, attended TMDL meetings, participated in the Grasses for the Masses program, organized youth and adult fishing tournaments, etc. I personally feel regulations regarding water quality from industry, farm land and local government need to be firmly put in place so our waterways can begin to heal. I also feel that tax breaks should be given to any landowner that creates properly designed riparian buffers along their stream/river banks. For that matter anyone that can properly control water runoff from their property should somehow be rewarded.

This cycle of algae diminishes both my use and my enjoyment of the Shenandoah River system. As a result I would like to ask that DEQ include the three sections of the Shenandoah River on 305B/303D list, determine why we are having these algae problems, and to develop and implement a plan to make them go away.
Thank you for considering my comments,

Mr. Kirk Comer
917 Junior Avenue
Shenandoah, VA 22849
Email: kirkcomer@gmail.com
Phone: 540-742-1323

Jeff Kelble <jeff@potomacriverkeeper.org>

Form submission from: Take Action!- Help the Shenandoah Riverkeeper Get Rid of Algae

Potomac Riverkeeper <sarah@potomacriverkeeper.org> Sat, Apr 14, 2012 at 6:32 PM
To: jeff@potomacriverkeeper.org

Submitted on Saturday, April 14, 2012 - 6:32pm
Submitted by anonymous user: [173.79.213.221]
Submitted values are:

Your Letter:
14 April 2012

John M. Kennedy
DEQ Office of Water Monitoring and Assessment
P.O. Box 1105
Richmond, Virginia 23218-1105

Dear Mr. Kennedy,

I'm writing this to let you know the Commonwealth *definitely* has a problem with algae growth in the Shenandoah River, caused by pollution that stimulates growth.

I used to fish the Shenandoah frequently, particularly the part around Luray but also downstream as far as the US Route 50 bridge. I greatly enjoyed Virginia's wonderful scenery combined with the opportunity to fish for smallmouth bass. I have essentially stopped fishing the Shenandoah because of the effects of pollution on both of these that the state government should have stopped. I'm grateful that some action is underway regarding chicken waste but much more remains to be done to return this river to the state where fishing is a rewarding activity for the citizens of the Commonwealth.

The Shenandoah used to be recognized as one of the great scenic rivers and smallmouth streams of the United States. Now I must drive to West Virginia to find water clean enough to support a healthy smallmouth sport fishing population.

Please do your job and enforce the water quality laws.

J. Mark Pullen
Name: J. Mark Pullen
Email: mpullen@netlab.gmu.edu

The results of this submission may be viewed at:
http://www.potomacriverkeeper.org/node/428/submission/822

April 5, 2012
John M. Kennedy
DEQ Office of Water Monitoring and Assessment
P.O. Box 1105
Richmond, Virginia 23218-1105
RE: Draft 2012 305(b)/303(d) Water Quality Assessment Integrated Report

Dear Mr. Kennedy,
I am writing to request that Virginia's Department of Environmental Quality add the North Fork, South Fork and Main Stem of the Shenandoah as impaired waters on thee 2012 305(b)3/303(d) Water Quality Assessment Integrated Report because of the severe algae problems the river experiences every year.

I kayak, canoe, fish and hike at various locations on the Shenandoah North Fork, South Fork, and Main Stem, as well as the Potomac rivers. Over the past several years I have observed a decline in both rivers clearly linked to the algae problem in the Shenandoah. As a result my use and enjoyment of all three of the above-referenced portions of the Shenandoah has diminished drastically, and my use and enjoyment of the Potomac below Harper's Ferry has also been seriously diminished. Last year my family canceled an annual June canoe outing on the South Fork of the Shenandoah due to unpleasant conditions and poor fishing resulting from the annual algae bloom. The number of trips that I make to the Shenandoah has dropped off from approximately six per year 10 years ago to one or two per year over the past three years. While I return occasionally myself to see how the river is doing, it is no longer a place I am proud to take out-of-town guests for an outing.

When my wife and I were dating in the 1980s we found several locations along the North Fork of the Shenandoah in the Strasburg area where we would picnic, wade and fish. For a number of years we occasionally returned to these spots, such as the VA Rt. 55 crossing. When we visited that area in the summer of 2011, the river bottom and water conditions were drastically changed from a decade earlier. There was abundant "snot weed" and a lack of the grasses that were previously in the river. The river bottom was not visible and what was previously an attractive river for wading was not at all inviting. What should have been a pleasant outing remembering good times from earlier years turned out to be very disappointing and left us concerned that future generations will not realize what a lovely resource the Shenandoah once was.

Of course the Shenandoah flows into the Potomac and the problems of the Shenandoah have seriously impacted the Potomac below Harper's Ferry. A couple of years ago a number of us who fish and paddle the Potomac from Harper's Ferry to Little Falls began noticing cloudy water and extremely slow fishing from early in the season into July. In 2011, it seemed that the Potomac never attained its usual midsummer clarity. This aerial photo, taken by a member of the Shenandoah Riverkeeper organization on August 30, 2011, explains why. It shows the green, algae-stained water of the Shenandoah flowing into the relatively clear water of the Potomac at Harper's Ferry. I know from paddling and fishing the river at this location and all the way downstream to Little Falls that the algae-stained water basically "takes over" not far below the Rt. 340 bridge and affects use of the Potomac by thousands, if not tens of thousands, of people, as well as billions of organisms that live in the river. This photo confirms that the problems of the Shenandoah are having a dramatic impact on the Potomac and eventually on the Chesapeake.

I could cite many more specific instances, but clearly the algae blooms of the Shenandoah have diminished my use and enjoyment of the Shenandoah River system and the Potomac. As a result I would like to ask that DEQ include the three sections of the Shenandoah River on 305B/303D list, determine why we are having these algae problems, and develop and implement a plan to eliminate them.

Thank you for considering my comments,

Mark R. Myers
12128 Quorn Lane
Reston, VA 20191
Email: markrobertmyers@yahoo.com
Phone: 703-328-5843

April 10, 2012
John M. Kennedy
DEQ Office of Water Monitoring and Assessment
P.O. Box 1105
Richmond, Virginia 23218-1105
RE: Draft 2012 305(b)/303(d) Water Quality Assessment Integrated Report

Dear Mr. Kennedy,
I am writing to request that Virginia's Department of Environmental Quality add the North Fork, South Fork and Main Stem of the Shenandoah as impaired waters on thee 2012 305(b)3/303(d) Water Quality Assessment Integrated Report because of the severe algae problems the river experiences every year. As a frequent fisherman and paddler on the water, I see the huge impacts currently impeding the fishing on the Shenandoah system when I fish there. The contrast with other rivers like the Susquehanna, Rappahannock, Potomac, North Anna, James, and New is all the more striking, as those other flows do not show the same levels of algae that I find every year on the Shenandoah watershed. Even when weather and water flow conditions are similarly low, only the waters of the Shenandoah system show the severe different algal impacts at different times of the year that combine to make enjoying the use of this beautiful river impossible anymore.

I used to fish the North Fork, South Fork and Main Stem of the Shenandoah extensively. For much of the year, however, large sections of each river seems to experience a series of noxious algae blooms that seriously diminish my use and enjoyment of the rivers. I now spend my time on less polluted rivers in other areas, both in Virginia and other states such as Pennsylvania, Maryland, and West Virginia, rather than spend my valuable time on an unenjoyable body of water.

In each of the past six years during late winter a planktonic algae blooms, usually after a high water event, but not always. Over the course of the year I use the entire river system and have seen this on all three rivers from their most upper reaches (Port Republic and Cootes Store) to the Confluence with the Potomac River. This bloom turns the river a dark murky green color, like green paint, from late winter until about July. The impact is so severe that even when fishing the Potomac below Harpers Ferry, the Virginia bank is consistently heavily stained and unfishable, while the Maryland shore had noticeably clearer water with better fishing. Above the mouth of the Shenandoah, this problem does not exist on the Potomac, and the difference in water color and clarity is clearly visible from elevated areas around Harpers Ferry if you want to go take a look. I'm sure it will be back again this year, probably sooner rather than later given the recent weather. Something needs to be done.

When the algae blooms I cannot swim or fish on these rivers, and I will not travel to the Shenandoah Valley to fish the any of the parts of the Shenandoah because the trip is not worth the time or cost. Local merchants lose my business because of the water quality issues. When I do fish with friends, I find the fishing is poor and I don't enjoy the experience as much. Whenever the river is this murky color, it's disturbing to fish. They are usually lethargic and often they don't feed at all. Activity in the river drops to near zero. For the past two years I simply could not fish the lower south fork or the entire MainStem from July through August because of the plankton green water.

By mid May or June each year as the upper river clears the planktons seem to die back. But then a filamentous algae (I call it snot grass because of the consistency and color. You get the picture.) takes hold covers the river bottom and the native grasses which are trying to emerge. The native grasses turn brown and lose their leaves and remain bare the rest of the year. Additionally, a floating bubbly type of algae (I'm told its Cynabacteria or Blue Green Algae) comes at this time. Clumps rise up to and float on the water surface and then accumulates on anything sticking out of the river and in back eddies. This algae smells like sewage or rotting broccoli. When these algaes are blooming I literally do not want to be

anywhere near the river and I often choose to fish the Rappahannock, which does not have the severe water quality issues above the fall line than the Shenandoah basin does. Unfortunately, it often is too low to float in summer because of a lack of rain when the Shenandoah is still kayakable. In that case, I travel to better fishing destinations that are often out of our state.

The fishing on the Shenandoah is frustrating because I cannot fish without fouling my line on the algae, and the fish won't bite lures or bait with algae sticking to it. Imagine wiping Ghostbusters-like slime from your lure and line after every cast. Sound enjoyable? It smells, literally. In fact, just being around the river is an assault on the olfactories due to the very unpleasant odors. I found this problem in the following areas last year from July through August; Broadway, Lupton, Deer Rapids, Edinburg, Woodstock, Strasburg, Front Royal, Goods Mill, Luray Dam, 211, Alma, Port Republic, Warren Dam, Shenandoah, Route 50, and Route7 and at places in between while floating between accesss points. Clearly pollution is having an impact when such large stretches of river are all impacted by the same poor water quality.

Finally, in the fall each year the native grasses die back and a bright green filamentous algae again covers the entire bottom of the river from Broadway to Strasburg. When fishing this time of year the water is usually clear from the green paint algal blooms, so I can see the bottom. The fish avoid areas of the river with the dead and dying native grasses. They should be thriving here, feeding on baitfish hiding in the cover the aquatic vegetation provides, but they are clumped and clustered into small pockets where the current manages to keep the green algae from collecting, which are very few. On a six mile float from State Park to Karo in early November, the majority of the river bottom was covered in this gunk. All three of these algaes make it nearly impossible to fish and again diminishes and sometimes eliminates my ability to enjoy the river.

This cycle of algae diminishes both my use and my enjoyment of the Shenandoah River system. As a result I would like to ask that DEQ include the three sections of the Shenandoah River on 305B/303D list, determine why we are having these algae problems, and to develop and implement a plan to make them go away. Thank you for considering my comments.

Sincerely,

Mr. Peter Pfotenhauer
10604 Wakeman Drive
Fredericksburg, VA 22407
Email: ppfoten211@aol.com
Phone: 540 498 0966

Jeff Kelble <jeff@potomacriverkeeper.org>

Alge blooms on Shenandoah

Evans Ron <riverbound29@yahoo.com> Thu, Apr 12, 2012 at 1:49 PM
Reply-To: Evans Ron <riverbound29@yahoo.com>
To: Jeff Kelble <jeff@shenandoahriverkeeper.org>

----- Forwarded Message -----
From: Evans Ron <riverbound29@yahoo.com>
To: Jeff Kelble <jeff@shenandoahriverkeeper.org>
Sent: Wednesday, April 11, 2012 10:33 AM
Subject: Alge blooms on Shenandoah

Dear Shenandoah Riverkeeper,
I am writing you this letter in concern for the water quality of the Shenandoah River. Seems like "here we go again" is my thought as I float down the river. I lived waterfront on the mainstem just downriver from Berryville,VA for 2 years, and moved when all this started.
I am a full-time firefighter who has guided for smallmouth in the past for years. I currently do not guide however I have friends and family out on the river at least once a week. Guiding ceased for me with the fishkills earlier this decade.
Seems like once it starts warming up about this time every spring, we are plagued by a blue-green alge that smells HORRIBLE. We also notice dead fish prodominatly through the march-sept months. My concerns are for the health of the river and the fish that live in it...however my concern is evern greater for those that spend time in and around the river, and for those that get water from the river. Its discouraging to be out there, and it worries me are we getting exposed to fertilizers and chemicals from farms and chicken operations that might harm us our our families. It makes us decide to go to other rivers in other states sometimes. I certianly don't feel comfortable wading, or throwing the ball for my dog to swim in it like she always has. This also hurts the economy and businesses we spend money with to be there in the first place.
I fish many different areas of the Shenandoah...North Fork, South Fork, and theMain Stem. I used to enjoy the runs from Luray down, and from Bentonville down. On the North fork I liked to spend time from Edinburg to Woodstock. The mainstem I spent time from Route 50 down to WV. However I see the problem on all stretches. I am contacting you because I see on your website that you are politicing for the health of the Shenandoah. Please forward my letter with any others you recieve through the appropriate channels. I know with the economy and budget things are tough for all agencies...however the Shenandoah is a true gem and I would call it arguably the prime resource for the state of Virginia....and it would be an absolute shame to see the health of the river get ANY worse. The DEQ and VDGIF need to do all they can possibly do to restore and protect this precious landmark of Virginia.
This expected low water year could be the death of this river. Nows the time to act.
I would strongly urge the people in the right positions to put the Shenandoah on the 303D list as soon as possible. Help protect and restore VA's prime resource.
Contact me if I can help in any way.
Thank-You!
Ron Evans
(240)421-8198

John M. Kennedy

DEQ Office of Water Monitoring and Assessment

P.O. Box 1105

Richmond, Va 23218-1105

RE: Draft 2012 305(b)/303(d) Water Quality Assessment Integrated Report

Mr, Kennedy,

My wife and I live on the west slope of the Blueridge equidistant between Rt. 7 and Rt. 50. We literally can coast down to the Shenandoah River once we hit the paved road (Va #649) that heads downhill to Va #605 aka The River Road. When I retired in 2001 and decided to bring my wife back home to the D.C. area we chose our home based for a large part on the nearness of the Shenandoah River. We both love canoeing, recreational kayaking, and I am an avid smallmouth bass fisherman. At the time of our move in 2001, the Shenandoah was rated as one of the top 10 smallmouth bass fisheries in the United States. We were very excited about renovating the old farmhouse we had bought and living near one of the prettiest rivers in America.

Since we have lived here the river has undergone some pretty "nasty" events. First were the fish kills on both forks and the main stem in the past ten years. In spring of 2002 I caught my first bragging size smallmouth, a beautiful 19" fish. Beautiful that is except for the lesions on its tail that looked like cigar burns. But that's another story.

Algae bloom. That's the big story now. For much of the year the river is turned a murky, nasty looking green from the algae. The water doesn't smell all that good as well. Last June I canoed the South Fork of the Shenandoah from Bentonville to Front Royal with a group of friends. It was one of the most unpleasant experiences of the year. Worse than the record-breaking heat was the appearance, smell, and an almost slimy feeling of the water. It was disgusting. We stayed overnight on a sand bar. I usually take a swim before I bed down to remove the day's dirt and sweat. Not that night. I choose not to expose myself to the water anymore than needed. We reveled the next day when a thunderstorm hit. We all dashed for cover on the bank but would run out into the rain risking being lightning rods to rinse our bodies. Another group of friends out on the main stem that same weekend said they experienced the same conditions i.e. nasty looking water, lethargic fish, and a general feeling of "yuk."

As a result of my experience my wife canceled a float trip for the next weekend. A group of her girl friends had planned to come out to our home, do a day trip on the main stem of the Shenandoah, and then wine and dine at a restaurant in Upperville. Instead my wife drove to D.C. Her and her friends spent a lot of money in the District rather than out in country where the small business owners are hurting. I suspect that other family and friend groups have canceled trips thus denying needed income for fishing guides, canoe outfitters, and other businesses in the Shenandoah River valley.

Please find a solution for this condition. Recreation and tourism is an important cog in the economic engine that runs this fair state. Let's not let green water stop that flow of needed income into the valley. Perhaps a good start would be to include all three sections of the Shenandoah River on the 2012 303B/303D list. I believe our beautiful river is endangered and impaired. Let's stop whatever is causing the bloom and start getting the water clean. No one wants to expose themselves or their children to unclean water. Think about it.

Sincerely,

Stan Ikonen

2479 Morgans Mill Road

Bluemont, Va 20135

Email:texfinn@hughes.net

Phone:540-550-3555

March 27, 2012
John M. Kennedy
DEQ Office of Water Monitoring and Assessment
P.O. Box 1105
Richmond, Virginia 23218-1105
RE: Draft 2012 305(b)/303(d) Water Quality Assessment Integrated Report

Dear Mr. Kennedy,
I am writing to request that Virginia's Department of Environmental Quality add the North Fork, South Fork and Main Stem of the Shenandoah as impaired waters on thee 2012 305(b)3/303(d) Water Quality Assessment Integrated Report because of the severe algae problems the river experiences every year.

I am a retired professional sanitary engineer with a keen interest in water quality and the health of our rivers. For the past 50 years I have been an avid bass fisherman. I grew up fishing the James River. As a result of being retired and having more free time I routinely fish most of the major smallmouth bass rivers in Virginia. I have fished the North Fork, South Fork and Main Stem of the Shenandoah for decades and for all that time observed noxious algae blooms that seriously diminish my use and enjoyment of the rivers. In fact, due to the very poor water quality I see much of the year and the serious fish kills and resulting steep decline in the quality of the angling I use the river much less. Of all the many rivers I recreate on in Virginia the water quality in the Shenandoah system is by far the worst to the point that it seriously diminishes the recreational experience.

The most extensive and troublesome problems are the planktonic algae blooms that I often see after a high water events. I have seen this on all three rivers from Port Republic down to Front Royal, from Broadway to Front Royal and from Front Royal to the Confluence with the Potomac River. This bloom turns the river a dark murky green color, like green paint, from late winter until about July. I do not fish the river when this condition occurs because fishing is generally poor. Activity in the river drops to near zero. At this same time water quality on other bass rivers is fine so I fish other streams. I am presently very concerned about recent the fact that for the past two years the algae blooms were so bad I could not fish the lower South Fork or the entire Main Stem from July through August.

It is almost impossible to escape the impacts of poor water quality and over enrichment of the Shenandoah River system on recreational angling. In May and June blue green and filamentous algae begins to appear and impact our native grasses as well as the ability to fish. They turn brown, lose their leaves and remain bare the rest of the year. Additionally, a floating bubbly type of blue green algae clumps rise up to and float on the water surface and then accumulates on anything sticking out of the river and in back eddies. The fishing is impossible because you cannot fish without fouling your line on the algae, the fish won't bite lures or bait with algae. I find this problem everywhere I fish in the river.

I would like to see improvements in water quality and be able to recreate more often on the Shenandoah river system. I request that DEQ include the three sections of the Shenandoah River on 305B/303D list, determine why we are having these algae problems, and to develop and implement a plan to make them go away.

Urbie Nash , P.E.
1232 Frederick Street
Waynesboro, Virginia 22980

Allan Thomson

709 Cargil Lane

Charlottesville

VA 22902

John M. Kennedy

DEQ Office of Water Monitoring and Assessment

P.O. Box 1105

Richmond, Virginia 23218-1105

RE: Draft 2012 305(b)/303(d) Water Quality Assessment Integrated Report

Dear Sir,

As a frequent user of the Shenandoah River, I have noticed that there is often in the spring and summer a slimy mat of algae covering the rocks and native grasses which makes the river not only unsightly but also hazardous to walk in. This is especially true in the North fork and the main stem north of Front Royal. I have been told that this algal bloom is caused by excess fertilizer and manure seeping into the waterway which is a severe deterrent from using the river for recreational purposes with friends and family.

I hope that there is something you can do about this to restore the river to being the wonderful resource for recreation and tourism that it should be.

Yours Sincerely

Allan Thomson

John M Kennedy
DEQ Office of Water Monitoring and Assessment
PO Box 1105
Richmond VA 23218-1105
RE: Draft 2012 305(b)/303(d) Water Quality Assessment Integrated Report

Dear Mr. Kennedy,

I am a long time resident and nature photographer in Shenandoah County. The Valley and the beautiful Shenandoah River have been something that I have enjoyed for decades. Several times a year, I will go to the river, either by myself or with friends and/or family to enjoy it. Canoeing is my favorite activity but also swimming, wading, wildlife watching and fishing are things that I enjoy with my daughter as I did when I was her age growing up in Mt. Jackson.

Problems affecting the health of the Shenandoah River have been around for a very long time. On many occasions over the past several years, algae blooms have been observed from Deer Rapids to the Strasburg town park. I live near Strasburg and this stretch of the river is one that I canoe many times. The algae on this section is worse during the summer months.

In 2009, I canoed the entire length of the North Fork and main stem from Fulks Run to Harper's Ferry at its confluence with the Potomac River. During that time on the river in April and early May, I witnessed something at the area known as Shenandoah Shores on the main stem below Front Royal. There were large clumps of green and brown stuff that were floating around. As I passed, the clumps had a sewage-like stench that could be smelled.

Due to the algae that has been occurring in the river for many years, I try to avoid the areas that are plagued by the algae during my visits or I just avoid going to the river altogether. I am only one person with one family and the algae has certainly diminished our enjoyment and our experience of the river to the point where we have cut back on our use of the river. I am sure many other people, locals and tourists, also feel this way and are affected in a similar fashion.

Because of the algae pollution that is affecting the quality of our environment, I am requesting the Virginia Department of Environmental Quality to have the Shenandoah River (North Fork, South Fork and the main stem) be included to the impaired waters 303D/305B list. I believe it is important that the DEQ study this unique algae problem found in the valley then solve it in order to clean it up.

The Shenandoah River has been romanticized and immortalized through songs, poems and verse. The health of the river is important to the wildlife that live within its ecosystem, to the local economy that depends upon the dollars that recreation on the river brings in and the communities that use it for consumption. We should clean and protect this great Virginia and national treasure that is our Shenandoah River.

Andrew Thayer
361 Little Sorrel Drive
Strasburg, VA 22657
(540) 465 – 8266

<<CONTINUED>>

Bernard Griswold
829 Hickory Lane
PO Box 267
Woodstock, Virginia, 22664
April 12, 2012

Mr. John M. Kennedy
DEQ Office of Water Monitoring and Assessment
PO Box 1105
Richmond, Virginia 23218-1105

RE: Draft 2012 30(b)/303(d) Water Quality Assessment Integrated Report

Dear Mr. Kennedy:

I am writing to request that Virginia's Department of Environmental Quality add the Shenandoah River system as impaired waters to the 2012 305(b)3/303(d) Water Quality Assessment Integrated Report because of severe and growing algae growth and blooms each year.

I have had riverfront property directly on the North Fork outside of Woodstock since 1991, first as a weekend retreat, then as a permanent new retirement home on expanded land holdings since 2002. Therefore, I have had intimate contact and use of the North Fork for over 20 years, mainly through floating, fishing, some swimming, and regular environmental monitoring both for water quality and benthos for the Friends of the North Fork of the Shenandoah River. Our home sits on a 25-foot bank with deck directly above the river affording a close up river view 24/7. My use of the river includes primarily the stretch from Edinburg to Strasburg. I am amazed at the changes in indicators of water quality over these 20 years, accelerating in the past 6 or 7.

The visible issue is primarily an increase in attached algae, both type and quantity. However, during dry low water spells, planktonic algae also increases dramatically to the point that it covers and clogs grass beds from shore to shore. Fifteen years ago, vegetation in this area consisted primarily of rooted grasses which provided cover and food for a variety of river creatures, especially from mid-June through early fall. Now, beginning in early June, rooted filamentous brown algae begins to coat rocks and rubble in pools and runs and increases by mid-August to provide floating clumps of brown gunk in such quantity as to collect in masses around any object at the surface. This has resulted, in recent years, in much reduced use of the river in late summer and early fall for all our activities and provides a real eyesore from our vantage point on shore. It also provides a severe odor problem during hot dry, low water periods in late summer. It is obvious, from these indicators over a 20 year time frame that significant changes are occurring in water quality that are not indicative of a healthy ecosystem.

Thus, I am asking that DEQ include the Shenandoah River in its 2012 Water Quality Assessment Report to study this issue carefully and develop a plan and strategy to reverse these issues. The Shenandeoah River is historically a national icon and conserving it as a healthy ecosystem should be paramount as an environmental goal.

Sincerely,
Bernard Griswold

April 8,2012
Mr. John M. Kennedy
DEQ Office of Water Monitoring and Assessment
P.O. Box 1105
Richmond, Virginia 23218

RE: Draft 2012 305(b)/303(d) Water Quality Assessment Integrated Report

Dear Mr. Kennedy,

I am writing this letter to request that your organization add the North Fork, South Fork and Main Stem of the Shenandoah River as impaired waters on your Water Quality Assessment integrated Report because of the very severe algae problems evident in the river every year.

I fish for small mouth bass in all portions of the river, particularly the North Fork near Edinburg, and the main stem down river from Route 50. I also fish the South Fork near Bentonville. I am experiencing a lot of frustration as the temperature rises during the summer months with regard to cloudiness of the water, and the algae blooms that cause the fishing to be difficult, and the river bottom to be slippery and the surface of the water to be unsightly. This lasts until the fall. It is getting to the point that I do not find it much fun anymore. I cannot even run a lure through the water because of this problem. Also, I am beginning to wonder if there are some things in the water that might affect my skin, should I continue to come in contact with it.

I keep hoping for some improvement, but over the years the problems continue. They continue to the point that it my use of the river will diminish accordingly. It is a shame because it is so beautiful but not so pristine, as it used to be.

Accordingly, I am repeating my request for you to include the waters of the Shenandoah on 305B/303D list to determine why the river is having these problems.

Thank you for taking the time to read this letter. Hopefully, if addressed a plan can be developed and implemented to make the problem go away and make the river more enjoyable for me and future generations.

Sincerely,

Carl Cisky

Carl Cisky
13113 Applegrove Lane
Herndon, VA 20171
Tel. 703-476-2391
Email: cfcisky@verizon.net

April 18, 2012
John M. Kennedy
DEQ Office of Water Monitoring and Assessment
P.O. Box 1105
Richmond, Virginia 23218-1105
2012 305(b)/303(d) Water Quality Assessment Integrated Report

Dear Mr. Kennedy,
I am writing to request that Virginia's Department of Environmental Quality add the North Fork, South Fork and Main Stem of the Shenandoah as impaired waters on thee 2012 305(b)3/303(d) Water Quality Assessment Integrated Report because of the severe algae problems the river experiences every year.

I was born and raised in Northern VA and lived in this wonderful state my whole life. When it came time for my wife and me to buy our first house we both decided that we would like to settle and make a home in the Shenandoah Valley. So we could be closer to the things we love, for me that was mostly the Shenandoah River. From the early spring to late fall I enjoy floating, fishing and swimming in the river. I take my dogs down to the river for a swim from time to time and I use to take my lab mix fishing with me before she got too old to handle a long day on the river.

I have seen in recent years when on the river that the water has an odd dark greenish color that seems almost like it could glow in the dark to it in the spring. I have witnessed this just last weekend on 4-15-12 when I had floated from Rt.50 to a takeout 4 miles downstream. Then on other floats I have taken in the summer between July and August on the main stem at the low water bridge at Morgan's Ford and between Rt.50 and Rt.7, as well as the South Fork between New Port and Alma also as far up river as Kara there are tons of large clumpy pods of algae that are so bad that it looks like you could walk across it. It is very hard to float this because basically you can't float on top of these algae clumps very well. It has also make it very hard to fish when every cast I'm clearing the algae from my line or lure. Also during the summer there is a strong smell that is not enjoyable at all. This smell seems to stay with you because it is on everything from my canoe and everything I take fishing. I have to spend hours cleaning my gear and bathing my dogs after a trip.

This cycle of algae has diminishes my use and my enjoyment of the Shenandoah River system. As a result I would like to ask that DEQ include the three sections of the Shenandoah River on 305(b)/303(d) list, determine why we are having these algae problems, and to develop and implement a plan to make them go away.
Thank you for considering my comments,

Mr. Charles V Loudermilk II
150 Travis Court
Winchester, VA 22602
Email: Charlie@winchestertu.org
Phone: 571-236-5640

April 15, 2012

John M. Kennedy
DEQ Office of Water Monitoring and Assessment
P.O. Box 1105
Richmond, Virginia 23218-1105

RE: Draft 2012 305(b)/303(d) Water Quality Assessment Integrated Report

Dear Mr. Kennedy,

I am writing to request that Virginia's Department of Environmental Quality add the North Fork, South Fork and Main Stem of the Shenandoah as impaired waters on thee 2012 305(b)3/303(d) Water Quality Assessment Integrated Report because of the severe algae problems the river experiences every year.

I have fished the North Fork, South Fork and sometimes the Main Stem of the Shenandoah frequently over the last forty plus years. In recent years large sections of each river get nasty algae blooms that seriously diminish my use and enjoyment of the rivers.

It seems that the problems now begin early in the spring of the year with a dark green, murky color to the water that lasts several months. The fishing suffers during this time and it barely goes away around June when the snot grass version comes on and covers everything in the water including the rocks and the native grasses. I used to do a lot of wade fishing in the summer, but because of this snot grass overlay on the whole river bottom, I can't. The fishing isn't as good and the bottom is slippery and dangerous….and it smells bad. I've tried to get away from this by going to different stretches of the rivers, but it seems more and more to cover almost all of both branches and the main stem.

Although I don't live on the river, enjoying it has been a long and valuable part of my and my family's life. And I know that's true for so many of the people I've met there over the years.

The degradation that is happening to the Shenandoah system is a shame and affects a lot of people like me personally. Its worthy of some focused attention by the State of Virginia and its DEQ. Therefore I ask that DEQ include the three sections of the Shenandoah River on 305B/303D list, determine why we are having these algae problems, and to develop and implement a plan to make them go away.

Thank you for considering these comments,

Francis C. Steinbauer
2501 Fowlers Lane
Reston, VA 20191

Jeff Kelble <jeff@potomacriverkeeper.org>

River letter

George Patterson <gpatterson@thedownstreamproject.org> Fri, Apr 13, 2012 at 12:28 PM

To: Jeff Kelble <jeff@shenandoahriverkeeper.org>

April 13, 2012
John Kennedy
DEQ Office Of Water Monitoring and Assessment
PO Box 1105
Richmond VA 23218
RE:Draft 2012 305(b)/303(d) Water Quality Assessment Integrated Report

Dear Mr. Kennedy,

As a resident of Clarke County, we are blessed to be bordered to our east by the Shenandoah and The Blue Ridge Mountains. I enjoy this stretch of river mostly in a kayak taking video and still pictures. I do this for fun and the love of the river. I also am trying to document the state of this iconic national treasure. I helped produce a documentary film released in 2007, titled "Shenandoah; Voices of The River." The film as told through the voices of people who had a stake in the rivers past and future, spoke about the rivers place in history, it's storied beauty, it's economic importance, brutal legacy environmental challenges, and most of all, the stake all of it's residents had in improving it's water quality as an example to others in the greater Chesapeake Bay watershed. I am concerned about what progress we are making.

My first actual, on stream, concern about an increased algae problem was in October 2009. We were on an educational trip canoeing with a group of students from Shenandoah University. Port Republic was our starting point, and after a presentation about ongoing efforts at Dupont in Waynesboro it was not long into our trip that mercury concerns turned into seeing algae blooms along the way; not typical in late October. The students took part in Benthic testing, with the help of The Chesapeake Foundation, near the Coors plant (good results). Virginia Department of Game and Inland Fisheries did a fish shock to show us the diverse aquatic population in the river. We toured landowner riparian restoration efforts and unfortunately saw the other end of the spectrum; terrible stream bank erosion on farms not employing the best of farm management practices. We finished at Andy Guest State Park with Shenandoah Riverkeeper Jeff Kelbe, along for the last 20 miles or so. The Bottom line for that whole 75 mile trip; we got to see many problems and hope for the river. Hope, being mostly in citizen participation and one of the largest problems; Algae.

Recently my experiences on the river have been kayaking during the summer months, 2008- 2012. I've also photographed different sections of the river ongoing since 2007. The algae in the summer goes from noticeable to annoying. The noticeable involves the encroachment this stuff has on grasses, fish nests, as well as many other unseen habitats; stated simply, you can't see the damage because of the algae. The annoying involves more the smell and actually having to paddle around the heavy blooms. Photographically, most places you go, a bad picture can be had. A picture is worth a thousand words, good or bad. Clean pictures are there, only they require a bit more work.

Recently, Spout Run, in our county, was added to the dirty waters list and our organization hopes to be a force in cleaning up this stream. It is a small watershed, but it provides a snap shot of problems in much larger watersheds. It has agricultural impacts, two new residential neighborhoods have runoff issues, educational opportunities at two schools that are bordered by it, a sewage treatment plant pipes into a tributary, a golf course shares it boundaries, Project Hope is located there as well as The University of Virginia's State Arboretum. So you see, with a conservation minded community here, we see a bright future to take this stream off the impaired list. What a great example that would be to others in the state; that it can be done. We plan to document this effort as well as help with outreach and support by, among other things, providing a website as a virtual meeting place for watershed information and citizen involvement.

Spout Run, as a small stream has a chance through citizen effort to be de-listed. The bigger picture now arises, pardon the metaphor, but does all that effort get washed away as soon as that stream flows into the Shenandoah River? I know quite a few people who care and work tirelessly for better water quality in this watershed. I think great consideration should be taken in helping them give the Shenandoah River and it major tributaries the same chance as its smaller stream and tributaries. Please consider adding them to the 305(b)/303(d) list of impaired or unhealthy waters.

Thank you and everyone at DEQ for what they do!

Thanks for your consideration and kindest personal regards.

All Best!

George F. Patterson

George F. Patterson
PO Box 1000 | Berryville, Virginia | 22611
Cell: (540) 550-2089 | Office: (540) 955-6066

Dr. Hays Blaine Lantz, Jr.
P. O. Box 744, 236 Ellis Road
Telephone 540-856-2452
hlantz4446@gmail.com

April 14, 2012

John M. Kennedy
DEQ Office of Water Monitoring and Assessment
P.O. Box 1105
Richmond, Virginia 23218-1105
RE: Draft 2012 305(b)/303(d) Water Quality Assessment Integrated Report

Dear Mr. Kennedy:

The purpose of this correspondence is to communicate my concerns about the increasing nuisance algae problem in the North Fork of the Shenandoah River and the tributaries that are part of this watershed. I have lived on Stony Creek, a tributary to the North fork, and Lake Laura in Bryce Resort for almost thirty years. My background is in biology (three degrees) with much work in aquatic ecology, and I along with Friends of the North Fork have conducted water quality research on the Lake Laura watershed for many years. In addition, I am most familiar with past research on the Lake Laura watershed, including three scientific studies conducted by Dr. Thomas Grizzard of Virginia Tech and Aquatic Resources, Inc. Stony Creek and Lake Laura are part of my daily existence as I can look upon them from the deck of my home. I fish, boat, and walk around Lake Laura and the North Fork a minimum of 40-50 days per year.

The algae problem in Lake Laura and Stony Creek began to be noticeable in the early 1980's and has gotten increasingly worse with each successive year. Tremendous algal blooms now occur yearly in Lake Laura and cover about 1/3 of the surface. This growth is well documented by the Virginia Department of Game and Inland Fisheries, Dr. Grizzard, and me. The algal blooms create an environment in which boating, fishing, and swimming are next to impossible in Lake Laura and Stony Creek. .The recreational value and use of Lake Laura is decimated each year in the months of July through October. My use and enjoyment of Stony Creek and Lake Laura diminish significantly each year during this period of time. It is difficult if not impossible for me to take water samples during these months in Lake Laura for my studies Because of the algal blooms and subsequent decay, the odors emanating from Lake Laura are almost unbearable, to the point where my wife and I cannot sit on our deck and enjoy the vista.

Because of this situation with Stony Creek and Lake Laura I am requesting that the North Fork, South Fork and main stem of the Shenandoah River be added to Virginia's impaired waters 303D/305B list. This problem is as significant, if not more so, as many other impairments.

Respectfully submitted,

Dr. Hays B. Lantz, Jr.

Jeff Kelble <jeff@potomacriverkeeper.org>

Algae Letter

Hays Lantz <hlantz4446@gmail.com> Mon, Apr 16, 2012 at 4:55 PM
To: Jeff Kelble <jeff@shenandoahriverkeeper.org>

Hi Jeff,

I just finished conducting a BMI study at Bergton and noted that algae was starting to grow in the North Fork near these headwaters.

I would like to continue our discussion regarding Lake Laura as it has to be a significant contributor to algae problems in Stony Creek and the North Fork.

[Quoted text hidden]
--
Dr. Hays B. (HB) Lantz, Jr.
236 Ellis Road, PO Box 744
Basye, VA 22810
540-856-2452 (Home)
540-624-9019 (Mobile)

"Success isn't how far you got, but the distance you traveled from where you started."

John M. Kennedy
DEQ Office of Water Monitoring and Assessment
P.O. Box 1105
Richmond, VA 23218-1105

Dear Mr Kennedy

I am writing to you to have you consider placing the Shenandoah River on the Virginia list of impaired waterways.

I am a frequent user of the river, an avid fisherman, outdoorsman, naturalist and photographer. I spend many days on our states rivers, but the beauty and proximity of the Shenandoah make it my favorite...until the last few years.

The last few years, starting about the end of June, I find long stretches of the river, choked with algae. This emerald green slime coats rocks in the river, making it dangerous to wade and then breaks off and becomes suspended in the water making it difficult to fish. This algae is not a 'normal' condition for the river. It's a direct result of excess nitrogen and phosphorus in the river. Cleaning a hookful of this stuff off every time you reel in a lure gets old very quickly. I do a lot of 'sight-fishing', looking for fish activity, or bait fish being chased, and bird activity that tells me larger fish are present in that area. When this algae moves in and coats the bottom, the fish move out because they can't survive in that area. There's no oxygen. Which also means the fish-eating birds I so enjoy photographing move out as well. I recall an area of the south fork near Andy Guest State Park that was almost completely coated in the emerald green goo. I did finally find a bare area2 miles downriver. This area was about the size of my dining room and there was at least 3 dozen large fish milling around it, trying to breath and find some food. But it's not just near the park. I have found it up beyond Luray near Newport, as well as Karo landing, Front Royal landing and on into the main stem. The North Fork gets almost completely clogged with this stuff due to its shallowness.

As the summer wears on, the blue-green algae begins to show up in the low-water eddies and backwaters of the river. This stuff not only smells horrendous, it is also toxic to people and animals. I can't imagine allowing my kids to come in contact with any water where blue-green algae is growing. I don't care to come in contact with it either. I find myself traveling further afield to other waterways, and other states even, to find water where I can fish and pursue my passions. But not before I spend a good deal of time scrubbing my kayaks and pontoons boat. I don't care to inflict this on anyone else's river.I would strongly request that DEQ place both forks and the main stem of the Shenandoah River on Virginia's impaired waters 303D/305B list so that a proper plan and funding for it's cleanup and improvement can be formulated. I think all Virginians would appreciate being able to point to such a nationally famous and historic river as a point of pride and not as a river with a pollution problem.

Herschel L. Finch
Conservation Chairman
Potomac River Smallmouth Club
310 Oakridge CT.
Front Royal , VA 22630
540-270-1590

April 6, 2012
John M. Kennedy
DEQ Office of Water Monitoring and Assessment
P.O. Box 1105
Richmond, VA 23218-1105
RE: Draft 2012 305(b)/303(d) Water Quality Assessment Integrated Report

Dear Sir:

I am writing you as I am deeply concerned about the declining water quality and overall condition of the South Fork of the Shenandoah River, particularly the stretch between the low water bridge at Bentonville and the now closed campground at Hazard Mill. I used to fish this particular stretch of the Shenandoah many years ago and have always considered the fabled Shenandoah as one of the premiere waterways, not only in the state of Virginia, but also in the entire Mid Atlantic region.

During the 1960s, I fished this stretch of the river at least once a week and sometimes would camp for days at a time at the Hazard Mill recreation area some five miles upstream from the low water bridge at Bentonville. I have fond memories of my early days on the river as I recalled the crystal clear waters and rocky river bottom broken up by occasional rocky ridges which held great numbers of beautiful smallmouth bass. I continued to fish the river during the 1970s and 1980s and in dismay watched the quality of the river decline markedly. I first noticed the algae blooms , which were practically nonexistent during my early years on the river, become quite prevalent as time wore on. The last time I fished the Shenandoah was sometime during the 1990s. The algae had become a real pain as you had to clean your lure after every cast and the algae had taken on a real noxious odor which greatly diminished what I had always considered a memorable visit to what was then one of Virginia's best waterways. Considering the money and effort the state of Virginia has spent creating the Andy Guest State Park, which I presume was meant to replace the old Hazard Mill recreation area, one would think that you would want a pristine river which would attract plenty of fishermen and other outdoor enthusiasts. Unfortunately, this does not seem to be the case. As a matter of fact, the last time I fished the Shenandoah, it was in the Guest state park.

Finally in 2008, I decided to visit the low water bridge at Bentonville to see if the river quality had improved since I last fished there in the 1990s. It was during a particularly hot period in July. I was shocked to see the amount of algae both upstream and downstream from the bridge. The slime that clung to the rocks was clearly evident and the noxious odors that I first noticed in the 1990s had become worse. It is a sad epitaph for a river that so many considered one of the premier rivers in the Mid Atlantic.

It is for this reason that I implore the Virginia Department of Environmental Quality to include the three sections of the Shenandoah River on 305B/ 303D list, determine why we are having these algae problems, and to develop and implement a plan to make them go away. It is imperative

that the state of Virginia take the necessary steps to insure that this wonderful waterway is restored to its past pristine quality. Thank you for considering my comments.

Sincerely,

John F. Ehrlich

John F. Ehrlich
522 N. Norwood St.
Arlington, VA 22203
E-mail: bluesally@verizon.net
Phone: 703 527-5806

March 27, 2012
John M. Kennedy
DEQ Office of Water Monitoring and Assessment
P.O. Box 1105
Richmond, Virginia 23218-1105
RE: Draft 2012 305(b)/303(d) Water Quality Assessment Integrated Report

Dear Mr. Kennedy,
I am writing to request that Virginia's Department of Environmental Quality add the North Fork, South Fork and Main Stem of the Shenandoah as impaired waters on thee 2012 305(b)3/303(d) Water Quality Assessment Integrated Report because of the severe algae problems the river experiences every year.

I run a fishing camp for children every summer. In the past 3 years we have taken over 400 children to fish the North Fork, South Fork and Main Stem of the Shenandoah a whole lot and I also use all three rivers personally to take my family canoeing and rafting and have done so since 1982. However, for much of the past few years, large sections of each river seems to experience a series of noxious algae blooms that seriously diminish my use and enjoyment of the rivers.

In each of the past six years during late winter a planktonic algae blooms, usually after a high water event, but not always. Over the course of the year I use the entire river system and have seen this on all three rivers from Port Republic down to Front Royal, from Broadway to Front Royal and from Front Royal to the Confluence with the Potomac River. This bloom turns the river a dark murky green color, like green paint, from late winter until about July. When the algae blooms, I opt to take the children to fish other bodies of water so they can catch more fish and swimming safely and comfortably. Last year was a particularly poor year for fishing for the children in my camps and they requested we spend more time elsewhere. Whenever the river is this murky color, it's disturbing to us and the animals that we encounter. The numbers of fish that we catch are lower and the fish are usually lethargic. Activity in the river drops to near zero. For the past two years I simply could not fish the lower south fork or the entire MainStem from July through August because of the plankton green water.

By mid May or June each year as the river clears the planktons seem to die back. But then a filamentous algae (rock snot) takes hold covers the river bottom and the native grasses which are trying to emerge. The native grasses turn brown and lose their leaves and remain bare the rest of the year. Additionally, a floating bubbly type of algae (I'm told its Cynabacteria or Blue Green Algae) comes at this time. Clumps rise up to and float on the water surface and then accumulates on anything sticking out of the river and in back eddies. This algae smells like sewage or rotting broccoli. When these algaes are blooming I literally do not want to be anywhere near the river and I often choose to go somewhere else. When I have no other place to go then it just takes all the enjoyment out of my day. The fishing is frustrating because you cannot fish without fouling your line on the algae, the fish won't bite lures or bait with algae sticking to it. I find being around the river very unpleasant due to the odors and annoyance. I found this problem in the following areas last year from July through August; Broadway, Lupton, Deer Rapids, Edinburg, Woodstock, Strasburg, Front Royal, Goods Mill, Luray Dam, 211, Alma, Port Republic, Warren Dam, Shenandoah, Route 50, Route7 among other places.

Finally, in the fall each year the native grasses die back and a bright green algae again covers the entire river. These algaes make it nearly impossible to fish and again diminishes and sometimes eliminates my ability to enjoy the river.

This cycle of algae diminishes both my use and my enjoyment of the Shenandoah River system. As a result I would like to ask that DEQ include the three sections of the Shenandoah River on 305B/303D list, determine why we are having these algae problems, and to develop and implement a plan to make them

go away.
Thank you for considering my comments,

Mr. John D. Lipetz
2717 Pioneer Lane
Falls Church, VA 22043
Email: jdlipetz@fishandexplore.com
Phone: 703-609-8083

John M Kennedy
DEQ Office of Water Monitoring and Assessment
PO Box 1105
Richmond VA 23218-1105
RE: Draft 2012 305(b)/303(d) Water Quality Assessment Integrated Report

Dear Mr. Kennedy,

My name is John Politz. I am a native and resident of Louisiana. Awhile back, I had the pleasure to live in the Shenandoah Valley for a few years and to have the opportunity to enjoy my favorite activities of fishing and hunting.

During the years of 2000 - 2003, I fished all over the Shenandoah River, the South Fork and the Main Stem. However, the North Fork was where I spent most of my time fishing. I saw problems with algae at all areas of the river.

Because of the problems with the algae, fishing became a hassle with having to avoid it for it would get tangled up on the line. There isn't much worse than having an enjoyable day of fishing ruined by some algae. I still return to the valley and visit old friends every few years. I like to take the opportunity while here to get some fishing in. I spend money at the local businesses because of fishing but if the algae continues to worsen, then fishing might not be on my list of things to do while here.

The problems of the river have made me decide to write the VA DEQ to say that the Shenandoah River needs to be included on the impaired waters 303D/305B list. The Shenandoah River is a great river and I tell people wherever I go that it has some of the best fishing in the country. Please don't make a liar out of me and take care of your river.

Respectfully,
John Politz
535 Broadmoor Blvd
Shreveport, LA 71105

Jeff Kelble <jeff@potomacriverkeeper.org>

Form submission from: Take Action!- Help the Shenandoah Riverkeeper Get Rid of Algae

Potomac Riverkeeper <sarah@potomacriverkeeper.org> Sun, Apr 15, 2012 at 11:56 AM

To: jeff@potomacriverkeeper.org

Submitted on Sunday, April 15, 2012 - 11:56am
Submitted by anonymous user: [76.120.244.178]
Submitted values are:

Your Letter:
Mr. Kennedy,
As an outdoorsman and father who recreates in the South Fork and main stem of the Shenandoah River 4 to 5 times a year, I urge you to add the Shenandoah River system (NF, SF, and main stem) to Virginia's impaired waters 303D/305B list due to reduced water clarity and increased amount of algae.

For over 20 years, I have camped, canoed and fished on the SF of the Shenandoah near Newport, VA every April and September. I float and fish the SF of the Shenandoah from Luray to Front Royal at least once a year. I also wade fish the main stem of the Shenandoah River near Harpers Ferry multiple times every summer for over 30 years. During that time, I have noticed a gradual reduction in water clarity and increases in algae in the river. During the most recent 10 or so years, these clarity and algae issues have increased dramatically to the point where these issues have diminished my enjoyment considerably on every outing on the river. Even the smell of the river in the summer makes me question wading in the Shenandoah. As a result, I now consider not going to the river when the opportunity arises. I also second guess recommending the Shenandoah River to new acquaintances, as their experience is likely to be marred by the algae and water clarity issues.

I have always been proud to be a Virginian and of the Shenandoah River. She is still visually beautiful from a distance and the valleys she flows through are still a delight to travel through. I still catch some fish when I do go, but not like in year's past. Her resiliency is great, characterized by the increasing, but lagging recovery from the fish kills a few years ago. Only diligent efforts by the citizens' of the Old Dominion's with the help of our great State, can we bring the Shenandoah River back to her former glory.

Thanks in advance for your consideration. I can be reached at:

Michael E. Dunn
21370 Ashburn Run Place
Ashburn, VA 20147

(703) 723-4443

Name: Michael E. Dunn
Email: med5987@comcast.net

The results of this submission may be viewed at:
http://www.potomacriverkeeper.org/node/428/submission/823

March 27, 2012
John M. Kennedy
DEQ Office of Water Monitoring and Assessment
P.O. Box 1105
Richmond, Virginia 23218-1105
RE: Draft 2012 305(b)/303(d) Water Quality Assessment Integrated Report

Dear Mr. Kennedy,
I am writing to request that Virginia's Department of Environmental Quality add the North Fork, South Fork and Main Stem of the Shenandoah as impaired waters on thee 2012 305(b)3/303(d) Water Quality Assessment Integrated Report because of the severe algae problems the river experiences every year.

I fish the North Fork, South Fork and Main Stem of the Shenandoah extensively and I also use all three rivers to take my family canoeing and camping. However, for much of the year, large sections of each river seems to experience a series of noxious algae blooms that seriously diminish my use and enjoyment of the rivers.

In each of the past six years during late winter a planktonic algae blooms, usually after a high water event, but not always. Over the course of the year I use the entire river system and have seen this on all three rivers from Port Republic down to Front Royal, from Broadway to Front Royal and from Front Royal to the Confluence with the Potomac River. This bloom turns the river a dark murky green color, like green paint, from late winter until about July. When the algae blooms I often choose not to swim or fish and I don't like to take my family swimming because they don't like the off-color murk water. When I do fish I find the fishing is poor and I don't enjoy the experience as much. Whenever the river is this murky color, it's disturbing to fish and the fish are usually lethargic and often they don't feed at all. Activity in the river drops to near zero. For the past two years I simply could not fish the lower south fork or the entire MainStem from July through August because of the plankton green water.

By mid May or June each year as the river clears the planktons seem to die back. But then a filamentous algae (rock snot) takes hold covers the river bottom and the native grasses which are trying to emerge. The native grasses turn brown and lose their leaves and remain bare the rest of the year. Additionally, a floating bubbly type of algae (I'm told its Cynabacteria or Blue Green Algae) comes at this time. Clumps rise up to and float on the water surface and then accumulates on anything sticking out of the river and in back eddies. This algae smells like sewage or rotting broccoli. When these algaes are blooming I literally do not want to be anywhere near the river and I often choose to go somewhere else. When I have no other place to go then it just takes all the enjoyment out of my day. The fishing is frustrating because you cannot fish without fouling your line on the algae, the fish won't bite lures or bait with algae sticking to it. I find being around the river very unpleasant due to the odors and annoyance. I found this problem in the following areas last year from July through August; Broadway, Lupton, Deer Rapids, Edinburg, Woodstock, Strasburg, Front Royal, Goods Mill, Luray Dam, 211, Alma, Port Republic, Warren Dam, Shenandoah, Route 50, Route7 among other places.

Finally, in the fall each year the native grasses die back and a bright green filamentous algae again covers the entire bottom of the river from Broadway to Strasburg. All three of these algaes make it nearly impossible to fish and again diminishes and sometimes eliminates my ability to enjoy the river.

This cycle of algae diminishes both my use and my enjoyment of the Shenandoah River system. As a result I would like to ask that DEQ include the three sections of the Shenandoah River on 305B/303D list, determine why we are having these algae problems, and to develop and implement a plan to make them go away.
Thank you for considering my comments,

Nick Elgas
13211 Sapphire ridge pl
Bristow VA, 20136
703-498-9723

WATERKEEPER® ALLIANCE

Potomac River Smallmouth Club

PO Box 1240
Vienna, VA 22183

Since 1988

April 1, 2012

John M. Kennedy
DEQ Office of Water Monitoring and Assessment
PO Box 1105
Richmond, VA 23218-1105

Regarding: Draft 2012 305(b)/303(d) Water Quality Assessment Integrated Report

Dear John:

As the President of the Potomac River Smallmouth Club, I represent the recreational angling interests of approximately 120 members. On their behalf, I want to express our deep, shared concern over the rapid decline of the Shenandoah in recent years. Don't let the name of our club fool you. During any given week of the spring or summer, many of my members use the Shenandoah for recreational angling and the club sponsors several trips a year to the South Fork.

Last year, my members complained consistently about the oppressive algae bloom that not only filled the Shenandoah, but spilled over into the Upper Potomac – impairing that fishery – as well. Beyond the algae, I have heard consistent, recurring complaints over odor and suspicious floating detritus of unknown origin that creates the urge to wash everything that came in contact with it immediately.

Without a doubt, water quality issues have diminished our enjoyment of the river and make it likely that we will diminish our use of the river in the coming year. This will have an unfavorable economic impact on the canoe outfitters we routinely use as well as associated restaurant and hotel businesses.

I urge you to take action now and include the North Fork, South Fork and the main stem on your impaired waters 303D/305B list. Please save our river!

Sincerely,

Steve Moore
President
703 638-0359
president@prsc.org

Steve Kimm
1933 Wilson Lane #T2
McLean, VA 22102

April 11, 2012

John M. Kennedy
DEQ Office of Water Monitoring and Assessment
P.O. Box 1105
Richmond, Virginia 23218-1105

RE: Draft 2012 305(b)/303(d) Water Quality Assessment Integrated Report

Dear Mr. Kennedy,

I am writing to request that Virginia's Department of Environmental Quality add the North Fork, South Fork and Main Stem of the Shenandoah as impaired waters on the 2012 305(b)3/303(d) Water Quality Assessment Integrated Report because of the severe algae problems the river experiences every year.

I fish the South Fork and Main Stem of the Shenandoah and I also use these rivers to take club fishing trips. However, for much of the year, large sections of each river seems to experience a series of noxious algae blooms that seriously diminish my use and enjoyment of the rivers.

In each of the past five years during late winter a algae blooms, usually after a high water event, but not always. This bloom turns the river a dark murky green color, like green paint, from late winter until about July. When the algae blooms I may still fish but I find the fishing is poor and I don't enjoy the experience as much. Whenever the river is this murky color, it's disturbing to fish and the fish are usually lethargic and often they don't feed at all. Activity in the river drops to near zero. For the past two years I simply could not fish the lower south fork or the entire Main Stem from July through August because of the plankton green water.

By mid May or June each year as the river clears the planktons seem to die back. But then a another algae (rock snot) takes hold and covers the river bottom and the native grasses which are trying to emerge. The native grasses turn brown and lose their leaves and remain bare the rest of the year. Additionally, a floating bubbly type of algae often comes about this time. Clumps rise up to and float on the water surface and then accumulates on anything sticking out of the river and in back eddies. This algae smells like sewage or rotting broccoli. When these algaes are blooming I literally do not want to be anywhere near the river and I am forced go somewhere else.

I personally found this problem in the following areas: Front Royal, Goods Mill, Luray Dam, 211, Alma, Port Republic, Warren Dam, Shenandoah, Route 50, Route7 among other places…

This cycle of algae diminishes both my use and my enjoyment of the Shenandoah River system. As a Virginian and a taxpayer I encourage DEQ to include the all sections of the Shenandoah River on 305B/303D list, determine why we are having these algae problems, and more importantly to develop and implement a plan to make them go away.

Respectfully,

Steve Kimm

John M. Kennedy
DEQ Office of Water Monitoring and Assessment
P.O. Box 1105
Richmond, Virginia 23218-1105
RE: Draft 2012 305(b)/303(d) Water Quality Assessment Integrated Report

Mr Kennedy,

My name is Tim England and I have lived here in Winchester VA since 1998. Before moving here, I was in the military and lived in TN, TX, AR, MT, MS and CA. I have fished and hunted in what I would consider some of the the finest areas of our country. I can say without hesitation that the best river that I ever fished was the Shenandoah River. That includes the Mississippi River, Tom Bigbee Waterway and the Missouri River in Montana. Some of my best memories were floating down the South Fork in 1999- 2001. That all came to a halt. SInce then, the combination of the fish kills and the algae on the river have taken this great resource and ruined it. The fishing has become so unproductive, that I have essentially stopped river fishing.

Usually April brings solid rainfall and great fishing. By June, due to low water and hot temperatures, the river becomes an eyesore and stops becoming an asset to the state. I don't know what is causing this, but this I do know. Other states like Montana (with Whirling Disease) become very proactive when evasive infestations threaten the waters. Even Maryland has become very forward thinking. They are aggressively battling the Snakehead, require watercraft exclusivity on lakes like Liberty and Prettyboy and have enacted a new law requiring rubber wading boots on rivers instead of felt to prevent invasive species.

The effects of weeds and algae have certainly gotten out of control in VA. Every year, the weed growth in Lake Frederick becomes thicker and deeper in the lake. Since I don't fish the Doah anymore, all I have to go by is the sight of algae on the river as I drive on route 7 on my way to work. I can tell it is getting worse, thicker and deeper each year.

Hopefully, the tide will turn for this great river which has become the example for all other VA rivers NOT to follow. Hopefully, it will return the glory years of not so long ago.

Thanks,
Tim England
92 Abbey Rd
Winchester VA 22602