Estuaries and Coasts (2011) 34:221–231
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 / Published online: 29 May 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

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).

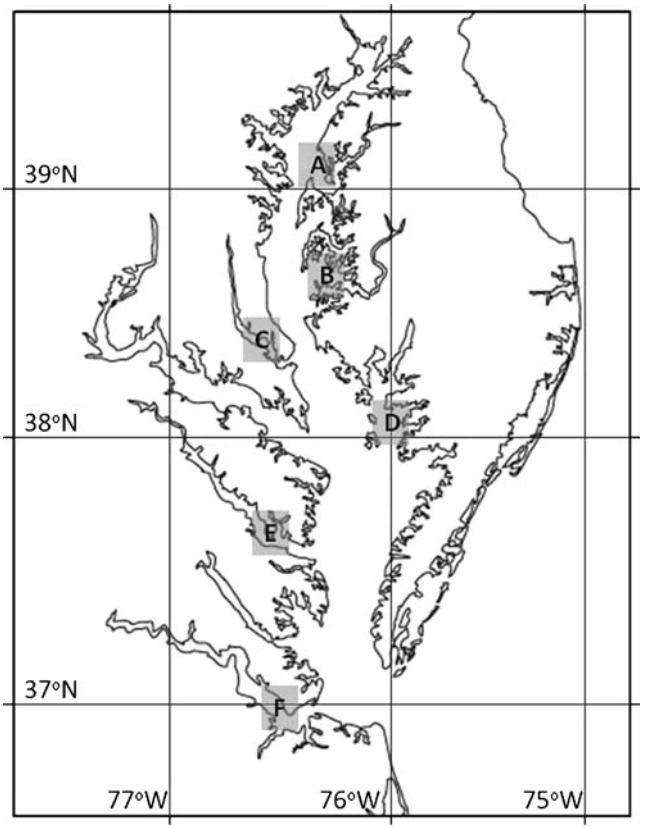


**Fig. 1** Map of Chesapeake Bay indicating areas of historically important oyster grounds. Letters correspond to mesohaline areas in which pH data were analyzed in Table 1, as follows: **a** Chester River, **b** Choptank River, **c** Patuxent River, **d** Tangier Sound, **e** Rappahannock, and **f** 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 ($\alpha$=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 $\alpha$=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

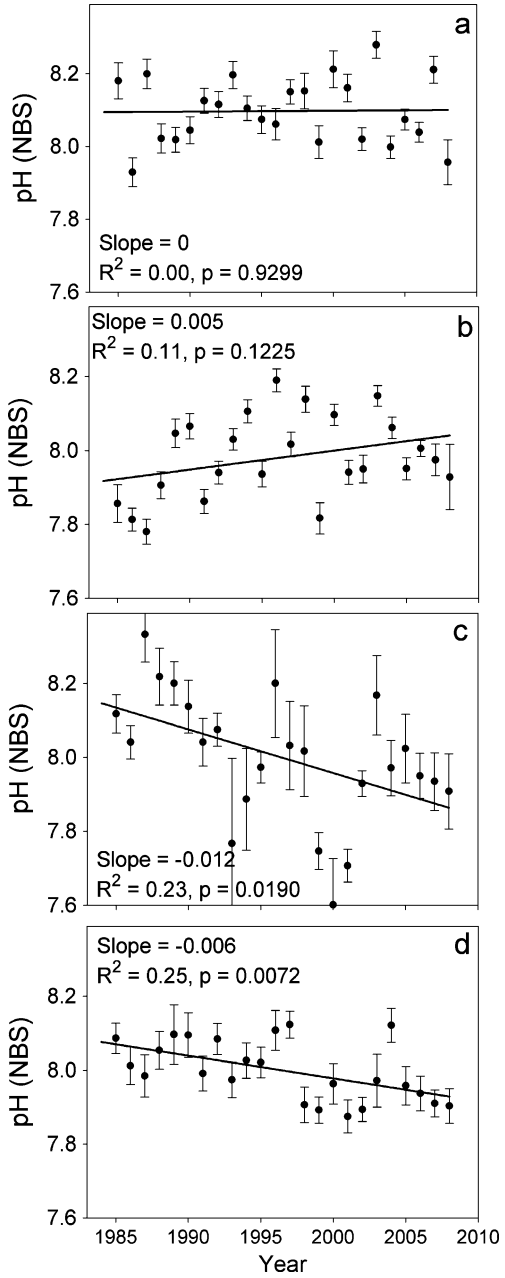


**Fig. 2** Annual trends in Chesapeake Bay surface water pH in the **a** Spring-Mesohaline, **b** Summer-Mesohaline, **c** Spring-Polyhaline, and **d** Summer-Polyhaline. Mesohaline includes salinities of 5–18, and polyhaline are salinities >18. Mean values are based on surface waters of 1 m or less, 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.

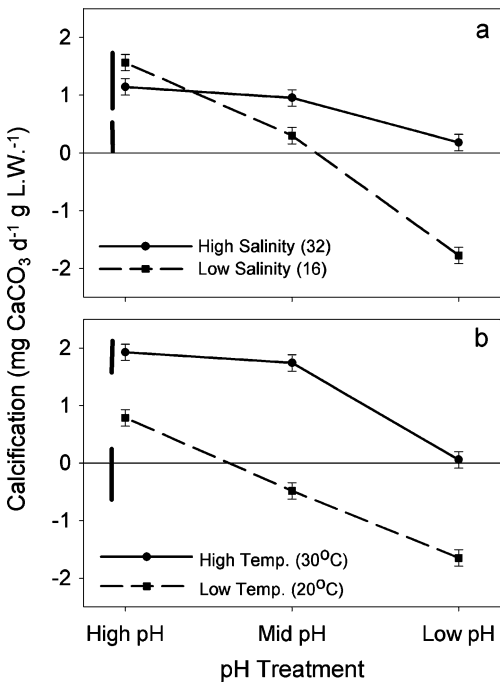


**Fig. 3** Biocalcification rates of *C. virginica* for treatment combinations of **a** salinity by pH and **b** temperature by pH estimated by least squares means from each two-way ANOVA. Units of calcification are milligram of calcium carbonate per gram oyster whole live weight per day. Treatment levels 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 $\alpha=0.05$

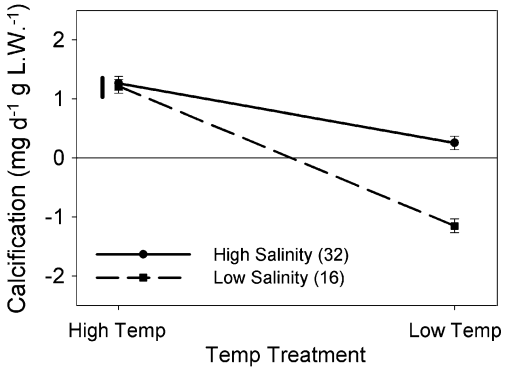


**Fig. 4** Biocalcification rates of *C. virginica* for treatment combinations of temperature and salinity estimated by least squares means from the two-way ANOVA. Units of calcification are milligram of calcium carbonate per gram oyster whole live weight per day. Treatment levels of salinity, temperature, 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 $\alpha=0.05$

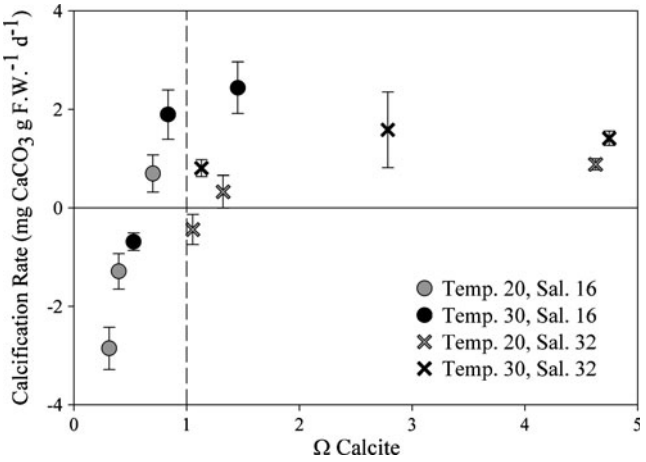


**Fig. 5** Biocalcification rates of *C. virginica* plotted against saturation state of calcite. Units of calcification are mg of calcium carbonate per gram oyster whole live weight per day. Temperature and salinity treatments are noted as follows: 20°C is in *gray*, 30°C in *black*, salinity 16 (*filled 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 predawn 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.