

Large Natural pH, CO₂ and O₂ Fluctuations in a Temperate Tidal Salt Marsh on Diel, Seasonal, and Interannual Time Scales

Hannes Baumann · Ryan B. Wallace · Tristen Tagliaferri · Christopher J. Gobler

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Abstract Coastal marine organisms experience dynamic pH and dissolved oxygen (DO) conditions in their natural habitats, which may impact their susceptibility to long-term anthropogenic changes. Robust characterizations of all temporal scales of natural pH and DO fluctuations in different marine habitats are needed; however, appropriate time series of pH and DO are still scarce. We used multiyear (2008–2012), high-frequency (6 min) monitoring data to quantify diel, seasonal, and interannual scales of pH and DO variability in a productive, temperate tidal salt marsh (Flax Pond, Long Island, US). pH_{NBS} and DO showed strong and similar seasonal patterns, with average (minimum) conditions declining from 8.2 (8.1) and 12.5 (11.4) mg l⁻¹ at the end of winter to 7.6 (7.2) and 6.3 (2.8) mg l⁻¹ in late summer, respectively. Concomitantly, average diel fluctuations increased from 0.22 and 2.2 mg l⁻¹ (February) to 0.74 and 6.5 mg l⁻¹ (August), respectively. Diel patterns were modulated by tides and time of day, eliciting the most extreme minima when low tides aligned with the end of the night. Simultaneous in situ pCO₂ measurements showed striking fluctuations between ~330 and ~1,200 (early May), ~2,200 (mid June), and ~4,000 µatm (end of July) within single tidal cycles. These patterns also indicate that the marsh's strong net heterotrophy influences its adjacent estuary by 'outwelling' acidified and hypoxic water during ebb tides. Our analyses emphasize

the coupled and fluctuating nature of pH and DO conditions in productive coastal and estuarine environments, which have yet to be adequately represented by experiments.

Keywords Flax Pond · Long Island Sound · Ocean acidification · Net heterotrophy · Hypoxia · Outwelling hypothesis

Introduction

Ongoing anthropogenic ocean acidification has heightened the need to better understand the sensitivity of marine organisms to low pH conditions (Branch et al. 2012; Denman et al. 2011; Doney et al. 2009; Fabry et al. 2009). Increasing oceanic CO₂ levels have so far reduced the average open ocean pH by ~0.1 units (Orr et al. 2005) and may cause further reductions by 0.7 units under 'business-as-usual' scenarios that predict CO₂ levels to increase from ~400 µatm (2013) to ~2,000 µatm CO₂ by the year 2300 (Caldeira and Wickett 2003; Le Quere et al. 2009). These predictions have also formed the basis for guidelines recommending realistic CO₂ levels for experiments that seek to challenge contemporary marine organisms with future high CO₂ (low pH) conditions in order to infer a species vulnerability (Ishimatsu et al. 2008; Riebesell et al. 2010). The result has been a complex, fast expanding picture of mostly negative responses to high CO₂ (Branch et al. 2012; Doney et al. 2009; Hendriks et al. 2010; Kroeker et al. 2010), particularly during the early life stages (Baumann et al. 2012; Kurihara 2008) and in calcifying invertebrates (Gazeau et al. 2007; Kleypas et al. 2006; Orr et al. 2005; Talmage and Gobler 2010), but also a range of neutral (Frommel et al. 2012; Hurst et al. 2012; McConville et al. 2013; Munday et al. 2011) or positive reactions to elevated CO₂ that often differ at the species level (Gooding et al. 2009; Lohbeck et al. 2012; Ries et al. 2009).

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H. Baumann (✉)
School of Marine and Atmospheric Sciences, Stony Brook
University, 123 Dana Hall, Stony Brook, NY 11794, USA
e-mail: hannes.baumann@stonybrook.edu

R. B. Wallace · C. J. Gobler
School of Marine and Atmospheric Sciences, Stony Brook
University, 239 Montauk Hwy, Southampton, NY 11968, USA

T. Tagliaferri
U.S. Geological Survey, New York Water Science Center, 2045
Route 112, Building 4, Coram, NY 11727, USA

Increasingly, the complexity of organism responses to elevated CO₂ appears to stem, in part, from insufficient knowledge and thus appreciation of the scales of natural pH variability experienced by marine organisms in their habitats (Hendriks et al. 2010; Hofmann et al. 2011; Hofmann and Todgham 2010; Kelly et al. 2013). This is particularly true for coastal environments, where the majority of ecologically and economically important marine species spend all or part of their life cycle (Harley et al. 2006; Hendriks et al. 2010) and which are generally more variable in pH than the open ocean, owing to seasonal cycles in biological productivity (Wootton et al. 2008), river discharge (Borges and Frankignoulle 1999; Salisbury et al. 2008), upwelling (Feely et al. 2008), or human influences along coastlines (Borges and Gypens 2010; Frankignoulle et al. 1996). However, to advance this general understanding, efforts to specifically characterize different marine habitats in terms of their short- and long-term pH variability are clearly needed (Provoost et al. 2010; Wootton et al. 2008). Hofmann et al. (2011) recently documented the increasing short-term variability in pH when moving from open ocean to coral reef, to upwelling, and to estuarine sites, suggesting that organisms in the latter experience diel pH changes greater than the predicted pH decrease in the average open ocean within the next 100 years (see also O'Boyle et al. 2013; Wootton et al. 2008). Similarly, organisms in coastal upwelling areas regularly experience elevated CO₂ levels of ~1,000 μ atm (Feely et al. 2010; Feely et al. 2008). Levels of pH in an urbanized estuary in the Baltic Sea fluctuate seasonally between 8.05 and 7.45 (Melzner et al. 2012), which exceeds the predicted changes in open ocean pH within the next 300 years. Duarte et al. (2013) concluded that short-term pH variability in coastal habitats is far greater than in the open ocean, while long-term coastal pH conditions often show larger declines (Provoost et al. 2010; Waldbusser et al. 2011; Wootton et al. 2008) or no clear trend (Duarte et al. 2013). Hence, ocean acidification may in fact be an 'open-ocean syndrome', while a more comprehensive paradigm is required to include the entirety of processes affecting pH in the coastal marine environments (Duarte et al. 2013). This paradigm shift, however, is still hindered by the comparative scarcity of published, high-resolution pH time series across the range of different coastal habitats.

Because coastal pH fluctuations are primarily caused by variable metabolic rather than atmospheric CO₂ (Wootton et al. 2008), they are necessarily accompanied by changes in dissolved oxygen (DO) concentrations (Cai et al. 2011; Melzner et al. 2012; Waldbusser et al. 2011). Net heterotrophy decreases DO while releasing metabolic CO₂, which forms carbonic acid (H₂CO₃) dissociating into HCO₃⁻, CO₃²⁻, and H⁺ ions (with an increase in the latter measured as a decrease in pH). Conversely, net autotrophy increases DO and pH in a system, while decreasing CO₂ concentrations. The large empirical knowledge on low oxygen effects on marine organisms

predates the more recent interest in ocean acidification, and often the two stressors are still considered separately (Diaz and Rosenberg 2008; Ekau et al. 2010; Officer et al. 1984; Spitzer et al. 1969; Widdows et al. 1989). In reality, low pH and low oxygen conditions are inseparable in most aquatic environments, and the concurrent effects of 'oceans twin stressors' on marine life have yet to be thoroughly studied (Cai et al. 2011; Gobler et al. 2014). Such investigations would benefit from a more comprehensive understanding of the scales and magnitudes of concurrent pH and DO fluctuations in coastal habitats.

Here, we add to this baseline understanding by fully characterizing the pH and DO variability in a temperate tidal salt marsh. Salt marshes are common worldwide and constitute the dominant intertidal habitat along the US east coast and the Gulf of Mexico (Pennings and Bertness 2001). They provide highly valuable ecosystem services such as sheltering coasts from erosion, filtering sediments and nutrients from the water column, and supporting fisheries by acting as important spawning and nursery habitats (Bertness 2007; Boesch and Turner 1984; Pennings and Bertness 2001; Raposa and Roman 2001). Ecologists have long been fascinated by the extreme nature and extraordinary productivity of tidal salt marshes, which accumulate a greater amount of organic matter per meter squared than any crop except cultivated sugar cane (Odum 1961). This productivity is utilized by fish, shellfish, and crustaceans, while also influencing adjacent estuaries via 'outwelling' of dissolved organic matter and nutrients (Koch and Gobler 2009; Odum 1969; Odum et al. 1995; Valiela et al. 1978). Hence, organisms in or near tidal salt marshes likely experience highly variable salinity, temperature, water level, as well as oxygen and pH conditions that typify this habitat.

The goal of this study was to characterize the patterns and magnitudes of diel, seasonal, and interannual fluctuations in pH and DO in an undisturbed tidal salt marsh adjacent to Long Island Sound, using a multiyear, high-frequency data set. Given the marsh's productivity, we hypothesize that pH and DO variations are strongly correlated and much larger than those in the open ocean or even most other coastal marine habitats characterized thus far. This study has implications for assessing marsh species sensitivities to low and highly variable pH and DO conditions, particularly with respect to the magnitude of projected long-term changes.

Materials and Methods

Diel to seasonal fluctuations in pH and DO were analyzed in Flax Pond (40° 57.78'N, 73° 8.22'W, Fig. 1), a ~1 km² tidal salt marsh on the north shore of Long Island, NY, USA, connected to Long Island Sound by a single inlet (Richard 1978). The mean tidal range is 1.8 m (Richard 1978), with ebb tides approximately 2 hours longer than flood tides due to a

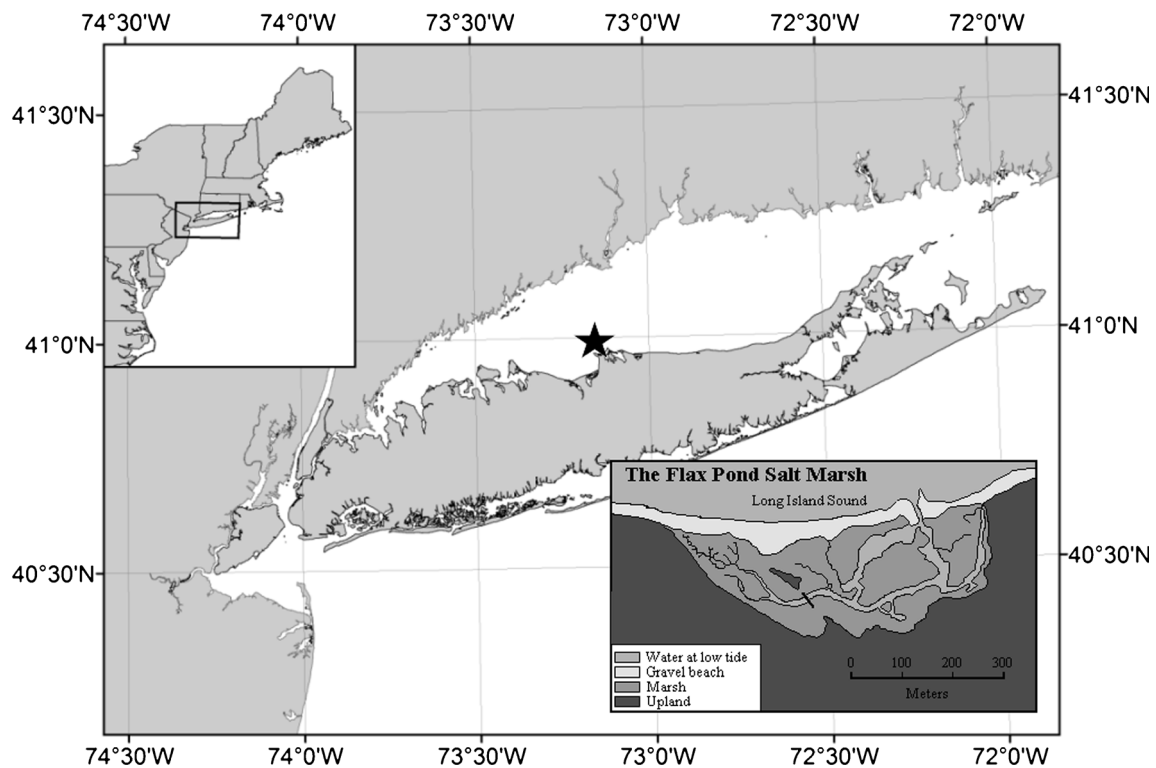


Fig. 1 Map of Long Island and Long Island Sound at the US Atlantic coast, with inset and star showing the Flax Pond salt marsh and its main topographic features

sill at the channel entrance (Woodwell and Pecan 1973). The surface of the marsh receives negligible freshwater input (Woodwell and Pecan 1973) and is a mosaic of primarily *Spartina alterniflora* stands (46 % in 1980), open water, upland islands, and bare mud (Houghton and Woodwell 1980). Flax Pond has been protected from development and degradation since 1965, thereby facilitating pioneering studies on physical and biological processes of temperate tidal salt marshes (Houghton and Woodwell 1980; Hovel and Morgan 1997; Richard 1978; Woodwell and Pecan 1973).

Since April 2008, high frequency monitoring (6-min intervals) of temperature ($^{\circ}\text{C}$), pH (National Bureau of Standards, NBS), dissolved oxygen (DO , mg l^{-1}), and tidal elevation (m) among other parameters has been carried out at Flax Pond by the US Geological Survey (USGS, site # 01304057) in collaboration with the New York State Department of Environmental Conservation (NYSDEC) and the Nature Conservancy. DO and pH sensors are located approximately 0.5 m above sandy bottom in the main channel of the marsh. Over the course of the time series, pH was measured with probes specific to the YSI 6 series, i.e., models 6579 (Hemispherical glass probe) and 6589 (Fast-Response Probe). Each probe had a resolution and accuracy of 0.01 and ± 0.2 units, respectively, and final monitoring data were provided to the public with a precision of 0.1 units. Dissolved oxygen was measured using a ROX[®] Optical Dissolved Oxygen sensor with a precision of 0.1 mg l^{-1} . Routine maintenance on the

device was performed monthly in the summer, every 6 weeks in the spring and fall, and bimonthly in the winter, when probes were cleaned and checked for biofouling and electronic drift (Wagner et al. 2006). Fouling error was determined from readings of a clean, calibrated field monitor submersed in a bucket of environmental water relative to readings of the monitoring probe before and after cleaning. Electronic drift was determined by checking values against NBS standards, with recalibration performed if readings were outside the pH calibration criteria (± 0.2 pH units). In general, the deployed pH probes did not drift considerably and required recalibration only about twice per year. Due to temporary discontinuations of pH and DO monitoring, the time series analyzed here are for periods of April 2008–November 2012 (temperature and pH) and April 2008–March 2011 (DO).

To complement the pH and DO records from Flax Pond, direct measurements of pCO_2 in the water were conducted on four occasions between May and September 2012. A HydroC[™] (Contros GmbH) in situ pCO_2 sensor was tethered next to the USGS pH and DO sensors at the same depth, measuring pCO_2 every 5 s for approximately 48 h during each deployment. The instrument utilizes infrared technology and provides measurements of pCO_2 in coastal systems consistent with the traditional measurements made on discrete samples using standard wet chemical methods (Fiedler et al. 2012; Fietzek et al. 2014). During this study, we compared measurements of pCO_2 with the HydroC[™] to the levels measured in

discrete water samples. Discrete water samples were collected at depth using a Van Dorn water sampler and were carefully transferred without bubbling to acid washed, borosilicate glass BOD bottles (330 ml). During sample transfer, water was allowed to overflow by at least one full sample volume and at least 1 % headspace remained within each bottle to allow for sample expansion and preservation. Samples were preserved by adding 100 μl of a saturated mercuric chloride (HgCl_2) solution. Glass bottle stoppers were coated with vacuum grease and twisted when inserted into bottle necks to form an air-tight seal. A cable-tie collar was mounted tightly around the bottle neck with a rubber band attached over the top to keep the stopper secure during storage. Samples were stored at 4 °C and analyzed within 4 weeks of sample collection. Total dissolved inorganic carbon measurements were made using an EGM-4 Environmental Gas Analyzer® (PP Systems) after acidification and separation of the gas phase from seawater using a Liqui-Cel® Membrane (Membrana). This instrument was calibrated using standards made from sodium bicarbonate and generally provided a methodological precision of $\pm 1.05\%$ for replicated measurements of total dissolved inorganic carbon. As a quality assurance measure, certified reference material provided by Dr. Andrew Dickson's lab (University of California San Diego, Scripps Institution of Oceanography; Batch 102=2013 $\mu\text{mol DIC kg seawater}^{-1}$) was analyzed in quadruplicates immediately before and after the analysis of every set of samples and yielded full recovery (measured values= $104 \pm 4\%$ of certified values). pH was measured on discrete samples with an automated probe (Honeywell Durafet III® pH electrode) and spectrophotometrically using *m*-cresol purple (Dickson et al. 2007). Levels of pCO_2 were calculated based on measured levels of total inorganic carbon, pH ($\text{mol kg seawater}^{-1}$, total scale), temperature, salinity, and first and second dissociation constants of carbonic acid in seawater according to Millero (2010) using the program CO2SYS (<http://cdiac.ornl.gov/ftp/co2sys/>). Levels of pCO_2 measured with the HydroCTM were consistent with and not significantly different from levels measured on discrete samples: HydroCTM measured $\text{pCO}_2 = 0.93 * \text{pCO}_2$ (calculated within discrete samples) $-15 \mu\text{atm}$ ($n=16$; $R=0.93$; $p<0.001$).

Data Analysis

Minima, maxima, means, and amplitudes (max–min) of temperature, pH, and DO were calculated for each day of each month and year of the time series. To characterize diel fluctuations, daily amplitudes were averaged by month across all years (Fig. 2; Table 1). Mean seasonal patterns of temperature, pH, and DO were estimated as monthly averages of daily minima, means, and maxima across all years (Fig. 2), whereas annual amplitudes were estimated as the difference between each year's maximum and minimum monthly average (Table 1). Annual averages of pH and DO were calculated to characterize interannual pH and DO variability (pH=3 full years, DO=2 full

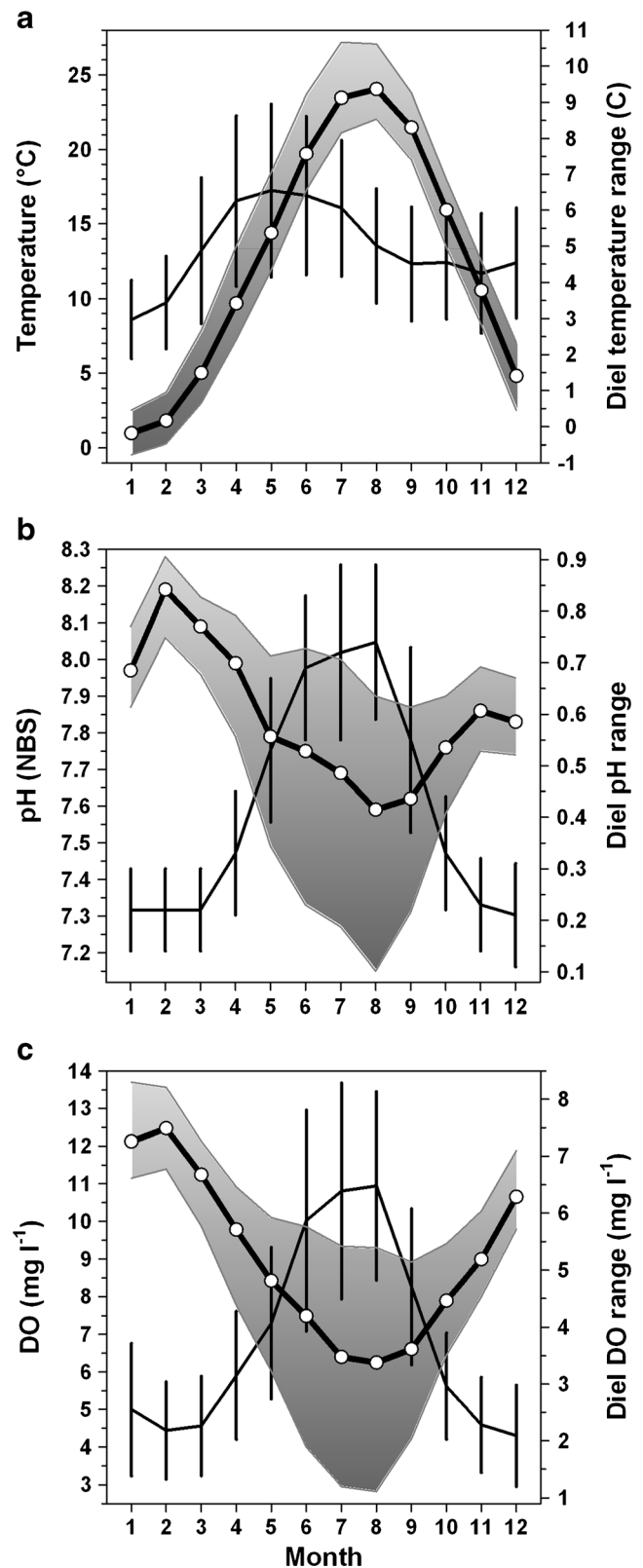


Fig. 2 Diel and seasonal temperature **a**, pH **b**, and DO **c** variability at Flax Pond salt marsh (2008–2012). Monthly means, white circles and thick black lines; monthly averages of daily minima and maxima, dark grey lines and shading; monthly average of diel amplitudes ± 1 SD, thin black line and error bars

Table 1 Summary of the diel, seasonal, and interannual amplitudes of pH, dissolved oxygen (DO), and CO₂ at the Flax Pond salt marsh on Long Island Sound

Temporal scale	min Δ pH–max Δ pH	min Δ DO–max Δ DO (mg l ⁻¹)	Min–max CO ₂ (ppm)
Day	0.22 ^a –0.74 ^b	2.18 ^a –6.48 ^b	331–3,996 ^c
Season	0.51–0.70 ^c	5.68–6.50 ^f	
Year	0.08 ^c	0.04 ^f	

^a February mean^b August mean^c on 29 July 2012^e 2009–2011^f 2009–2010

years; Table 1). To explore the relationship between pH, DO, and tidal dynamics, we estimated Pearson correlations coefficients (R) separately for day and night and for each month across all years. Tidal elevation was measured directly by the USGS monitoring site, while sunset/sunrise times were retrieved from online tables of the US Naval Observatory (http://aa.usno.navy.mil/data/docs/RS_OneYear.php) and used to discern day (sunrise–sunset) from night values (sunset–sunrise). To correlate pH (USGS) to CO₂ measurements (Contros), pCO₂ data were averaged over the same 6-min intervals, followed by fitting an exponential relationship to the data [$\text{pH} = a \cdot \exp(b \cdot \text{pCO}_2)$]. If not specified otherwise, all averages are given ± 1 standard deviation. All statistical analyses were conducted using SPSS (V.17.0) IBM™.

Results

The Flax Pond salt marsh was characterized by very large fluctuations in temperature, pH, and DO conditions on both diel and seasonal time scales (Fig. 2, Table 1). Monthly mean temperature ranged from 0.97 ± 1.94 °C in January to 24.04 ± 1.81 °C in August, while average diel fluctuations increased from 2.97 ± 1.09 °C in January to 6.55 ± 2.40 °C in May (Fig. 2a). Average pH was consistently highest and least variable in February (8.19 ± 0.10) followed by a steady decrease in average pH with increasing variance throughout spring and summer until reaching a seasonal minimum in August (7.59 ± 0.25). Thereafter, average pH gradually reverted back to initial conditions during fall and early winter months (Fig. 2b). Diel pH fluctuations followed a similar pattern, with average daily pH amplitudes of 0.22 ± 0.08 in January, February, and March increasing more than threefold to 0.69 ± 0.13 , 0.72 ± 0.17 , and 0.74 ± 0.59 in June, July, and August, respectively (Fig. 2b). Between June and September, diel fluctuations equal or exceeding one unit of magnitude (i.e., $\Delta \text{pH} \geq 1$) occurred with an incidence of 5–7 %. Patterns

of diel and seasonal DO variability closely resembled those of pH, with a seasonal maximum in average DO in February (12.47 ± 0.88 mg l⁻¹) followed by a strong decline in average DO with increasing DO variance until reaching the annual minimum in August (6.25 ± 1.83 mg l⁻¹, Fig. 2c). Diel DO fluctuations were smallest in February and March (2.18 ± 0.86 and 2.26 ± 0.88 mg l⁻¹, respectively, Table 1) and largest in July and August (6.39 ± 1.90 and 6.48 ± 1.66 mg l⁻¹, respectively, Table 1); during these summer months, daily DO fluctuations exceeded one unit of magnitude ($\Delta \text{DO} \geq 10$ mg l⁻¹) with an incidence of 1–6 %.

Both diel and seasonal pH and DO patterns were significantly and positively linearly related ($p < 0.001$), but correlations were strongest between May and November ($R > 0.7$, Fig. 3c). Short-term patterns were significantly influenced by tidal elevation and day versus night hours. The correlations between tides versus pH (Fig. 3a) and tides versus DO (Fig. 3b) peaked during summer months (maximum in June) and were consistently stronger at night than during the day (tide vs. pH, $R_{\text{June, night}} = 0.65$, $R_{\text{June, day}} = 0.48$; tide vs. DO, $R_{\text{June, night}} = 0.74$, $R_{\text{June, day}} = 0.40$, all $p < 0.001$). However, the seasonal increase in correlation strength is expected given the higher pH and DO variability in summer than winter. Although high tides were associated with high pH throughout the year, DO conditions between October and January were negatively correlated to tides (e.g., $R_{\text{Dec, day}} = -0.45$, $p < 0.001$, Fig. 3b). To illustrate the main diel and seasonal patterns in short-term pH and DO variability, Fig. 4 shows four representative periods between February and August 2010. In February 2010, values of pH and DO were high and least variable (pH; 8.1–8.4, DO; 12–14.5 mg l⁻¹, Fig. 4a), whereas average pH and DO conditions in May were lower and more variable than in February (pH; 7.6–8.1, DO; 5.6–10.6 mg l⁻¹, Fig. 4b). pH and DO fluctuations in July 2010 (Fig. 4c) were more extreme than those depicted in August, illustrating how

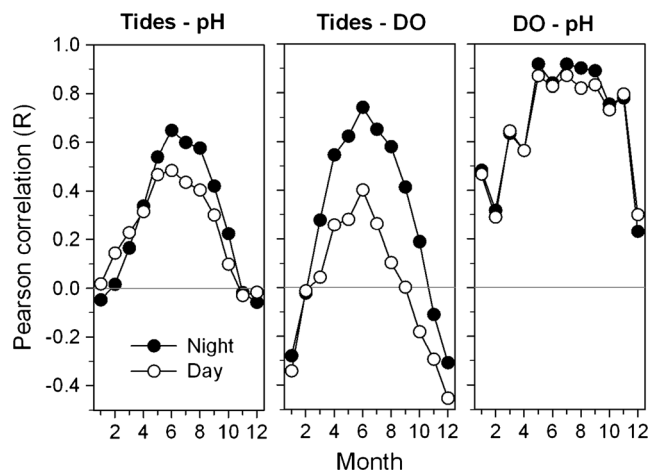


Fig. 3 Correlations between pH and tidal elevation **a**, DO and tidal elevation **b**, and pH and DO **c** by month during day (white circles) and night (black circles) at Flax Pond

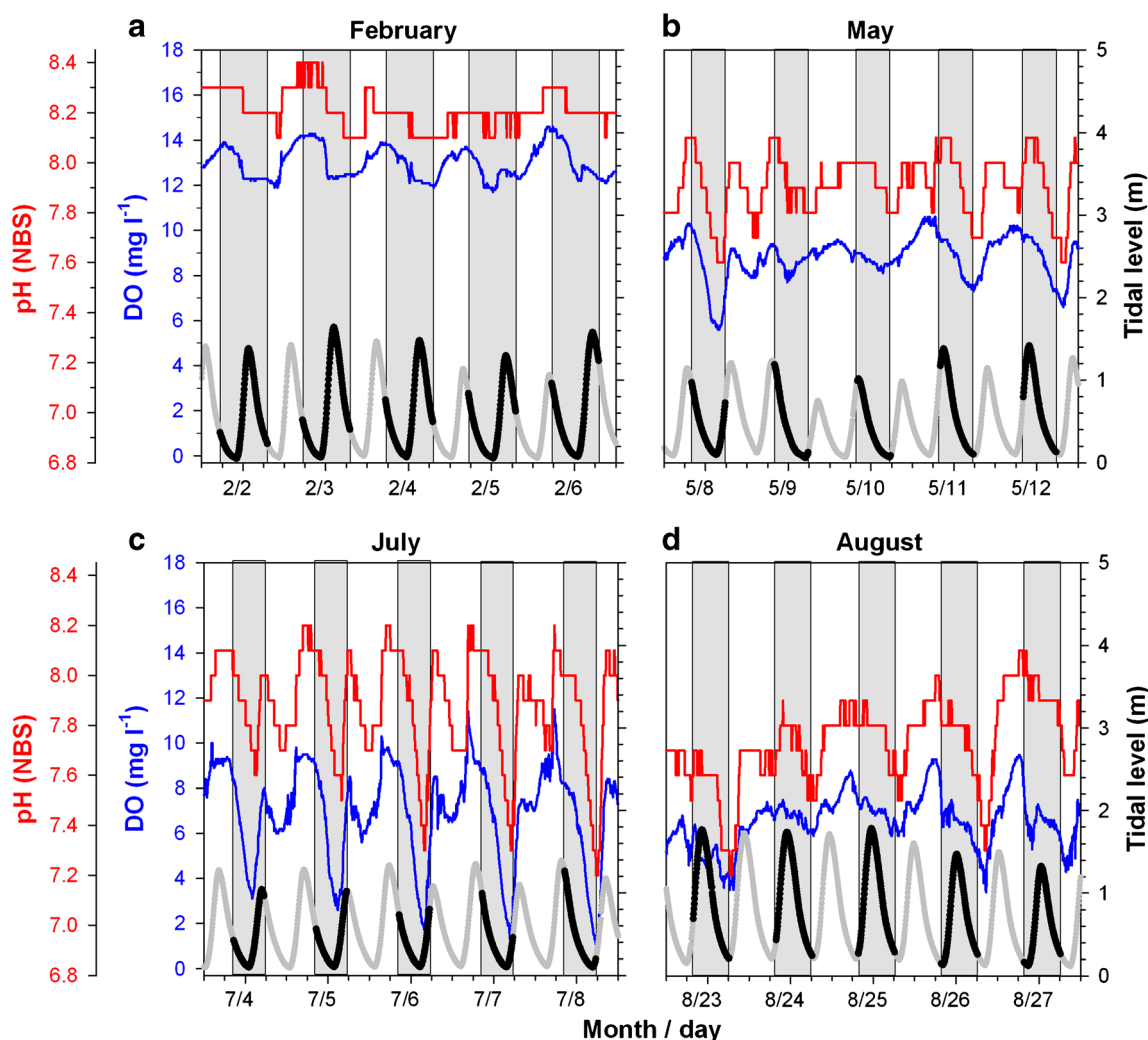


Fig. 4 Four representative examples of short-term pH (red line) and dissolved oxygen (DO, blue line) variability at Flax Pond salt marsh, each encompassing 120 h (~10 tidal cycles) in **a** February, **b** May, **c** July, and **d**

August 2010. Shaded areas depict times between sunset and sunrise; tidal elevations are given as grey (day) and black (night) lines. DO and pH fluctuations are tightly correlated and depend on season, tide, and day/night

different tidal alignments with day or night can influence diel variability. In general, the most extreme pH and DO fluctuations were observed, when low tides coincided with the end of the night, whereas night-centered high tides attenuated diel variability.

In 2012, short-term in situ $p\text{CO}_2$ measurements between May and September revealed (i) a close negative relationship between pH and $p\text{CO}_2$ [$p\text{H} = 1.78\text{E}10 \cdot \exp(-2.22 \cdot p\text{CO}_2)$, $R = -0.85$, $p < 0.001$], (ii) a general seasonal pattern of increasing $p\text{CO}_2$ mean and variance during spring and summer, and (iii) the extreme nature of diel $p\text{CO}_2$ fluctuations in the Flax Pond salt marsh during summer months (Fig. 5). During each of three consecutive nights at the end of July, we recorded $p\text{CO}_2$ fluctuations between ~350 μatm and nearly 4,000 μatm within one tidal cycle. Substantial regular $p\text{CO}_2$ spikes, albeit lower, were also recorded in early May (>1,200 μatm), mid-June (>2,200 μatm), and September (>2,900 μatm).

Interannual variability in monthly temperature, pH, and DO conditions at Flax Pond was considerable (Fig. 6), with monthly anomalies ranging from -2.0 to 2.6 $^{\circ}\text{C}$, -0.18 to 0.20 pH units, and -0.60 to 0.45 mg l^{-1} , respectively. Temperature anomalies were weakly negatively correlated to DO ($p = 0.05$), but not significantly correlated to pH anomalies ($p = 0.12$), whereas pH and DO anomalies were significantly correlated ($p < 0.01$, Fig. 6). Anomalously high temperatures in 2012 coincided with anomalously low pH conditions in the marsh.

Discussion

Diel, Seasonal, Interannual pH and DO Variability

We characterized the patterns and different temporal scales of pH and DO variability in a tidal salt marsh on Long Island

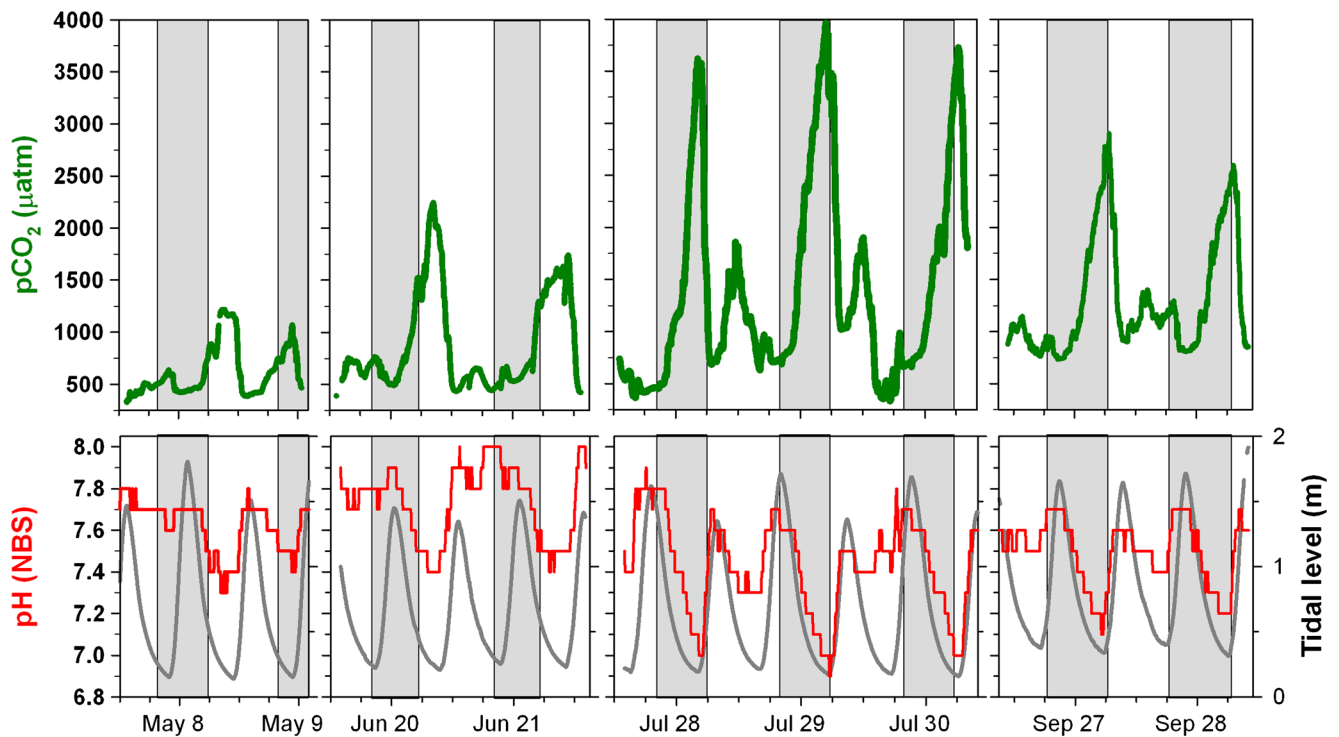
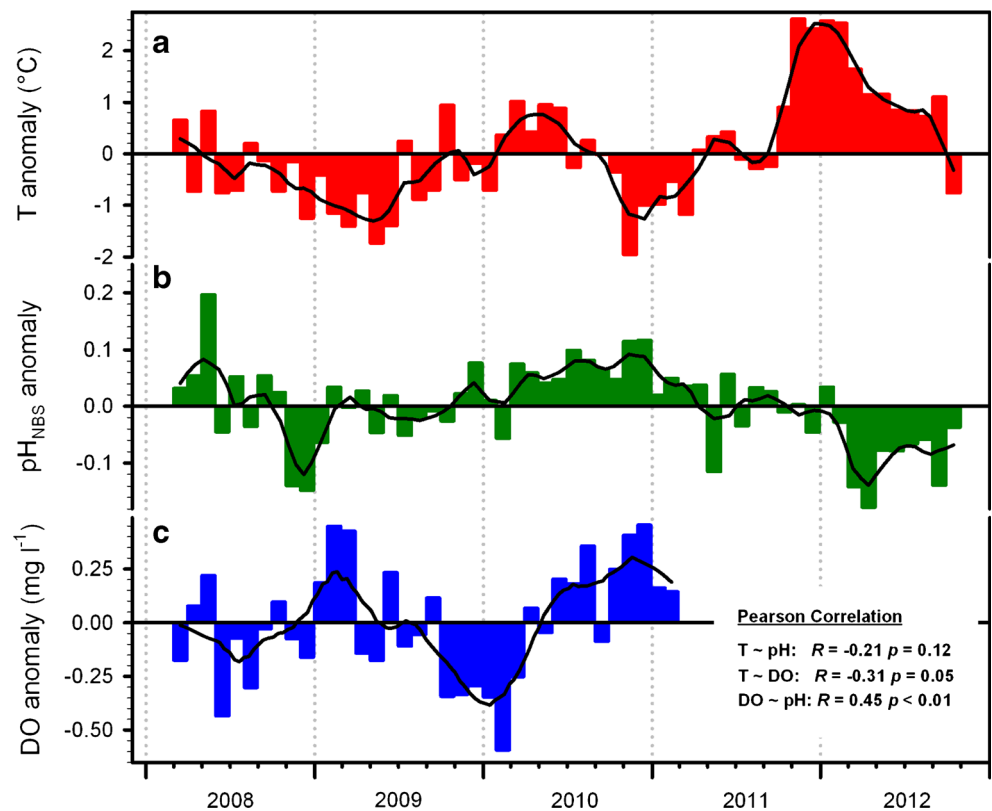


Fig. 5 Correspondence of pH and $p\text{CO}_2$ variations in Flax Pond during four measurement periods in May, June, July, and September 2012. Upper panels (green lines); in situ $p\text{CO}_2$ measurements (every 5 s for

38–70 h), lower panels (red lines); pH measurements (every 6 min, USGS) and tidal elevation (grey lines). Shaded sections correspond to the time between sunset and sunrise

Fig. 6 Monthly anomalies of **a** temperature (T), **b** pH, and **c** dissolved oxygen (DO) and their correlations (lower right corner) in the Flax Pond salt marsh between April 2008 and November 2012. Black lines represent nonparametric local fits (Loess, bandwidth=6 months)



Sound, using high-frequency monitoring data collected between 2008 and 2012. We showed that average pH conditions in this system decline from early spring until late summer by approximately 0.6 units and that average diel pH fluctuations exceed 0.7 units and commonly approach one unit of magnitude in July and August. These patterns are mimicked by diel and seasonal DO fluctuations, and together place the Flax Pond salt marsh among the most extreme marine habitats described to date in terms of natural pH and DO variability (Duarte et al. 2013; Hofmann et al. 2011). This is of interest particularly in the context of current acidification and hypoxia research on marine organisms; by emphasizing (i) the coupled nature of both stressors (Burnett 1997; Gobler et al. 2014; Melzner et al. 2012) and (ii) the fact that many contemporary coastal marine organisms experience short-term pH and DO variations far beyond the predicted changes in the average open ocean for the next centuries (Duarte et al. 2013; Hendriks et al. 2010; Hofmann et al. 2011; Kelly et al. 2013).

Similar to other aquatic habitats (Borges et al. 2003; Burnett 1997; Cai et al. 2011; Melzner et al. 2012), the strong covariance of pH and DO indicates that *diel* fluctuations are the result of changing net heterotrophy in the system, modulated by tides (O'Boyle et al. 2013). This is also indicated by the strong negative relationship between pH and $p\text{CO}_2$ observed during parallel sensor deployments in 2012. During night time, thus, community respiration leads to the observed sharp decline in pH and DO starting around the peak of each high tide. During the day, photosynthetic organisms release O_2 and assimilate CO_2 , thereby countering community respiration and attenuating the pH and DO decline during the outgoing tide. Incoming tides almost always raise pH and DO levels in the system, because of the lower biological productivity in the open, Central Long Island Sound, thus modulating conditions within the marsh. This is supported by Collins et al. (2013), who reported maximum diel DO fluctuations of 0.64 mg l^{-1} ($20 \text{ }\mu\text{M}$) in May 2010 in Central Long Island Sound, which is one unit of magnitude smaller than DO variability in the marsh during the same period ($\sim 6 \text{ mg l}^{-1}$). Incoming tides in winter, on the other hand, can decrease dissolved oxygen values in the marsh, likely because the shallow marsh waters are colder and thus have higher oxygen solubility than those in Long Island Sound.

The short-term patterns in observed pH and DO strongly suggest that acidified and hypoxic waters are tidally exported to Long Island Sound during spring, summer, and fall. This is consistent with Wang and Cai (2004), who demonstrated that highly productive salt marshes adjacent to Sapelo Island (Georgia, USA) 'outwell' both organic and inorganic carbons to the adjacent estuary, thereby contributing to its apparent net heterotrophy. Odum's original 'outwelling hypothesis' (Odum 1969) concerned the export of primarily nutrients and organic matter from marshes to estuaries (Dame et al. 1986; Valiela et al. 1978; Woodwell et al. 1977), promoting microbial

respiration (Koch and Gobler 2009) and thus estuarine net heterotrophy despite high levels of pelagic autotrophy (Caffrey 2004). Hence, expanding upon the original outwelling hypothesis, our findings indicate that in addition to nutrients and organic matter, salt marsh systems also export acidified and hypoxic waters to estuaries, thereby directly influencing the pH and DO conditions that estuarine, non-marsh organism's experience. This is supported by Caffrey (2004), who reported that salt marsh dominated estuaries have significantly higher rates of net oxygen consumption (and thus are more net heterotrophic) than open water estuaries.

The *seasonal* pH and DO patterns in Flax Pond are wholly consistent with annual changes in light and temperature conditions driving biological productivity in temperate latitudes (Odum 1961; Officer et al. 1984). The onset of the strong seasonal decline in pH and DO occurs right after their annual maxima in February when water temperatures have already increased from their minimum in January and the pace of day length increase is fastest. This stimulates biological production, with the resulting increases in biomass and thus community respiration decreasing average pH and DO conditions. As temperatures continue to rise, biological productivity in the marsh builds further in spring and summer until reaching its maximum in August, which coincides with the most extreme diel fluctuations and annual minima in average pH and DO in the system. Thereafter, decreasing temperature and day length trigger a gradual reduction in biomass and a return of the system to its winter state of high and least variable pH and DO conditions. It is noteworthy that the seasonal signal is a composite of two overlaying processes (Fig. 2): a smaller yet substantial seasonal pattern in background pH (i.e., Long Island Sound), as represented by high tide conditions in the marsh ($\text{pH}_{\text{max, Feb}}=8.28$, $\text{pH}_{\text{max, Aug}}=7.87$), and a much larger seasonal amplitude due to increasing and decreasing respiration in the marsh itself ($\text{pH}_{\text{min, Feb}}=8.06$, $\text{pH}_{\text{min, Aug}}=7.15$).

Compared to other coastal habitats (Borges et al. 2003; Hofmann et al. 2011), the magnitudes of pH and DO variations observed at Flax Pond are extreme on both diel and seasonal time scales, consistent with the extraordinary productivity of tidal salt marshes (Bertness 2007; Odum 1961; Pennings and Bertness 2001; Wang and Cai 2004). For example, diel pH variability measured in September/October 2008 in Elkhorn Slough, a tidal marsh on the Californian coast, showed fluctuations between 7.9 and 8.3, save for two stochastic records as low as 7.4 (Hofmann et al. 2011). O'Boyle et al. (2013) assessed summer pH and DO variability in 90 estuarine locations along the Irish Coast, finding average pH fluctuations of 7.86–8.28 in saltwater-dominated sites (>10) with the largest pH and DO amplitudes related to eutrophic waters. Wootton et al. (2008) documented typical diel pH fluctuations of 0.25 units at a coastal Pacific site (Tatoosh Island, Washington, USA), while Barton et al. (2012) measured diurnal fluctuations between 7.69 and 8.15 in a small Pacific bay (Netards Bay,

Oregon, USA). To our knowledge, larger short-term pH fluctuations than those at Flax Pond have only been reported at sites influenced by volcanic CO₂ vents (up to 1.6 pH units, Hall-Spencer et al. 2008).

Full seasonal characterizations of concurrent pH and DO variability are rarer; however, comparable fluctuations have been reported for an urbanized estuary of the Baltic Sea (Kiel Fjord, Melzner et al. 2012), where long-term average pH conditions in bottom waters (20–28 m) fluctuate annually between 8.05 (April) and 7.45 (September), coupled with an equally strong seasonal decline in DO (11.2–<1.6 mg l⁻¹). In the temperate coastal Pacific (Tatoosh Island), pH varies seasonally by up to 1 unit (Wootton et al. 2008), whereas sites along the southern North Sea coast have been shown to exhibit long-term mean seasonal pH variations of up to 0.5 pH units (Provoost et al. 2010).

Biological Implications

Although tidal salt marshes comprise extreme coastal environments with respect to pH and DO variability (Pennings and Bertness 2001), they are undoubtedly teeming with life. Apart from the success of its main founding angiosperm, *Spartina alterniflora*, common aquatic invertebrate and vertebrate species utilizing the Flax Pond marsh include ribbed mussels (*Geukensia demissa*), crabs (*Seasarma reticulatum*, *Uca pugnax*, *Uca pugilator*, and *Dyspanopeus sayi*), and forage fish like Atlantic Silversides, *Menidia menidia*, and Killifish, *Fundulus* spp. (Hovel and Morgan 1997), which comprise major prey items for transient predators like striped bass or bluefish (Tupper and Able 2000). Clearly, such marsh organisms and their offspring cope with frequent periods of high CO₂ and low oxygen conditions in their habitat, as well as with large diel to seasonal fluctuations in both parameters. Hence, many tidal salt marsh organisms and—more generally—coastal marine species may prove to be largely insensitive to elevated CO₂ levels (Frommel et al. 2012; Hendriks et al. 2010; Hurst et al. 2013), particularly levels mimicking the predicted increase in average open ocean conditions over the next 300 years (i.e., up to 2,000 µatm; Riebesell et al. 2010). On the other hand, such organisms could already live close to the edge of their physiological potential and may thus respond negatively to any further changes that increase the environmental extremes in their habitat (Hofmann et al. 2011; Pörtner 2010). Indeed, mollusks are well-known foundational species in coastal ecosystems (Newell 2004), but many species, and particularly early life stages, appear highly sensitive to experimentally reduced levels of pH within the range we report for Flax Pond (Gazeau et al. 2013; Gazeau et al. 2007; Talmage and Gobler 2010). A study using multi-species models to investigate the link between interannual pH variations and benthic community structure found a pronounced shift from calcifying to non-calcifying species during years of

predominantly low pH conditions and vice versa (Wootton et al. 2008). Moreover, during summer extremes, when levels of pCO₂ were high and pH was low in our study system, the most common forms of calcium carbonate were undersaturated (e.g., during the July measurements of pCO₂ and pH, $\Omega_{\text{aragonite}}$ and $\Omega_{\text{calcite}} < 1$, CO₂SYS). Collectively, these observations imply that exacerbating pH and DO conditions in coastal oceans will have consequences, despite the experimental tolerance or theoretical adaptation potential of many coastal marine species (Kelly et al. 2013; Parker et al. 2011; Skelly et al. 2007; Sunday et al. 2011).

Many temperate marine species exhibit prolonged spawning seasons that coincide with the seasonal decline in coastal pH and DO levels, hence, exposing early born offspring to a substantially different pH and DO environment than offspring born later in the season. To illustrate this for Flax Pond (Fig. 7), we have calculated the seasonal change in hours per day (hpd) that aquatic offspring spend in waters below three pH and DO levels: 8.1 (~500 µatm pCO₂) and 12 mg l⁻¹, 7.8 (~1,000 µatm pCO₂) and 8 mg l⁻¹, and 7.5

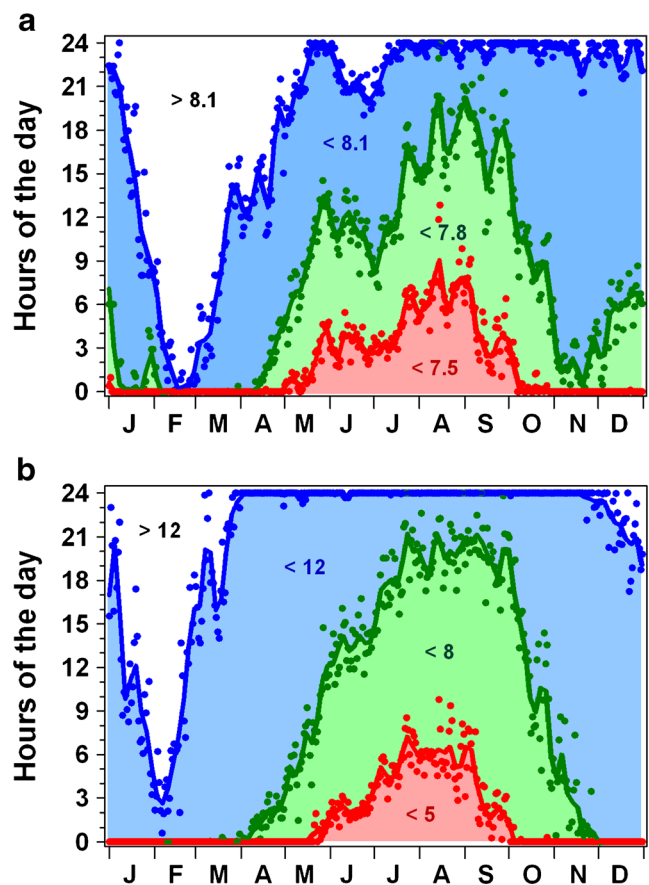


Fig. 7 Seasonal changes in daily **a** pH and **b** DO exposure of a hypothetical organism in Flax Pond salt marsh. Dots represent the average time per day (h), when aquatic organisms would experience conditions below pH 8.1 and 12 mg l⁻¹ (blue), below pH 7.8 and 8 mg l⁻¹ (green), and below pH 7.5 and 5 mg l⁻¹ (red). Lines represent nonparametric local fits (Loess, bandwidth=11 days)

($\sim 2,200 \mu\text{atm pCO}_2$) and 5 mg l^{-1} , respectively. This revealed that offspring in April would experience approximately 11 hpd above and 13 hpd below pH 8.1 (24 h below 12 mg l^{-1}), whereas at the end of May, exposures below pH 8.1 prevail during the entire day with already ~ 12 hpd below pH 7.8 and 8 mg l^{-1} , and 4 hpd below pH 7.5 (Fig. 7). In July and August, exposures below pH 7.5 and 5 mg l^{-1} occur for cumulatively 6–9 hpd (Fig. 7). Seasonal exposure maps like these may be useful to design more realistic experiments for organisms living in dynamic coastal habitats. For example, the minimum pH levels measured in Flax Pond have been shown to yield enhanced mortality in both finfish and shellfish (Baumann et al. 2012; Gazeau et al. 2013), and during summer months, these conditions can cumulatively persist for more than half of a day (Fig. 7). However, nearly all experimental studies examining the effects of acidification and hypoxia on marine animals have assessed static levels. The impacts of strong diel variations in pH such as those observed during this study have not been investigated. Experiments have shown that low pH and DO levels affect mainly or exclusively the early life stages of many marine species (Baumann et al. 2012; Talmage and Gobler 2010) and that these sensitivities may be modulated by parental experience (Miller et al. 2012; Parker et al. 2012). However, whether offspring sensitivities to low pH or DO indeed change over the course of the reproductive season has yet to be demonstrated.

The co-occurrence of low pH and low DO during summer in Flax Pond emphasizes the intimate linkage between these stressors. There is a growing recognition that low oxygen regions within the coastal ocean are also acidified, a condition that will intensify with climate change (Cai et al. 2011). In this time series, we observed a clear link between anomalously high winter and spring temperatures in 2012 and anomalously low pH and (by inference) oxygen conditions, potentially portraying future scenarios resulting from climate change. Presently, however, the concurrent effects of low oxygen and acidification on marine organisms are largely unknown, as most prior studies of marine hypoxia have not considered pH levels. Our recent research has demonstrated that hypoxia and acidification can have additive and synergistic negative effects on the growth, survival, and metamorphosis of early life stage bivalves (Gobler et al. 2014). As such, comprehensive evaluation of the combined effects of low oxygen and acidification on marine life is critical for understanding how ocean ecosystems respond to these conditions both today and under future climate change scenarios.

Conclusions

Highly productive tidal salt marshes like Flax Pond are characterized by extreme and concurrent pH and DO fluctuations on diel to seasonal time scales. The magnitude of these

fluctuations is modulated by tides and the time of the day, with the most acidic and hypoxic conditions occurring during low tide at the end of the night. Tidal salt marshes support ecologically and economically important coastal organisms, which have likely evolved mechanisms to cope with large environmental variability, which need to be better understood (e.g., alternative metabolic pathways, advanced acid–base regulation, and parental conditioning). These organisms may largely tolerate the CO_2 levels predicted to occur within the average open ocean during the next 300 years. They may, however, already live close to their tolerance threshold and thus react negatively to anthropogenic changes in coastal pH unrelated to atmospheric CO_2 increases (e.g., due to eutrophication). Our study encourages novel experimental approaches that mimic fluctuating pH and DO environments of coastal habitats to better understand the concurrent effect of both stressors in more realistic laboratory settings.

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