Estuaries and Coasts
DOI 10.1007/s12237-014-9805-6

On the Response of pH to Inorganic Nutrient Enrichment in Well-Mixed Coastal Marine Waters

Scott W. Nixon · Autumn J. Oczkowski · Michael E. Q. Pilson · Lindsey Fields · Candace A. Oviatt · Christopher W. Hunt

Received: 30 August 2013 / Revised: 20 December 2013 / Accepted: 10 March 2014

Abstract Recent concerns about declining pH in the surface ocean in response to anthropogenic increases of carbon dioxide (CO_2) in the atmosphere have raised the question of how this declining baseline of oceanic pH might interact with the much larger diel and seasonal variations of pH in coastal marine ecosystems. Nutrient enrichment, which can amplify both production and respiration, has the potential to reduce or exacerbate the impacts of ocean acidification in coastal waters. Here, we present results from a multi-year experiment in which replicate phytoplankton-based mesocosms with a 5-m deep well-mixed water column (salinity = 27–31) and intact benthic community were exposed to a gradient in daily inorganic nitrogen (N), phosphorous (P), and silica (Si) addition. We show that the response of water column pH to nutrient enrichment was the greatest during the autotrophic winter-spring period, and there was no significant decline in pH across treatments during the heterotrophic summer-fall period. We believe that the differences in response lie in the seasonal cycles of production and respiration, where spring production peaks are large and discrete, and respiration is more temperature-driven but occurs diffusely throughout the year. The observed basification associated with enhanced nutrient inputs may have consequences for phytoplankton community structure, some species of submersed aquatic vegetation, cycling of Si, and perhaps other ecological processes.

Keywords Coastal acidification · pH · Nutrient enrichment · Eutrophication · Mesocosms · Narragansett Bay

Communicated by Hans W. Paerl

S. W. Nixon · M. E. Q. Pilson · L. Fields · C. A. Oviatt
Graduate School of Oceanography, University of Rhode Island, South Ferry Rd., Narragansett, RI 02882, USA

A. J. Oczkowski (✉)
Atlantic Ecology Division, U.S. EPA, 27 Tarzwell Dr., Narragansett, RI 02882, USA
e-mail: oczkowski.autumn@epa.gov

C. W. Hunt
Ocean Process Analysis Laboratory, University of New Hampshire, 8 College Rd., Durham, NH 03824, USA

Introduction

For over 75 years, scientists have known that pH can vary in the surface waters of the open ocean by a tenth of a unit or more in response to the biological production and consumption of CO_2. Likewise, it is known that larger variations in pH occur in more highly metabolic coastal areas such as estuaries (e.g., Sverdrup et al. 1942). As a practical matter, however, this knowledge did not stimulate the measurement of pH in open ocean or coastal waters, and the application of pH to questions of biological metabolism in marine ecology was slow and halting until recently (Hinga 2002).

The few early measurements cited in Sverdrup et al.'s classic 1942 text were all based on colorimetric techniques that were only accurate to about 0.1 unit (Szabadváry 1960). The modern electronic pH meter was invented by Kenneth Goode in the 1920s and manufactured commercially in Europe and the USA beginning in the mid 1930s (Hines and de Levie 2010). The accuracy of specially built electronic pH meters improved rapidly from 0.1 in 1922 to about 0.002 units in 1931, though such high precision was not available commercially until decades later (Hines and de Levie 2010). In his early review of techniques for measuring primary productivity in aquatic systems, Ryther (1956 p. 77) was not optimistic about the use of pH-CO_2 methods because "With the equipment available in the average laboratory (i.e., a Beckman pH meter) a change of 0.1 pH unit probably represents the limit of sensitivity." Since this was equivalent to about 0.5 g C m^{-3} of seawater, pH seemed a dull tool for marine ecologists. With a

Published online: 31 March 2014

few exceptions (e.g., Park et al. 1958 used pH-CO_2 relationships to estimate diel metabolism in lagoons along the coast of Texas); Ryther's assessment casts a long shadow. Combined with the rapid adoption of Steemann Nielsen's (1952) highly sensitive ^{14}C technique for measuring phytoplankton production, attention turned away from emerging opportunities to measure total system inorganic carbon metabolism with the rapidly increasing capability of commercial pH meters.

Interest in the pH of marine ecosystems began to increase in the early 2000s, particularly after June 2005 when the issue of a UK Royal Society report described a declining pH in the surface 100 m of the world oceans and linking the post-Industrial Revolution decline of about 0.1 unit with increasing emissions of anthropogenic CO_2 to the atmosphere. The apparent surprise with which many in the marine sciences community greeted this announcement reflected the lack of attention that the previously unfashionable metric of pH had been receiving. The increasing concentrations of CO_2 in the atmosphere had been documented for many decades, and the chemical constants necessary to calculate changes in the accompanying equilibrium pH in the surface ocean had been known for decades as well. Nevertheless, the increasing frequency of reports like those put out by the Royal Society and others (like Caldeira and Wickett 2003) stimulated research and historical analyses of ocean and coastal pH data as well as added another dimension to concerns over increasing atmospheric CO_2 levels (e.g., Doney et al. 2009).

The growing unease over ocean acidification also caught the attention of coastal and estuarine ecologists who had developed a renewed interest in the total ecosystem metabolism of coastal systems and their role as net sources or sinks for atmospheric CO_2 (e.g., Nixon and Pilson 1984; Oviatt et al. 1986a; Smith 1991; Smith and Hollibaugh 1993; Kemp et al. 1997; Gattuso et al. 1998; Borges 2005; Laruelle et al. 2010; Cai 2011; Kemp and Testa 2011; Duarte et al. 2013). While other factors, particularly temperature, salinity, and alkalinity, influence the pH of coastal waters, it is high rates of photosynthesis and respiration, and associated uptake and release of dissolved inorganic carbon that commonly produce diel and seasonal changes in pH that are much larger than the secular changes observed to date in the surface ocean. For example, during summer Park et al. (1958) measured diel pH changes of 0.5 or more in some Texas lagoons. When the annual cycle of pH in the waters of lower Narragansett Bay (RI, USA), the site of the experiment that forms the basis of this paper, was first reported (Hinga 1992); it varied from a low of ~7.65 in late summer to a maximum of 8.5 during the winter-spring bloom for an annual range of 0.85. While salinity and alkalinity vary relatively little at this site (Frithsen et al. 1985a), an annual variation in water temperature from about 1 to 22 °C contributes to this seasonal variation, but it is difficult to make a precise assessment. Hinga (2002) calculated that pH levels due only to equilibrium with atmospheric CO_2 varied from a winter minimum of ~8.15 to a summer maximum of ~8.24, a 0.1 range that was completely out of phase with the observed pH cycle. As Hinga (2002) noted, the fact that the observed pH is virtually always considerably above or below this range of equilibrium pH, is evidence that metabolic exchanges of CO_2 within the bay are much faster than the physical exchanges of CO_2 across the air-water interface and the exchange of bay water with offshore waters. Temperature also appears to have a fairly minor, or at least secondary, impact on the pH variations observed in Narragansett Bay. For the western side of the bay, the maximum seasonal pH change that could be attributed to temperature is about 0.27, approximately a third of the range of 0.85 observed in 1989–1990 (Hinga 2002). Since there must be some exchange of CO_2 with the atmosphere, and the bay is supersaturated with CO_2 for much of the year (Oczkowski et al. 2010), the remaining annual pH change of 0.58 during that period is an underestimation of the annual pH cycle due to metabolism within the bay.

If we accept that variations in coastal pH are largely due to the production and consumption of organic matter, questions naturally arise about the potential impact of nutrient enrichment on pH changes in coastal waters (e.g., Howarth et al. 2011). To the extent that fertilization stimulates primary production, it will increase pH, while the subsequent respiration and consumption of that organic matter will reduce the pH. The respiration of organic matter carried into coastal systems from land drainage also has the potential to contribute to the acidification of coastal systems, as does CO_2 produced in the watershed and carried into coastal waters by land drainage. The contribution of wetland and terrestrial organic matter to estuarine respiration has been extensively and recently reviewed by Cai (2011) and Kemp and Testa (2011), and the link between allochthonous C inputs, the release of CO_2, and its potential effect on increasing coastal acidification seems straightforward once the issues of mass balance and accessibility of the organic C are known (neither of which are easy matters). However, the net effect of inorganic nutrient enrichment on pH in coastal systems is less straightforward, and may be strongly influenced by how well-mixed the system is. In some stratified water bodies, there have been reports of nutrient enrichment exacerbating the larger scale decline in oceanic pH (e.g., Cai et al. 2011; Duarte et al. 2013) while multi-decadal numerical simulations using complex, coupled river and shelf physical and biogeochemical models of the southern North Sea have shown "that the increase in primary production due to eutrophication could counter the effects of ocean acidification on surface water carbonate chemistry in coastal environments." (Borges and Gypens 2010, p. 352).

Our purpose here is to present experimental data that address the impact of inorganic nutrient enrichment on the annual cycle of pH in a well-mixed temperate coastal system. To this end, we revisit the results of a relatively long-term

(June 1981 through September 1983) inorganic nutrient enrichment experiment carried out using the large (13 m^3, 5 m deep) mesocosms at the Marine Ecosystems Research Laboratory (MERL) at the University of Rhode Island's Graduate School of Oceanography adjacent to the lower West Passage of Narragansett Bay. The purpose of the experiment was to establish a wide gradient of daily inorganic nutrient (nitrogen (N), phosphorous (P), silica (Si)) enrichment to mesocosms containing a well-mixed water column and an intact benthic community, and to observe the response of numerous biological and chemical variables.

Fortunately, pH was among those variables, as were temperature, salinity, and total alkalinity. Dawn-dusk-dawn changes in these parameters were analyzed during the last 9 months of the experiment to calculate daily net total ecosystem production and respiration through changes in total CO_2 for comparison with diel net metabolism calculated from changes in O_2 and water column production measured by ^{14}C uptake (Oviatt et al. 1986b). Aside from that exercise, the pH and alkalinity data collected weekly or biweekly throughout the experiment have largely been neglected, though many other results of the experiment have been published (e.g. Gearing et al. 1984; Hobbie and Cole 1984; Sullivan and Ritacco 1985; Berounsky and Nixon 1985; Grassle et al. 1985; Oviatt et al. 1986a; Nixon et al. 1986; Hunt and Kelly 1988). More than 30 years later, we revisit the MERL mesocosm experiments, now paying careful attention to those pH and alkalinity measurements, to improve our understanding of the impact of inorganic nutrient additions on acidification in well-mixed estuarine systems. We provide additional evidence for the importance of eutrophication and seasonal variability of production and respiration on pH values and suggest that these drivers may overprint climate change-associated ocean acidification in well-mixed estuaries.

Materials and Methods

The MERL Mesocosms

Descriptions of the MERL mesocosms are given in detailed data reports from the nutrient enrichment experiment (e.g., Frithsen et al. 1985a, b) as well as in publications cited above. Briefly, the facility consisted of 14 cylindrical fiberglass reinforced non-toxic polyester insulated mesocosms 5.5 m high and 1.8 m in diameter. Only nine mesocosms were used in the experiment discussed here. The mesocosms were white on the inside to maximize light and maintained out of doors in natural sunlight. Silt-clay sediments and associated organisms were carefully collected at the start of the experiment from mid-Narragansett Bay using a 0.25 m^2 box corer so as to maintain the vertical structure of the community as much as possible. The sediments were gently dropped into grid cells in 2.5 m^2 circular trays. When the trays were full, the grids were removed and the trays were placed at the bottom of the MERL mesocosms. The mean thickness of the sediments was about 35 cm. A 5-m deep water column (~13 m^3) was maintained over the sediments using water pumped (using a diaphragm pump) from a depth of 2 m off the dock at the Graduate School of Oceanography on the lower west side of Narragansett Bay (approximately 41.5° N, −71.4° W). Bay water was input daily at a rate sufficient to provide a mean water residence time in each mesocosm of 27 days, the long-term mean for Narragansett Bay (Pilson 1985). The depth of the water was sufficient that the sediments were below the 1 % light depth and remained heterotrophic as they are over all but the shallowest parts of the bay (mean depth 8.6 m). Glass heat exchangers were used to keep the water temperatures in the mesocosms within 2 °C of the bay. The water column in each mesocosm was mixed using a weighted rotating plunger (50 cm diameter) in a 60-cm elliptical orbit at 5 rpm for 2 out of every 6 h. The bottom of the orbit was 60 cm off the sediment surface. This provided mean suspended loads of about 4 g m^{-3}, similar to those in the adjacent bay, but produced greater vertical advection and lower horizontal advection compared with the bay (Nixon et al. 1980). Slight vertical temperature gradients developed during the four non-mixed hours during some days in the summer, but overall, the water columns were well mixed. The walls of the mesocosms were cleaned twice weekly during the summer and weekly in the rest of the year using pneumatically driven rotating brushes. The organic matter scrubbed from the walls remained in the mesocosms. Easy access to the mesocosms was provided by an elevated walkway attached to a dedicated laboratory on a hillside adjacent to the mesocosms.

Analytical Methods

Analytical methods used in this and many other MERL experiments are detailed in Lambert and Oviatt (1986). Of particular interest here are the measurements of pH and alkalinity described by Sampou (1989) which are more easily accessible in summary form in Oviatt et al. (1986b). Briefly, pH was measured using an Orion Ross Combination Electrode and a Beckman Model 71 Meter with a precision of ±0.02 units. All of the pH data presented are given in the National Bureau of Standards scale. Buffers (pH 4, 7, and 9) for standardization were made up monthly and placed in a water bath set at the temperature of the mesocosms. A working buffer (pH=7) was used to check the accuracy of the probe prior to sample analysis. Water samples were collected (usually at dawn and dusk) by siphon into 250 ml jars, overflowed twice, and sealed. Samples were quickly transferred to the water bath set at mesocosm temperature and read with the pH meter, then removed and allowed to come to room temperature.

Alkalinity was determined using the method outlined by Parsons et al. (1984). Acidified samples were titrated after at

least 1 h to assure degassing. Stored acidified samples were titrated within 7 days.

Experimental Design

The MERL nutrient addition experiment was designed to examine the response of water columns and associated benthic communities to a wide gradient of inorganic nutrient enrichment. Three mesocosms were run as unenriched controls which only received background inorganic nutrients that were added each day in the water from the bay and direct deposition of dissolved inorganic nitrogen (DIN) from the atmosphere on the water surface within the mesocosms. Atmospheric deposition is not a significant source for P or Si. Nutrient concentrations in the inflowing bay water were measured weekly and interpolated linearly to estimate daily inputs from this source. Atmospheric deposition of DIN, including NO_3^-, NH_4^+, and dry deposition as vapor and aerosol were taken from Fraher (1991) who reported fluxes made on nearby Prudence Island, in mid-Narragansett Bay. Additional mesocosms were assigned randomly to a geometric gradient of enrichment of 1×, 2×, 4×, 8×, 16×, and 32× where × was the estimated N loading per square meter to Narragansett Bay as a whole from sewage effluent. In this paper, we focus on the control, 1×, 2×, and 4× mesocosms as they span the feasible range of nutrient inputs to this ecosystem. Dissolved inorganic P and Si were also added in the molar ratio of 13N:1P:1Si, a characteristic of urban sewage entering the upper bay (Frithsen et al. 1985a, b). The area specific inorganic nutrient inputs to the control mesocosms averaged:

$$\mathrm{DIN} = 1.1\ \mathrm{mmol\,m^{-2}d^{-1}}$$
$$\mathrm{DIP} = 0.2\ \mathrm{mmol\,m^{-2}d^{-1}}$$
$$\mathrm{DSi} = 1.6\ \mathrm{mmol\,m^{-2}d^{-1}}$$

The enriched mesocosms received the same background input plus multiples of the 1× treatment which amounted to as follows:

$$\mathrm{DIN} = 2.88\ \mathrm{mmol\,m^{-2}d^{-1}}$$
$$\mathrm{DIP} = 0.22\ \mathrm{mmol\,m^{-2}d^{-1}}$$
$$\mathrm{DSi} = 0.22\ \mathrm{mmol\,m^{-2}d^{-1}}$$

Nutrients were added daily during a mixing cycle as concentrated solutions of reagent grade salts (NH_4Cl, KH_2PO_4, and $Na_2SiO_3{\cdot}9H_2O$) in distilled water. The mesocosms were filled with sediments and water in April 1981, and regular additions of bay water and the standard mixing regime began at that time. Nutrient additions began in June 1981, and the experiment was terminated in September of 1983. While nutrients were added in an N:P ratio of 13:1 across treatments, the water column N:P ratio was often lower than this. For example, the average summer N:P ranged from 4 in the control tanks to 12 in the 2× tank, suggesting greater N limitation. However, these nutrient ratios do not appear to have had much of an impact on the phytoplankton community. In general, diatoms and ciliates increased with increasing nutrient enrichment while monads and flagellates (<10 μm) did not. Overall, species did not appear to change with nutrient treatment over the length of this experiment (Oviatt et al. 1989).

Salinity measurements were made only in the last year of the experiment (January to September 1983). Salinity values are needed to calculate pCO_2 and DIC concentrations, so we used the relationship between Narragansett Bay salinity and alkalinity demonstrated in Magnuson (1997) to estimate salinity for the duration of the experiment. As the conditions in the control mesocosms were the closest approximation to bay conditions, and salinity would not be altered by the nutrient inputs (but alkalinity would), we applied the salinity estimated from the control mesocosm alkalinity values to all of the mesocosms. This is in agreement with the available data, where salinity values were similar across treatments, but alkalinity values were not.

Both the total carbon dioxide (TCO_2) and pCO_2 were calculated from daily values of total alkalinity (titration alkalinity), pH, temperature, and estimated salinity (Pilson 2013).

Calculations and Statistics

By using the relationship $\mathrm{pH} = -\log[H^+]$, and then dividing by the hydrogen activity coefficient (f_H), we were able to calculate the concentration of H^+ in mole per kilogram water (Pilson 2013).

A daily carbon mass balance for each mesocosm was taken as the difference between inputs and exports and was calculated by first assuming that the TCO_2 concentration of seawater added to the fertilized mesocosms was equivalent to the TCO_2 measured in the control mesocosms. Then, the total input to each mesocosm was determined by multiplying the TCO_2 concentration in the control mesocosm by the volume of water in the mesocosm (13.1 m^3) to get a stock, then multiplying the stock by the daily seawater exchange rate of 3.8 % to get an estimate of the daily TCO_2 input to each of the enriched mesocosms. The export, via loss of dissolved TCO_2 from water leaving the mesocosm, was calculated by multiplying the mesocosm TCO_2 concentrations by the mesocosm water volume and the 3.8 % loss rate to estimate the dissolved TCO_2 loss.

Air-sea CO_2 fluxes (F, mmol m^{-2} d^{-1}) were roughly estimated as there has been no theoretical evaluation that adequately addresses the conditions involved with air-sea transfer of gases in the MERL mesocosms. The mesocosms are protected by a small lip above the surface of the water, and there is no fetch for the wind to gain a purchase upon the water. While very high winds would surely have some effects, there is no experimental evidence available. Low and average winds do not ruffle the surface of the water, and probably

serve only to replace the air above. The air-water exchange of gas must be controlled by the level of turbulence introduced by the mixer.

Bopp et al. (1981) evaluated the exchange coefficients for several gases, some of which were biologically inert, and employed a formulation based on the diffusion constant of the gas and the calculated thickness of the "unstirred" boundary layer at the surface of the water:

$$\text{Flux} = \frac{D}{z}\left([G]_I - [G]_W\right) = e_G\left([G]_I - [G]_W\right) \quad (3)$$

where e_G is the exchange velocity (m d^{-1}, or cm h^{-1}, etc.); $[G]_I$ is the concentration of gas exactly at the interface, assumed to be in equilibrium with the gas in the air above; $[G]_w$ is the concentration of gas in the bulk water; D is the diffusion constant of the gas; and z is the thickness of the so-called unstirred boundary layer.

If z is evaluated by measuring the flux for one gas, the calculated flux for another gas can be estimated by inserting the appropriate diffusion constant. Bopp et al. (1981) obtained a value of z=560±90 μm with radon at 20 °C and 600 μm (no uncertainty provided) with CF_2Cl_2 and with CH_4 at 10 °C. The salinity was about 30 ‰ in both cases. Diffusion constants of CO_2 can be simply incorporated into Eq. 3 via a simple algorithm. The diffusion constant for CO_2 is given:

$$D = 5019e^{\left(\frac{-19510}{RT}\right)}$$

where D is in units of 10^{-9} m^2 s^{-1}, R is 8.3145 J mol^{-1} K^{-1}, and T is in Kelvin.

To evaluate potential differences in pH and H^+ concentrations across treatments, data from 2-month windows during periods of peak production and respiration were compared using a one-way ANOVA. Differences among the three treatment means were tested by Fisher's least significant difference (LSD). All analyses were performed using SAS 9.3 statistical software. The probability for significance was $p<0.05$ for all statistical analyses.

Results

Consistent with the annual cycle in the lower West Passage of Narragansett Bay (Hinga 2002), the highest pH values in the mesocosms occurred during the winter-spring bloom, especially in 1981–1982 (Fig. 1). Measurements of phytoplankton standing crop (measured as chlorophyll), primary production (using ^{14}C uptake), production per unit of chlorophyll, and annual production all also increased along the nutrient enrichment gradient (Keller 1986).

As noted earlier, the 2× and 4× enrichments were a significant nutrient addition over the controls (which received only

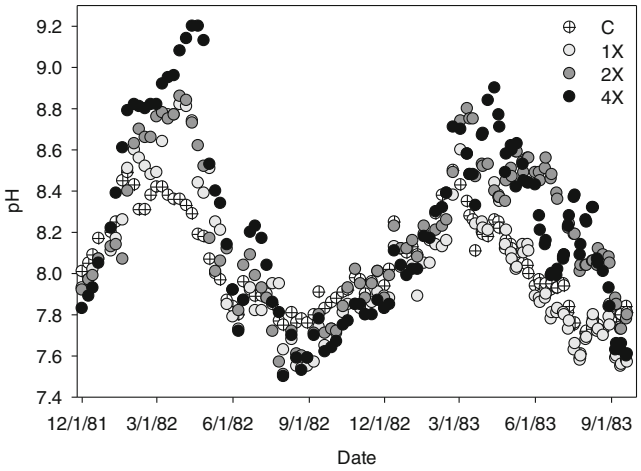

Fig. 1 Dawn pH in triplicate control and individual fertilized mesocosms

the nutrients in the lower West Passage inflow water) and the 1× treatment that received a DIN input per unit area equals to that from rivers and sewage effluent pro-rated over Narragansett Bay as a whole. The 2× enrichment approximated the increase in total N loading to Narragansett Bay from direct atmospheric deposition, land drainage, and sewage discharges between the early 1900s and 2005 (Nixon et al. 2008). The 4× treatment received a total DIN input of about 4.6 mol N m^{-2} y^{-1}, a fertilization rate greater than many agricultural crops and estuaries, including the Chesapeake Bay, the Potomac estuary, the Pamlico estuary, the Mobile Bay, and the Wadden Sea (Nixon et al. 1986).

Before presentation of pH data with more temporal resolution, it seems useful to document the close coupling of total system metabolism in the mesocosms as reflected by contemporaneous changes over an annual cycle in pH and inorganic nutrient concentrations (Fig. 2). Increases in pH are mirrored by declines in DIN, DSi, and DIP (latter not shown), while decreases in pH are similarly reflected in increases in the nutrients as organic matter is respired. These linkages are not only clear in the broad seasonal cycles but in a short intense diatom bloom (increase in pH) in late February to early March that was accompanied by sharp declines in DIN and DSi which were then reversed with the decline in pH following the end of the bloom.

The relationship between nutrients and pH in this experiment was apparent not just seasonally, with the rise and fall of the phytoplankton community, but also across the mesocosms with nutrient enrichment, where higher nutrient inputs generally led to higher pH values. During the 2-month period bracketing the spring peak in pH values, there were significant differences among the C, 1×, 2×, and 4× mesocosms in 1982 (February 1 to April 1, 1982) and between C, 1×, and the more enriched mesocosms in 1983 (2× and 4×, March 1–May 1, 1983, Table 1). However, the reverse does not seem to be true. During periods of respiration in the late summer and early fall, when the pH values were at their lowest, the consistent pattern between enrichment and pH disappears. The control mesocosm

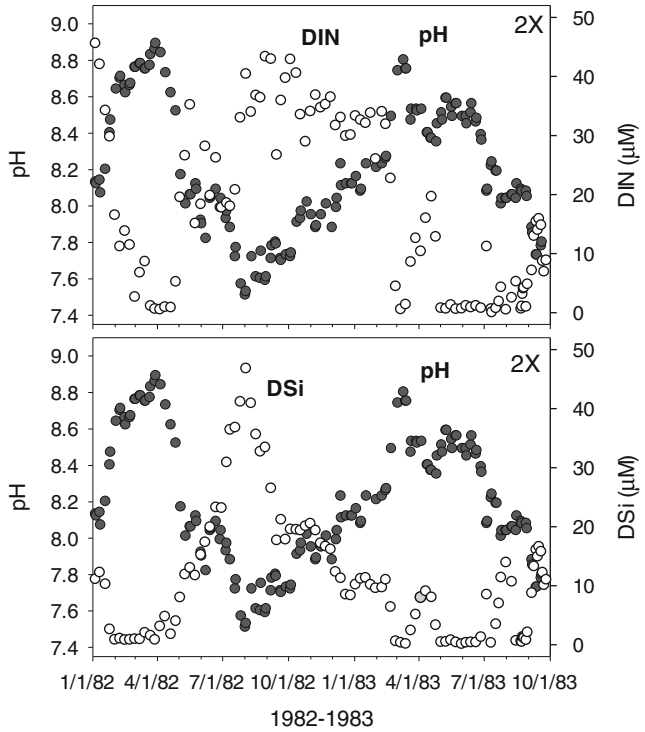

Fig. 2 Dissolved inorganic nitrogen (*top panel*) and dissolved silica (*bottom panel*) plotted with pH in the 2× mesocosm

has a slightly higher pH (7.8) than the 1×–4× mesocosms (~7.7), suggesting that the CO_2 concentrations in the tanks did not have enough time to equilibrate with atmospheric CO_2.

The dramatic appearance of the high pH values during spring production is partially attributable to the asymmetric response of pH to CO_2, which occurs because pH is measured on a log scale (Fig. 3). To see if the treatment effects were present in the actual H^+ concentrations, the pH data from the C, 1×, 2×, and 4× mesocosms were converted to H^+ concentrations (mol kg^{-1}) and, using a 2-month window to bracket the periods of lowest (February 1–April 1, 1982, March 1–May 1, 1983) and highest (July 15–September 15, 1982) H^+ concentrations, *t* tests (LSD) results indicate that the four mesocosms were significantly different from one another during the bloom of 1982 (February 1–April 1, 1982) and, while significantly greater than the C and 1× treatments, the 2× and 4× mesocosms were not distinguishable the following year (March 1–May 1, 1983). In contrast, during the late summer period, characterized by respiration (July 15–September 15, 1982), only the control mesocosm was distinguishable from the other treatments.

Our TCO_2 mass balance estimates indicate that the enriched mesocosms were largely net sinks for TCO_2, especially in the spring and early summer. But, sometimes, they were slight net sources in the fall and winter (Fig. 4). These trends are just opposite to source/sink patterns in oxygen concentration (Oviatt et al. 1986a). The TCO_2 balance appears to lag the pCO_2 in the mesocosms. After periods of high respiration (as indicated by high pCO_2), such as in August and September 1982, the mesocosm flux values become more positive and, in some cases, were net sources of inorganic C to the atmosphere. The mass balance was driven largely by the dissolved C inputs and outputs to and from the mesocosms; the CO_2 loss to the air was about 0.26 % of the total inorganic C loss from the mesocosms (atmosphere + outflow).

Discussion

Concern over the impact of increasing levels of atmospheric CO_2 on the acidification of marine waters has spread from the open ocean to our estuaries and coasts. Coastal ecologists have begun addressing this issue through observational and manipulative studies, where pH levels are decreased, and the resultant effects on flora and fauna are observed (Doney et al. 2009; Hendriks et al. 2010). While the purposes of these studies are to examine the sensitivity of various ecosystem components to reductions in pH and to determine thresholds at which deleterious effects are observed, the probability of such scenarios is, understandably, unclear. Although collected 30 years ago, prior

Table 1 Summary of results of post hoc multiple comparison tests for pH and H^+ data during the spring bloom periods of February 1, 1982–Aptil 1, 1982 and March 1, 1983–May 1, 1983 and late summer high respiration period of July 15, 1982–September 15, 1982

Treatment	Spring 1982 (*n*=9)				Spring 1983 (*n*=12)				Summer (*n*=9)			
pH												
C	8.37	A			8.22	A			7.8	A		
1×	8.63		B		8.34		B		7.68			B
2×	8.73			C	8.54			C	7.65			B
4×	8.89			D	8.63			C	7.67			B
H^+ (mol kg^{-1})												
C	5.27×10^{-9}	A			7.74×10^{-9}	A			2.14×10^{-9}	A		
1×	3.03×10^{-9}		B		6.36×10^{-9}		B		2.93×10^{-9}			B
2×	2.34×10^{-9}			C	4.05×10^{-9}			C	3.08×10^{-9}			B
4×	1.64×10^{-9}			D	3.14×10^{-9}			C	2.95×10^{-9}			B

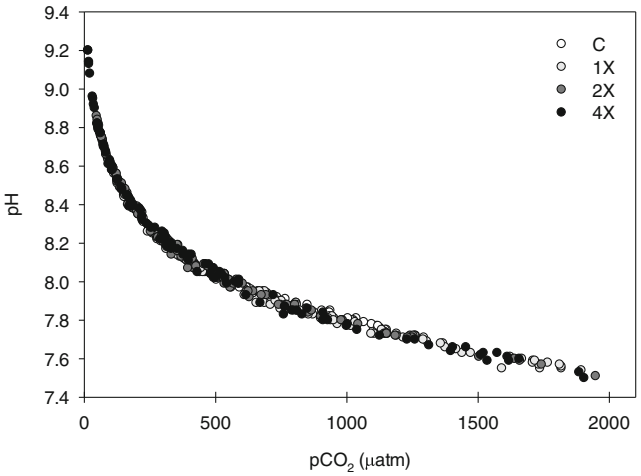

Fig. 3 Dawn pH (*top panel*) measurements are plotted against calculated dissolved pCO_2 concentrations for all treatment mesocosms

to the widespread concern about global climate change, the MERL data set provides additional context to this body of knowledge. Designed to replicate mid-Narragansett Bay conditions, the mesocosms were vertically well mixed, and salinity varied little throughout the year. As is typical of coastal systems (e.g., Hinga 1992), the magnitude of pH variation in the mesocosms throughout the year was greater than is measured in the open ocean (about 7.6–8.6 in the control mesocosm vs. <0.1 annually in the surface of the open ocean; Duarte et al. 2013). Furthermore, during the winter-spring bloom, the pH values rose in accordance with increasing nutrient inputs, with values in the 4× mesocosm reaching >9.2.

In contrast, there was no consistent separation among mesocosms during periods of low pH, which occurred in the late summer, during maximum respiration. Incremental nutrient enrichments across mesocosms resulted in increased production, and increased respiration (Oviatt et al. 1986a), but the trends in pH observed during seasons of high production were not mirrored during seasons dominated by respiration. This trend, and subsequent lack of trend, is particularly apparent because of the asymmetric response of pH to pCO_2, where an incremental change in pCO_2 when pCO_2 concentrations are low has a much larger effect on pH than when pCO_2 concentrations are high. While the $pCO_2 - pH$ relationship may appear to buffer the coastal waters at high dissolved inorganic carbon concentrations, the exponential decay shape of the relationship is attributable to pH being measured on a log scale. Because pH values increase exponentially, the response of actual H^+, reported as pH, to the spring bloom across treatments is exaggerated, and the range in H^+ concentrations during late summer/early fall respiration is minimized. Thus, we looked for statistically significant differences in 2 month windows when H^+ concentrations were at a minimum (and pH at a maximum, i.e., the spring bloom) and during the late summer when respiration was at maximum (and H^+ concentrations a minimum). Overall, the patterns observed in pH are consistent with those in H^+. The H^+ concentrations are significantly different across all four treatments in spring 1982, and the C and 1× mesocosms separate out from the more enriched treatments (2× and 4×) in spring 1983. In contrast, only the C mesocosm is distinguishable from the others during the summer/fall period of high respiration. While pH may appear to amplify the effects of nutrient enrichment during high ecological productivity, observations and trends observed in the pH data hold when examining the H^+ concentration directly. Perhaps, these trends are not evident when pH is low (and H^+ high) because pH reflects net metabolism and respiration occurs more diffusely throughout the year than the relatively more discrete spring bloom, which is bound on one end by the easement of light limitation and on the other by grazing and nutrient limitation (Pratt 1965; Oviatt et al. 2002). Taken together, these experiments suggest that, in addition to large seasonal changes in pH, increasing nutrient enrichment will drive pH values higher (and H^+ concentrations lower), but enrichment effects are not evident during

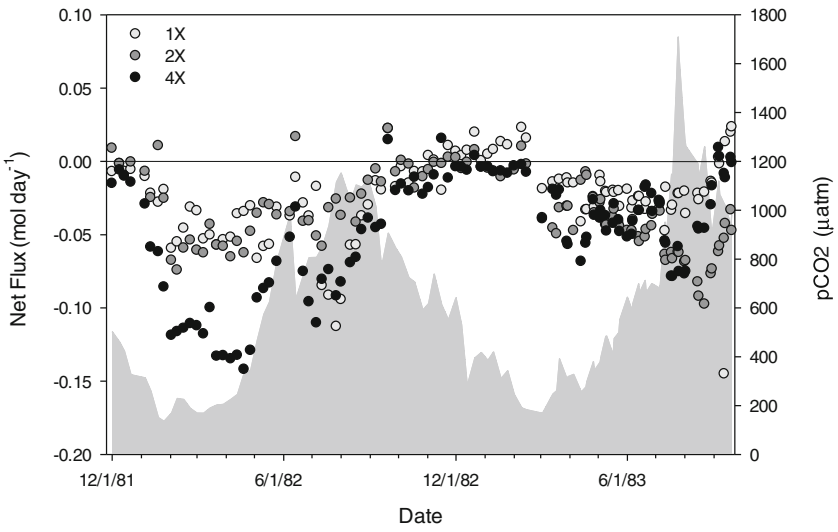

Fig. 4 Net exchange of TCO_2 in the enriched mesocosms (*circles*). *Values above zero* indicate that the mesocosms were net sources of C to the atmosphere, while *negative values* indicate a net sink. The pCO_2 values for the control mesocosm (indicated by the *shaded area*) are shown for reference

periods of low pH. This is certainly not to say that all estuaries are immune to climate change induced ocean acidification, but we believe that it is reasonable to suggest that, in vertically well-mixed systems like Narragansett Bay, other impacts of climate change, like warmer water temperatures or rising sea levels, may be more significant.

Given Narragansett Bay's 200-year history of intense fertilization (Nixon et al. 2008), the ranges in mesocosm data suggest that estuaries may have experienced much larger perturbations of pH (and $[H^+]$) in the past. Increased nutrient loads probably resulted from organic loading associated with land clearing, organic loading in urban estuaries from water and sewage systems, nutrient enrichment from sewage, fertilizer runoff, and nitrogen-enriched atmospheric deposition. Specifically, in Narragansett Bay, a fairly urban estuary (Nixon et al. 2008), watershed development, particularly during the industrial revolution, likely led to even greater annual pH excursions.

In larger, more stratified systems like the Gulf of Mexico, East China Sea, and Baltic Sea, the acidification of subpycnocline waters appears to be a much more significant issue (Cai et al. 2011; Sunda and Cai 2012). Cai et al. (2011) found drops in subsurface pH greater than could be predicted from increased respiration and rising atmospheric CO_2 levels alone. They attributed this lower than expected drop to a decreased buffering capacity of subsurface waters and cautioned that increasing nutrient inputs could exacerbate acidification. What makes these systems so different from Narragansett Bay, and perhaps more sensitive to perturbation, is essentially a decoupling of production and respiration by the pycnocline. In well-mixed Narragansett Bay, production and respiration co-occur as opposing forces, and the net balance varies seasonally. In contrast, in the Gulf of Mexico, excess production (eutrophication) drives pH more basic in the surface water, but respiration, and subsequent acidification, occurs largely below the pycnocline which acts, at least seasonally, as a CO_2 trap.

While the overall focus of the scientific community is on the impacts of future acidification on the marine environment, the effects of higher pH's associated with eutrophication, as observed in the MERL mesocosm experiments, should not be ignored. In highly productive systems, it is not unusual to measure pH values >9 during periods of high productivity, with values potentially exacerbated by the timing and delivery of increased nutrient inputs (e.g., Buskey 2008; Macedo et al. 2001; Hansen 2002). It seems that phytoplankton ecologists have taken the lead on exploring these impacts, and there is substantial documentation of the influence of high pH on the phytoplankton community. When pH values approach and exceed a pH of about 9, phytoplankton species richness declines, and in many cases, declines precipitously (Pedersen and Hansen 2003a, b; Weisse and Stadler 2006; Buskey 2008). Similarly, zooplankton abundance also declines as pH values reach and exceed 9.5, and there is some suggestion that these extremely high pH values may have a negative impact on larval fish and shellfish (Pedersen and Hansen 2003a; Buskey 2008; Salisbury et al. 2008). The combination of high nutrient concentrations (particularly ammonium) and high pH values (≥9) can result in severe necrosis and mortality of eelgrass within only a few days, particularly when the eelgrass density is already low (van der Heide et al. 2008). This may be especially relevant in Narragansett Bay, where Fulweiler et al. (2011) observed an inverse relationship between nitrification (the microbial conversion of ammonium to nitrate) and pH, where nitrification rates decreased as pH increased. In turn, during the spring bloom, microbes oxidize ammonium more slowly, not just exposing eelgrass to more ammonium during a vulnerable period, but also potentially decreasing the rate at which denitrification (the conversion of bioavailable N to inert N_2 gas) may take place.

Further, high pH values affect more than just the N cycle. At pH values >8.5, rates of phosphate desorption increase, increasing the amount of bioavailable phosphorous in the water column during biologically active periods (when pH typically does exceed 8.5; Spiteri et al. 2008). Similarly, rates of biogenic silica dissolution also exponentially rise with pH, increasing dissolved silica concentrations in the water column, particularly at the sediment water interface, during bloom periods (Van Cappellen and Qiu 1997; Loucaides et al. 2008). Overall, there is a positive feedback between primary productivity and increasing pH, which increases the bioavailability of limiting nutrients (N, P, Si) in these well-mixed systems.

As recently addressed by Duarte et al. (2013), climate change impacts, layered with the ebb and flow of cultural eutrophication, will present in complex, and perhaps conflicting, ways in coastal ecosystems. While there is emerging evidence that subsurface pH is rapidly declining in stratified systems (e.g. Cai et al. 2011), data from mesocosms designed to replicate well-mixed mid-Narragansett Bay conditions reflect the non-linear response of pH to changes in dissolved CO_2, where the pH of the water column, particularly during the growing season, is less sensitive to increases in CO_2.

Acknowledgments The observations described in this manuscript were discovered by S. Nixon. He began writing the text of the manuscript and, after his untimely death, his coauthors completed the manuscript and published it posthumously in his honor. We would like to thank the scientists and technicians who worked on the MERL mesocosm experiments, and upon whose careful measurements, this manuscript is based. We are grateful to Alana Hanson for some statistical support. The MERL nutrient addition experiment was supported by the Environmental Protection Agency Centers Program, Cooperative Agreements 807795–02 and 810265–01. Support for this work was also partially provided by the NSF Awards 12010 and OCE851447. This is ORD tracking number ORD-003895 of the Atlantic Ecology Division, National Health and Environmental Effects Research Laboratory, Office of Research and Development, US Environmental Protection Agency. Although the research described in this article has been funded in part by the US Environmental Protection Agency, it has not been subjected to Agency review. Therefore, it does not necessarily reflect the views of the Agency.

References

Berounsky, V., and S.W. Nixon. 1985. Eutrophication and the rate of net nitrification in a coastal marine ecosystem. *Estuarine, Coastal and Shelf Science* 20: 773–781.

Bopp, R.F., P.H. Santschhi, Y.-H. Li, and B.L. Deck. 1981. Biodegradation and gas exchange of gaseous alkanes in model estuarine ecosystems. *Organic Geochemistry* 1: 9–14.

Borges, A.V. 2005. Do we have enough pieces of the jigsaw to integrate CO_2 fluxes in the coastal ocean? *Estuaries* 28: 3–27.

Borges, Alberto V., and Nathalie Gypens. 2010. Carbonate chemistry in the coastal zone responds more strongly to eutrophication than to ocean acidification. *Limnology and Oceanography* 55: 346–353.

Buskey, Edward J. 2008. How does eutrophication affect the role of grazers in harmful algal bloom dynamics? *Harmful Algae* 8: 152–157.

Cai, Wei-Jun. 2011. Estuarine and coastal ocean carbon paradox: CO_2 sinks or sites of terrestrial carbon incineration? *Annual Review of Marine Science* 3: 123–145.

Cai, Wei-Jun, Xinping Hu, Wei-Jen Huang, Michael C. Murrell, John C. Lehrter, Steven E. Lohrenz, Wen-Chen Chou, Weidong Zhai, James T. Hollibaugh, Yongchen Wang, Pingsan Zhao, Xianghui Guo, Kjell Gundersen, Minhan Dai, and Gwo-Ching Gong. 2011. Acidification of subsurface coastal waters enhanced by eutrophication. *Nature Geoscience* 4: 766–770.

Caldeira, Ken, and Michael E. Wickett. 2003. Oceanography: anthropogenic carbon and ocean pH. *Nature* 425: 365.

Doney, S.C., V.J. Fabry, R.A. Feely, and J.A. Kleypas. 2009. Ocean acidification: the other CO_2 problem. *Annual Review of Marine Science* 1: 169–192.

Duarte, Carlos M., Iris E. Hendriks, Tommy S. Moore, Ylva S. Olsen, Alexandra Steckbauer, Laura Ramajo, Jacob Carstensen, Julie A. Trotter, and Malcolm McCulloch. 2013. Is ocean acidification an open-ocean syndrome? Understanding anthropogenic impacts on seawater pH. *Estuaries and Coasts* 36: 221–236.

Fraher, J. 1991. Atmospheric wet and dry deposition of fixed nitrogen to Narragansett Bay. M.S. Thesis in Oceanography. University of Rhode Island. Narragansett, RI.

Frithsen, J.B., A.A. Keller, and M.E.Q. Pilson. 1985a. Effects of inorganic nutrient additions in coastal areas: a mesocosm experiment data report. Volume 1. MERL Series, Report No. 3. University of Rhode Island. Kingston, RI.

Frithsen, J.B., P.A. Lane, A.A. Keller, and M.E.Q. Pilson. 1985b. Effects of inorganic nutrient additions in coastal areas: a mesocosm experiment data report. Volume 2. MERL Series, Report. No. 4. The University of Rhode Island, Kingston, RI.

Fulweiler, Robinson W., Hollie E. Emery, Elise M. Heiss, and Veronica M. Berounsky. 2011. Assessing the role of pH in determining water column nitrification rates in a coastal system. *Estuaries and Coasts* 34: 1095–1102.

Gattuso, J.-P., M. Frankignoulle, and R. Wollast. 1998. Carbon and carbonate metabolism in coastal aquatic systems. *Annual Review of Ecology and Systematics* 29: 405–434.

Gearing, J.N., P.J. Gearing, D. Rudnick, A.G. Requejo, and M. Hutchins. 1984. The isotopic variability of organic carbon in a phytoplankton-based, temperate estuary. *Geochimica et Cosmochimica Acta* 48: 1089–1098.

Grassle, J.F., J.P. Grassle, L.S. Brown-Leger, R.F. Petrecca, and N.J. Copley. 1985. Subtidal macrobenthos of Narragansett Bay. Field and mesocosm studies of the effects of eutrophication and organic input on benthic populations. In *Marine biology of polar regions and effects of stress on marine organisms*, ed. J.S. Gray and M.E. Christiansen, 421–434. New York: Wiley.

Hansen, P.J. 2002. Effects of high pH on the growth and survival of marine phytoplankton: implications for species succession. *Aquatic Microbial Ecology* 28: 279–288.

Hendriks, I.E., C.M. Duarte, and M. Alvarez. 2010. Vulnerability of marine biodiversity to ocean acidification: a meta-analysis. *Estuarine, Coastal and Shelf Science* 86: 157–164.

Hines, Wallis G., and Robert de Levie. 2010. The early development of electronic pH meters. *Journal of Chemical Education* 87: 1143–1153.

Hinga, K.R. 1992. Co-occurrence of dinoflagellate blooms and high pH in marine enclosures. *Marine Ecology Progress Series* 86: 181–187.

Hinga, K.R. 2002. The effects of pH on coastal marine phytoplankton. *Marine Ecology Progress Series* 238: 281–300.

Hobbie, J.E., and J.J. Cole. 1984. Response of a detrital foodweb to eutrophication. *Bulletin of Marine Science* 35: 357–363.

Howarth, Robert, Francis Chan, Daniel J. Conley, Josette Garnier, Scott C. Doney, Roxanne Marino, and Gilles Billen. 2011. Coupled biogeochemical cycles: eutrophication and hypoxia in temperate estuaries and coastal marine systems. *Frontiers in Ecology and the Environment* 9: 18–26.

Hunt, C.D., and J.R. Kelly. 1988. Mn cycling in coastal regions: response to eutrophication. *Estuarine, Coastal and Shelf Science* 26: 527–558.

Keller, A.A. 1986. Modeling the productivity of natural phytoplankton populations using mesocosm data along a nutrient gradient. Ph.D. Dissertation. University of Rhode Island, Kingston, Rhode Island.

Kemp, W.M., and J.M. Testa. 2011. Metabolic balance between ecosystem production and consumption. In *Treatise on estuaries and coastal science, vol. 7*, ed. E. Wolansky and D. McLusky. Oxford: Elsevier Ltd.

Kemp, W.M., E.M. Smith, M. Marvin-DiPasquale, and W.R. Boynton. 1997. Organic carbon-balance and net ecosystem metabolism in Chesapeake Bay. *Marine Ecology Progress Series* 150: 229–248.

Lambert, C.E., and C.A. Oviatt. 1986. Manual of biological and geochemical techniques in coastal areas. MERL Series, Report No. 1, 2nd edn. The University of Rhode Island, Kingston, RI.

Laruelle, Goulven G., Hans H. Dürr, Caroline P. Slomp, and Alberto V. Borges. 2010. Evaluation of sinks and sources of CO_2 in the global coastal ocean using a spatially-explicit typology of estuaries and continental shelves. *Geophysical Research Letters* 37, L15607. doi: 10.1029/2010GL043691.

Loucaides, Socratis, Philippe Van Cappellen, and Thilo Behrends. 2008. Dissolution of biogenic silica from land to ocean: role of salinity and pH. *Limnology and Oceanography* 53: 1614–1621.

Macedo, M.F., P. Duarte, P. Mendes, and J.G. Ferreira. 2001. Annual variation of environmental variables, phytoplankton species composition and photosynthetic parameters in a coastal lagoon. *Journal of Plankton Research* 23: 719–732.

Magnuson, Andrea. 1997. Chemical variability in coastal and mixed layer waters. Ph.D. dissertation. The University of Rhode Island, Kingston, RI.

Nielsen, Steemann E. 1952. The use of radio-active carbon ^{14}C for measuring organic production in the sea. *Journal du Conseil International pour l'Exploration de la Mer* 18: 117–140.

Nixon, S.W., and M.E.Q. Pilson. 1984. Estuarine total system metabolism and organic exchange calculated from nutrient ratios: an example from Narragansett Bay. In *The estuary as a filter*, ed. V.S. Kennedy, 261–290. New York: Academic.

Nixon, S.W., D. Alonso, M.E.Q. Pilson, and B.A. Buckley. 1980. Turbulent mixing in aquatic microcosms. In *Microcosms in ecological research*, ed. J. Giesy, 818–849. U.S. Department of Energy, CONF-781101.

Nixon, S.W., C.A. Oviatt, J. Frithsen, and B. Sullivan. 1986. Nutrients and the productivity of estuarine and coastal marine ecosystems. *Journal of Limnological Society of South Africa* 12: 43–71.

Nixon, S.W., B. Buckley, S. Granger, L. Harris, A. Oczkowski, R. Fulweiler, and L. Cole. 2008. Nutrient (N and P) inputs to Narragansett Bay: past, present, future. In *Science for ecosystem based management*, ed. B. Costa-Pierce and A. Debonnet, 101–176. New York: Springer Verlag.

Oczkowski, A.J., M.E.Q. Pilson, and S.W. Nixon. 2010. A marked gradient in $\delta^{13}C$ values of clams *Mercenaria mercenaria* across a marine embayment may reflect variations in ecosystem metabolism. *Marine Ecology Progress Series* 414: 145–153.

Oviatt, C.A., A.A. Keller, P.A. Sampou, and L.L. Beatty. 1986a. Patterns of productivity during eutrophication: a mesocosm experiment. *Marine Ecology Progress Series* 28: 69–80.

Oviatt, C.A., D.T. Rudnick, A.A. Keller, P.A. Sampou, and G.T. Almquist. 1986b. A comparison of system (O_2 and CO_2) and C-14 measurements of metabolism in estuarine mesocosms. *Marine Ecology Progress Series* 28: 57–67.

Oviatt, C.A., P. Lane, F. French III, and P. Donaghay. 1989. Phytoplankton species and abundance in response to eutrophication in coastal marine mesocosms. *Journal of Plankton Research* 2: 1223–1244.

Oviatt, C.A., A. Keller, and L. Reed. 2002. Annual primary production in Narragansett Bay with no bay-wide winter-spring phytoplankton bloom. *Estuarine, Coastal and Shelf Science* 54: 1013–1026.

Park, K., D.W. Hood, and H.T. Odum. 1958. Diurnal pH variation in Texas bays, and its application to primary production estimation. *Publication of the Institute for Marine Sciences, Texas* 5: 47–64.

Parsons, T.R., Y. Maita, and C.M. Lalli. 1984. *A manual of chemical and biological methods for seawater analysis*. Oxford: Pergamon Press.

Pedersen, Maria Fenger, and Per Juel Hansen. 2003a. Effects of high pH on the growth and survival of six marine heterotrophic protists. *Marine Ecology Progress Series* 260: 33–41.

Pedersen, Maria Fenger, and Per Juel Hansen. 2003b. Effects of high pH on a natural marine planktonic community. *Marine Ecology Progress Series* 260: 19–31.

Pilson, M.E.Q. 1985. On the residence time of water in Narragansett Bay. *Estuaries* 8: 2–14.

Pilson, M.E.Q. 2013. *An introduction to the chemistry of the sea*, 2nd ed. Cambridge: Cambridge University Press.

Pratt, D.M. 1965. The winter-spring diatom flowering in Narragansett Bay. *Limnology and Oceanography* 10: 173–184.

Ryther, John H. 1956. The measurement of primary production. *Limnology and Oceanography* 1: 72–84.

Salisbury, J., M. Green, C. Hunt, and J. Campbell. 2008. Coastal acidification by rivers: a threat to shellfish? *Eos* 89(50): 513.

Sampou, Peter Andre. 1989. Effects of eutrophication on the biogeochemical cycling of carbon, oxygen, sulfur, and energy in coastal marine ecosystems. Ph.D. Dissertation. The University of Rhode Island.

Smith, S.V. 1991. Stoichiometry of C:N:P fluxes in shallow-water marine ecosystems. Chapter 13. In *Proceedings of the Third Cary Conference: Comparative analyses of ecosystems: patterns, mechanisms and theories*, eds. Jonathan Cole, Gary Lovett, Stuart Findlay, Julie C. Morgan, 259–286. Springer.

Smith, S.V., and J.T. Hollibaugh. 1993. Coastal metabolism and the oceanic organic carbon balance. *Reviews of Geophysics* 31: 75–89.

Spiteri, Claudette, Philippe Van Cappellen, and Pierre Regnier. 2008. Surface complexation effects on phosphate adsorption to ferric iron oxyhydroxides along pH and salinity gradients in estuaries and coastal aquifers. *Geochimica et Cosmochimica Acta* 72: 3431–3445.

Sullivan, B.K., and P.J. Ritacco. 1985. The response of dominant copepod species to food limitation in a coastal marine ecosystem. *Archiv für Hydrobiologie–Beiheft Ergebnisse der Limnologie* 21: 407–418.

Sunda, William G., and Wei-Jun Cai. 2012. Eutrophication induced CO_2-acidification of subsurface coastal waters: interactive effects of temperature, salinity, and atmospheric P_{CO2}. *Environmental Science and Technology* 46: 10651–10659.

Sverdrup, H.V., M.W. Johnson, and R.H. Flemming. 1942. *The oceans*. Englewood Cliffs: Prentice-Hall.

Szabadváry, Ferenc. 1960. History of analytical chemistry. Gordon and Breach Science Publishers. Reprinted in English in 1966 by Pergamon Press Ltd, London, U.K.

Van Cappellen, Philippe, and Linqing Qiu. 1997. Biogenic silica dissolution in sediments of the Southern Ocean. II. Kinetics. *Deep Sea Research II* 44: 1129–1149.

van der Heide, T., A.J.P. Smolders, B.G.A. Rijkens, E.H. van Nes, M.M. van Katwijk, and J.G.M. Roelofs. 2008. Toxicity of reduced nitrogen in eelgrass (*Zostera marina*) is highly dependent on shoot density and pH. *Oecologia* 158: 411–419.

Weisse, T., and P. Stadler. 2006. Effect of pH on growth, cell volume, and production on freshwater ciliates, and implications for their distribution. *Limnology and Oceanography* 51: 1708–1715.