

Community composition in mangrove ponds with pulsed hypoxic and acidified conditions

KERYN B. GEDAN,^{1,†} ANDREW H. ALTIERI,² ILKA FELLER,³ REBECCA BURRELL,⁴ AND DENISE BREITBURG³

¹*Biological Sciences, George Washington University, 800 22nd Street NW, Washington, D.C. 20052 USA*

²*Smithsonian Tropical Research Institute, Apartado 0843-03092, Balboa, Ancon, Panama*

³*Smithsonian Environmental Research Center, 647 Contees Wharf Road, Edgewater, Maryland 21037 USA*

⁴*Maryland Department of Natural Resources, 580 Taylor Avenue, Annapolis, Maryland 21401 USA*

Citation: Gedan, K. B., A. H. Altieri, I. Feller, R. Burrell, and D. Breitburg. 2017. Community composition in mangrove ponds with pulsed hypoxic and acidified conditions. *Ecosphere* 8(12):e02053. 10.1002/ecs2.2053

Abstract. The potential resilience of biological communities to accelerating rates of global change has received considerable attention. We suggest that some shallow aquatic ecosystems, where temperature, dissolved oxygen (DO), and pH can exhibit extreme variation on short timescales of hours or days, provide an opportunity to develop a mechanistic understanding of species persistence and community assembly under harsh environmental conditions. Extreme diel swings in DO and pH have been observed in eutrophic temperate ecosystems, and here, we describe a similar phenomenon consistently occurring across tropical sites that included relatively remote atolls on the Meso-American barrier reefs in Belize and oligotrophic coastal lagoons in Panama. In particular, we documented large daily swings in temperature, DO, and pH within shallow ponds of Caribbean mangrove forests. Water in seven of 13 ponds went hypoxic (<2 mg/L DO) during the multiday sampling period, and pH dipped nightly to low levels, falling below 7.0 in some ponds. Minimum pH and minimum DO were correlated, and showed a similar relationship in Belize and Panama, suggesting a common mechanism produced diel cycles. Remarkably, most ponds exhibited high abundance of macroalgae, macroinvertebrates, and fish, despite potentially stressful abiotic conditions. Although fish diversity was negatively correlated with pH range, our overall results from the ponds suggest that many species are sufficiently resistant such that a functionally complex community can persist in the midst of pulsed stressful conditions. We propose that the mangrove ponds could serve as a model ecosystem for investigating resistance and resilience of coastal marine communities to global change factors such as climate change, hypoxia, and ocean acidification.

Key words: acidification; diel cycle; environmental filtering; eutrophication; hypoxia; stress.

Received 17 May 2017; revised 11 September 2017; accepted 20 September 2017; final version received 17 November 2017. Corresponding Editor: Debra P. C. Peters.

Copyright: © 2017 Gedan et al. This is an open access article under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

† **E-mail:** kgedan@gwu.edu

INTRODUCTION

A useful approach to understanding and anticipating ecosystem-level responses to global environmental changes comes from natural ecosystems that can serve as model study systems (Andersson et al. 2015). For example, CO₂ vent communities and associated gradients in pH have helped us predict effects of ocean acidification on community

structure (Hall-Spencer et al. 2008, Gambi et al. 2016). Coastal marine ecosystems are simultaneously experiencing shifts in water temperature, pH, and dissolved oxygen (DO) due to climate change and eutrophication (Cai et al. 2011, Wallace et al. 2014, Altieri and Gedan 2015). Predicting the response of ecosystems to these changes requires consideration of the co-occurrence of stressors affecting coastal marine communities, particularly

because multiple stressors can mask or exacerbate the effects of a given stressor (Breitburg et al. 1998, 2015, Crain et al. 2008, Halpern et al. 2008, Kroeker et al. 2017). Identifying and studying natural systems where multiple global change stressors are particularly acute and co-occur can provide a mechanistic understanding of the structure and function of marine ecosystems under future conditions.

Current conditions in dynamic coastal environments may preview, over short durations, the potentially stressful environmental conditions predicted to occur in the ocean over larger scales as the climate changes. Recent comparisons of coastal marine and open water ecosystems have drawn attention to the extreme conditions that certain coastal ecosystems experience, in sharp contrast to the more stable character of oceanic environments (Duarte et al. 2013, Baumann et al. 2015, Hendriks et al. 2015). Local biological oxygen demand and respiration in semi-enclosed and/or eutrophic environments such as estuaries can drive oxygen depletion as well as CO₂ enrichment (Duarte et al. 2013, Breitburg et al. 2015, Gobler and Baumann 2016). As a consequence, temperature, pH, and DO can co-vary over seasonal (Cai et al. 2011, Wallace et al. 2014, Baumann et al. 2015, Gobler and Baumann 2016) or daily (Hofmann et al. 2011, Baumann et al. 2015, Burrell et al. 2015, Gobler and Baumann 2016) timescales, and large amplitude variation in water conditions in shallow environments can expose organisms to extreme temperatures (Helmuth and Hofmann 2001), DO (Burrell et al. 2015), and pH (Hendriks et al. 2015).

Mangrove forests are among the most common coastal environments, dominating many tropical and subtropical coastlines (Spalding et al. 2010). Ponds are a common feature of mangrove forests that can occupy a substantial portion of forest area (Rützler et al. 2004). Within our study regions in Panama and Belize, for example, mangrove ponds accounted for 4% and 15% of the area of mangrove forest, respectively (Fig. 1; Rodriguez and Feller 2004). Mangrove ponds are recognized for their diverse aquatic communities. For example, Taylor et al. (2007) observed over 30 species of fish in ponds in Belize, four of which were unique to pond environments. Despite this potential for high biological diversity, we predicted that ponds within

mangrove forests could experience large diel cycles in DO and pH because they have many of the characteristics that drive coastal acidification and formation of hypoxic conditions—they are productive, shallow, warm and have limited exchange with open water. Therefore, mangrove ponds have the potential to serve as a general model system for exploring the persistence of biological communities in the face of global change stressors.

This study pursued two aims under the overarching goal of developing mangrove ponds as a model system: first, to comprehensively describe the natural history of mangrove ponds, an important and understudied component of mangrove forests, and second, to understand the relationships between fluctuating water quality conditions and persistence of the biological community in ponds. To this end, we characterized macroalgal and macrofaunal community composition in mangrove ponds and investigated diel fluctuations in DO, temperature, and pH in two relatively undisturbed regions, offshore mangrove islands in Belize and a coastal, mangrove-dominated lagoon in Panama. We hypothesized that regular, diel-cycling temperature, pH, and hypoxia may act as environmental filters and selective pressures that shape community composition. Previous work in temperate coastal ecosystems has revealed that high temperature, low pH, and hypoxia can select for organisms that can tolerate these “hot, sour, and breathless” conditions (Wallace et al. 2014). In the case of hypoxia, shorter-term (episodic or diel) hypoxia stress can cause the selective loss of susceptible organisms or life history stages and can induce temporary migrations in mobile fauna that dodge short-term hypoxic areas (Breitburg 1992, Brady and Targett 2010). Low pH, hypoxia, and high temperature can also have indirect effects by, for example, affecting immune function and disease progression (Harvell et al. 1999, Breitburg et al. 2015, Keppel et al. 2015), or modifying predator–prey interactions (Breitburg 1992, Pihl et al. 1992, Altieri 2008, Long and Seitz 2008, Pincebourde et al. 2008).

Based on what is known about the conditions that can promote pH, hypoxia, and temperature stressors in aquatic ecosystems, and biological responses to those stressors, we made several general predictions about mangrove pond dynamics. We expected that (1) ponds would exhibit large

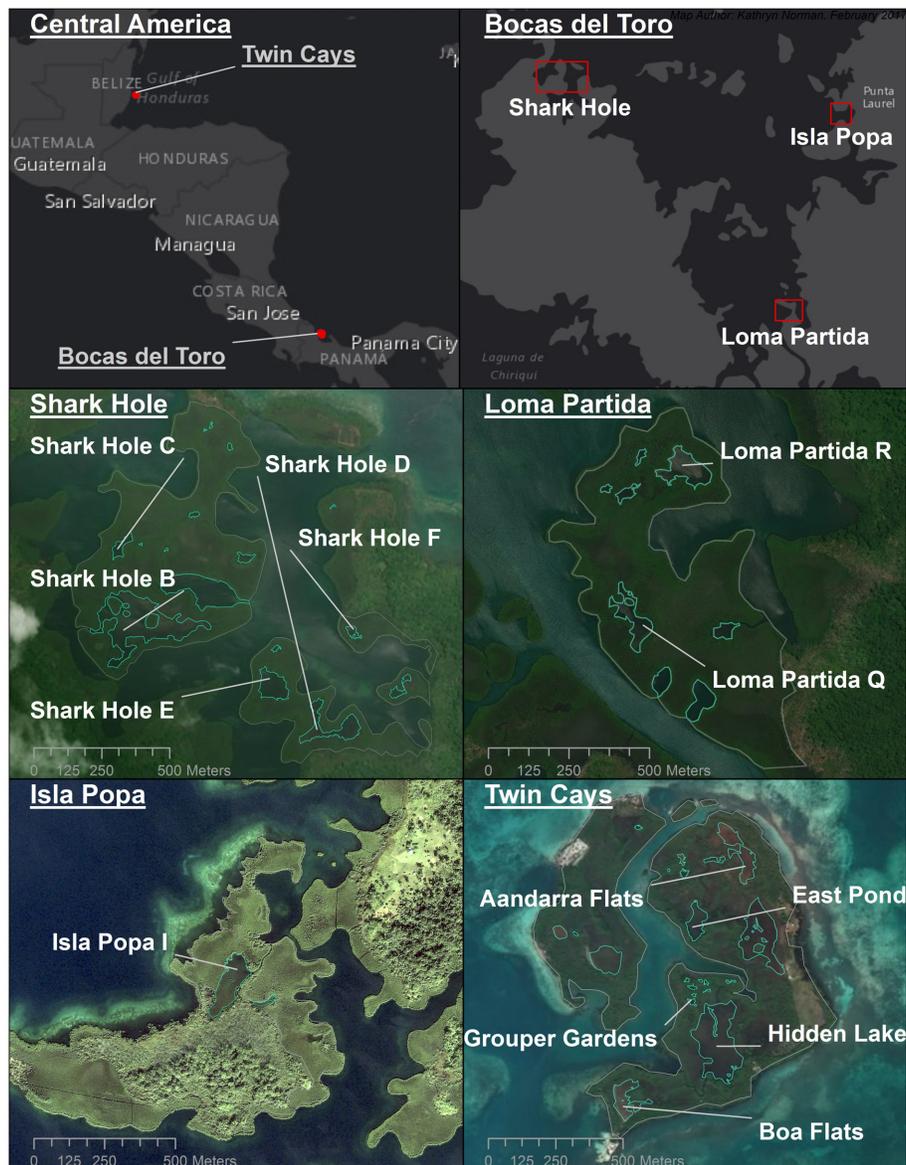


Fig. 1. Maps of study regions and sites. Top left: study regions in Panama and Belize. Top right: islands within the Bocas del Toro archipelago. Middle: mangrove pond sites in Shark Hole and Loma Partida, Panama. Bottom left: pond sites in Isla Popa, Panama. Bottom right: pond sites in Twin Cays, Belize. Ponds were outlined in ArcGIS 10.3.1.

fluctuations in pH, DO, and/or temperature conditions and exhibit potentially biologically stressful conditions, (2) the severity of physical conditions in ponds would vary spatially according to physical characteristics of pond size and depth and vary temporally on a diel cycle if

conditions are driven by local respiration, (3) a characteristic community of organisms would inhabit ponds, and that more stressful ponds would lack sensitive taxa, and (4) organisms that persisted in ponds would show signs of organismal stress such as higher rates of disease.

METHODS

Study sites

Belize.—We sampled water quality conditions in five mangrove ponds (Hidden Lake, East Pond, Grouper Gardens, Boa Flats, and Aandarra Flats) in Twin Cays, Belize, during the dry season in April–May 2011. Twin Cays is a 92-ha archipelago located ~20 km offshore of the southern coast of Belize (Fig. 1) and is part of the Meso-American barrier reef system with hypersaline to oceanic salinities and negligible terrigenous inputs of sediment or freshwater (Macintyre et al. 2004). All sampled ponds were located on the larger East Island. See Rützler et al. (2004) for a detailed description of the mangrove ponds and Twin Cays study area. Although we were not able to quantitatively survey macrofauna in Belizean mangrove ponds, we recorded a list of species observed in shoreline surveys and cast net samples during site visits (Appendix S1: Table S1).

Panama.—We sampled water quality conditions and macrobiota in eight mangrove ponds (assigned names: Shark Hole B, Shark Hole C, Shark Hole D, Shark Hole E, Shark Hole F, Popa I, Loma Partida Q, and Loma Partida R) in Bocas del Toro Province on the Caribbean coast of Panama (Fig. 1) during the dry season in January 2013. All mangrove ponds were located within northern Almirante Bay, which receives discharge from two mainland rivers and areas of coastal development. The Bay is, therefore, more terrestrially linked than the offshore mangrove ponds of Twin Cays, Belize. However, salinity, nutrient, and chlorophyll *a* levels in Almirante Bay are more similar to oligotrophic oceanic conditions than the adjacent Chiriquí Lagoon (D’Croze et al. 2005).

All study ponds were permanently submerged areas within red mangrove (*Rhizophora mangle*) forests, of shallow depth (<2 m), and at least 100 m² in area (Fig. 1). All ponds were mostly enclosed and had at least one channel connection that allowed water exchange. The underlying substrate in ponds was comprised of mangrove peat covered by unconsolidated sediments and detritus.

Water quality sampling

We deployed multi-parameter water quality sondes (YSI 6600V2-4, YSI Inc., Yellow Springs, Ohio, USA) at 10 cm from the bottom of each pond to collect data in 15-min intervals on water

temperature (°C), salinity (ppt), depth (m), pH (mV), chlorophyll *a* (µg/L), DO saturation (%), and DO concentration (mg/L) for a period of one to two days. The average collection period was 32 h in Belize and 49 h in Panama. Before and after deployments, the replicate water quality sondes were deployed at a common site and calibrated against standards to ensure consistent data quality. In each region, we also collected concurrent data on the same water quality parameters with a sonde at a well-flushed reference site of the research station dock, away from mangrove ponds.

At Panamanian ponds, we also measured total alkalinity of water samples. Total alkalinity (A_T) was determined via two-stage, open-cell, potentiometric titration using a Tazo Schott-Gerate piston burette titrator and a Corning pH Analyzer 350, following Dickson et al. (2007), using Scripps Institution of Oceanography Certified Reference Material to validate our measurements. The software program CO2SYS (Pelletier et al. 2007) was used to calculate pCO₂ values with measured salinity, temperature, pH, and alkalinity and using the constants of Dickson and Millero (1987).

Pond characterization

We measured the area of each pond in ArcGIS 10.3.1 (ESRI, Redlands, California, USA) using the polygon tool, and made in situ measurements of pond depth at 35 points. Pond volume was estimated by multiplying mean pond depth by pond area. Residence time, an indicator of tidal flushing, was approximated as the pond depth divided by the mean tidal range in the region (15 cm in Twin Cays, Belize [Kjerfve et al. 1982] and 25 cm in Bocas del Toro, Panama [Cubit et al. 1989]), similar to the tidal prism method of Oertel (2001).

Community composition of ponds

Benthic communities.—We observed a spatial gradient in macroalgal abundance within ponds and designed a sampling scheme to span that gradient. In each pond, two 30-m transects were placed on the more vegetated side of the pond, two in the less vegetated side, and one in the middle, for a total of five transects. We placed quadrats (0.5 m²) every 5 m along each transect ($n = 7$ points per transect) and visually estimated the percent cover of macroalgal species within

each quadrat. In addition, macroinvertebrates and burrow entrances within quadrats were counted and identified to the lowest taxonomic level possible in the field. At each quadrat location, we recorded the thickness of the algal mat when one was present or the thickness of flocculated sediment when an algal mat was absent. We also noted the presence of the purple sulfur bacteria *Chromatium* (Fig. 2D) in the surface sediment as a biogeochemical indicator of shallow, anoxic sediments (Madigan and Jung 2009).

The upside-down jellyfish, *Cassiopea* spp., is a common constituent of many ponds; in some places, they nearly cover the benthos (Fig. 2A). *Cassiopea* spp. densities were estimated in snorkel surveys in Panama. Along the five 30-m transects used for benthic sampling, *Cassiopea* were counted within 1.25 m of each side of transects (2.5×30 m transect). All field identifications were visual, to genus. In addition, we noted the presence of scyphomedusae and ctenophores in each pond during these surveys.

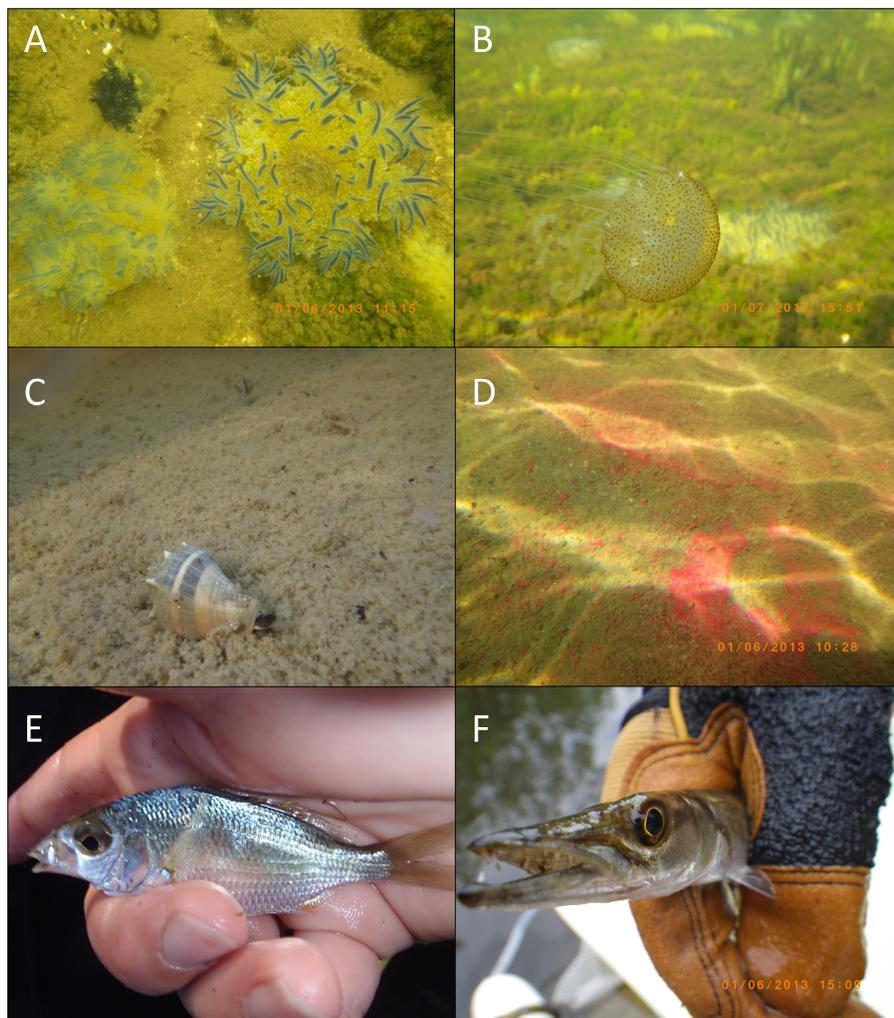


Fig. 2. Representatives of the biodiversity of Caribbean mangrove ponds. (A) Individuals of *Cassiopea* spp.; (B) an unidentified species of *Chrysaora*, with macroalgal mats and *Cassiopea* visible in the background; (C) *Melongena melongena* moving through the sediment floc layer; (D) a film of magenta *Chromatium* visible just below the sediment surface; (E) *Eucinostomus gula*; and (F) *Sphyraena barracuda*.

Fish.—Fish communities were sampled with a seine net (0.63 cm mesh size) pulled over an 8 × 8 m area. We collected two to four seine samples per pond, and fish were identified to the lowest taxonomic level possible. Fish were sampled between 10 am and 4 pm. Due to an injury, we were unable to collect fish samples from pond Shark Hole F.

Oyster disease assays.—We quantified the prevalence of *Perkinsus* sp. (Dermo) infection in oysters (*Crassostrea rhizophorae* and *Isognomen alatus*) of five Panamanian ponds (Shark Hole B, C, D, E, and F). Oysters of each species were collected at the mean low water line from *R. mangle* roots along each pond's margin ($n = 40$ individuals/species) and in the bay just outside of each pond ($n = 20$ individuals/species). All individuals were dissected, and infection was assessed using Ray's fluid thioglycollate media assay on rectal tissue (Ray 1952, 1954). Intensity of infections was scored using the Mackin scale, which ranges from 0 to 5 for each oyster (Mackin 1962). Prevalence within a pond oyster population was calculated as the number of infected oysters divided by the total number of oysters sampled. We determined the weighted prevalence (WP) of infection for each species in each pond as the mean infection intensity multiplied by the prevalence.

Statistical analyses

Water quality data were quality-assured by examining all parameters graphically for sudden transitions and outlier values that indicated possible probe malfunction. Suspect probe recordings were checked against post-deployment values from known standards, and erroneous data were removed.

We conducted an exploratory data analysis to detect correlations among abiotic and biotic variables, using pond as the level of replication ($n = 13$ for abiotic parameters and $n = 8$ for biotic parameters). To test the hypothesis that physical characteristics of ponds that affect gas dissolution and water exchange affect the magnitude of the diel cycle, we examined the correlation between pond characteristics (e.g., pond area, depth, volume, and residence time) and pond abiotic conditions that represent biological stressors (e.g., minimum pH, minimum DO, and maximum temperature) and those that indicate the magnitude of the diel cycle (e.g., range of pH, DO, and temperature).

We used linear regression to investigate the relationship between minimum pH and minimum DO saturation observed in Panama and Belize and to compare their relationship with that from another system with large diel cycles in pH and DO, the Chesapeake Bay (Maryland Department of Natural Resources 2017). Linear regression models of minimum pH and minimum DO saturation with and without the predictor of region (i.e., Panama, Belize, or Chesapeake Bay) were compared in ANOVA of the nested regression models.

To test the hypothesis that pond abiotic conditions affect pond community composition, we examined the correlation of minimum pH, minimum DO, pH range, and DO range with the diversity and abundance of algae, benthic invertebrates, and fish in replicate ponds. Diversity was described using the Shannon index of diversity. Abundance of algae, benthic invertebrates, and fish was indicated by percent cover, mean number per m^2 , and mean number per seine, respectively. Temperature was not included in this analysis because we observed a low level of variation in temperature among Panamanian ponds (Table 1).

When Pearson's correlations were strong (defined as $r \geq \pm 0.4$), we tested the significance of relationships with linear regression. To account for multiple comparisons and to reduce the likelihood of a false positive (type I error), we controlled for a false discovery rate of 5% within each family of tests using the technique of Benjamini and Hochberg (1995).

To more holistically visualize the effects of pH and DO extremes on the benthic macrofaunal community, we conducted a non-metric multidimensional scaling (NMDS) ordination on the dissimilarity of communities sampled in benthic transects, using the metaMDS function in the vegan package (Oksanen et al. 2015). The Jaccard index of dissimilarity, which is binary, was selected to enable an analysis that included cover and abundance data. We fitted surfaces from pond-level minimum pH and minimum DO data using the ordisurf function to observe the distribution of communities across these environmental gradients.

To test the hypothesis that pond abiotic conditions affect pond disease dynamics, we compared *Perkinsus* sp. infection prevalence, infection

Table 1. Water quality summary statistics of 13 mangrove ponds in Belize and Panama.

Site	Min pH	Max pH	pH range	Min DO (mg/L)	Max DO (mg/L)	DO range (mg/L)	Min T (°C)	Max T (°C)	T range (°C)	Mean salinity (ppt)	Mean chl a (µg/L)	Mean depth (cm)
Belize												
Grouper Gardens	7.19	7.87	0.68	0.91	5.72	4.81	29.02	31.80	2.78	38	3.66	79
Boa Flats	7.85	8.39	0.54	3.08	10.32	7.24	25.74	37.22	11.48	41	5.21	9
East Pond	7.54	8.03	0.49	0.42	8.84	8.42	28.65	35.20	6.55	38	2.78	75
Aandarra Flats	7.54	8.03	0.49	0.46	9.12	8.66	27.09	40.19	13.10	43	6.51	16
Hidden Lake	7.68	7.91	0.23	1.85	7.17	5.32	29.54	35.21	5.67	38	1.68	51
Dock (reference)	8.01	8.34	0.33	4.51	9.13	4.62	27.27	29.78	2.51	37	0.28	26
Panama												
Shark Hole C	6.77	8.24	1.47	0.05	7.61	7.56	26.15	28.78	2.63	31	1.23	50
Loma Partida Q	7.38	8.18	0.80	1.45	9.29	7.84	26.16	31.00	4.84	32	0.5	41
Popa I	7.36	8.02	0.66	3.26	6.12	2.86	25.58	31.17	5.59	31	1.72	44
Loma Partida R	7.5	8.11	0.61	2.99	7.4	4.41	27.14	30.81	3.67	31	1.22	47
Shark Hole D	7.47	7.98	0.51	2.15	8.16	6.01	28.11	32.62	4.51	30	0.31	46
Shark Hole F	7.34	7.84	0.50	1.71	5.2	3.49	27.07	31.50	4.43	31	0.68	47
Shark Hole E	7.55	7.96	0.41	3.28	7.2	3.92	28.65	31.78	3.13	30	1.16	34
Shark Hole B	7.53	7.87	0.34	3.38	7.57	4.19	27.16	31.14	3.98	30	1.56	59
Dock (reference)	7.62	8.28	0.66	3.5	9.41	5.91	26.20	28.98	2.78	32	1.27	62

Notes: DO, dissolved oxygen. Sites are ordered by decreasing pH range and with the reference site last.

intensity, and WP for each oyster species within each pond site with two-sided *t*-tests. We used two-sided paired *t*-tests to test for differences between infection levels within and outside of ponds. To detect differences in levels of disease between pond sites that might be explained by variation in abiotic conditions, we examined the main and interactive effects on infection intensity of oyster species, pond site, and location within or outside of ponds in a factorial ANOVA. *Perkinsus* sp. infection intensity was log-transformed to meet the assumptions of normality and homogeneity of variances required for ANOVA.

RESULTS

Pond characterization

Ponds had a mean area of 1.39 ha (range, 0.02–3.94 ha) in Belize and 1.42 ha (range, 0.17–4.98 ha) in Panama. Pond depth averaged 46 cm (range, 9–79 cm) in Belize and 46 cm (range, 34–59 cm) in Panama (Table 1). Estimated residence times ranged from less than one to over five tidal cycles, with a mean of 2.3 cycles (or 28.5 h).

Water quality

Ponds exhibited a strong diel cycle in water quality conditions (Fig. 3). The daily range,

maximum, and minimum values of DO, pH, and temperature varied between ponds and generally reached more extreme values than at reference sites (Table 1, Fig. 3). Pond salinity varied little within regions, with a range of 38–43 ppt in Belize and range of 30–32 ppt in Panama. In Panama, chlorophyll *a* concentrations in ponds ranged from 0.31 to 1.72 µg/L and were similar to the reference site, whereas in Belize, pond concentrations ranged from 1.68 to 6.51 µg/L and were higher than at the reference site (Table 1). Alkalinity in Panamanian ponds (excluding outlier Shark Hole C, see following page) ranged from 1928 to 2036 ueqHCO₃⁻/L, and calculated maximum pCO₂ reached levels as high as 4810 uatm, with an average maximum pCO₂ across all ponds of 2499 uatm (Appendix S1: Table S1).

Four of five ponds in Belize and three of eight ponds in Panama experienced DO levels <2 mg/L, but only at night, indicative of diel cycling. In contrast, DO concentrations at reference sites never dropped below this threshold. Extreme low nighttime DO levels of <1 mg/L were observed in three ponds in Belize and one pond in Panama. Ponds in Belize were regularly supersaturated (>100%) with oxygen during the day, whereas they were not in Panama, where maximum DO concentrations generally remained near 100% saturation (Fig. 3).

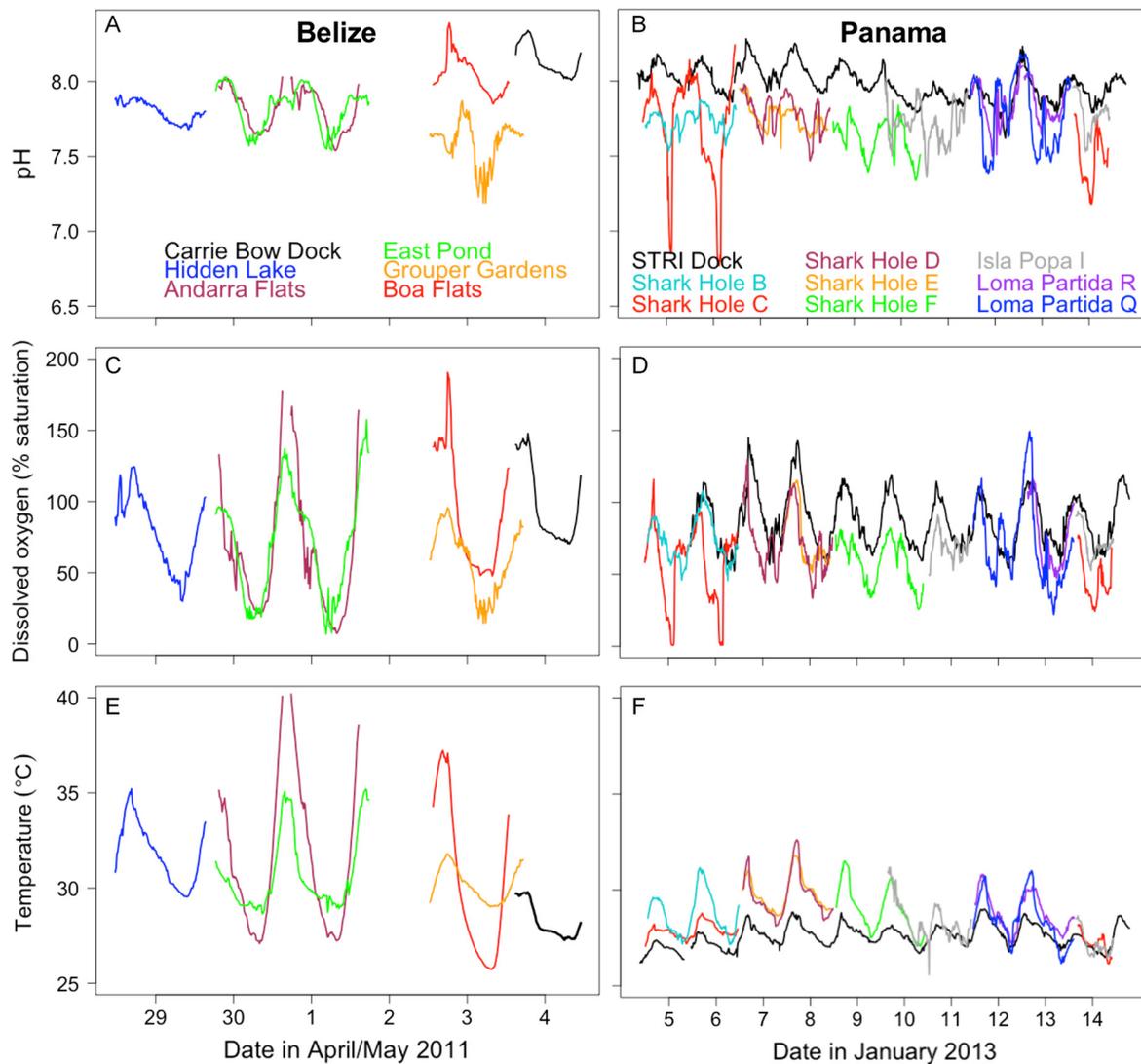


Fig. 3. Diel cycles in physical variables in mangrove ponds in Belize (five ponds and a reference site, left column) and Panama (eight ponds and a reference site, right column). (A) and (B) pH, (C) and (D) dissolved oxygen saturation, and (E) and (F) temperature.

There was also a clear diel cycle in pH in mangrove ponds (Fig. 3). Minimum pH varied significantly with minimum DO saturation (linear regressions: Belize $P = 0.022$, $R^2 = 0.68$; Panama: $P = 0.002$, $R^2 = 0.77$, Fig. 4). In Belize, nighttime pH in ponds fell as low as 7.54, whereas the minimum pH at the reference site was 8.01. In Panama, nighttime pH dipped below 7.40 in four ponds, and in one of those ponds (Shark Hole C), it fell to 6.77. We observed this drop at Shark Hole C on two consecutive nights. In

follow-up sampling of Shark Hole C, however, we observed a less pronounced diel cycle, with a minimum pH of 7.18 and a range of 0.6 pH units (red trace, Fig. 3B). Because DO and pH conditions at Shark Hole C were well outside of the range of other sites, and were not consistent on these two sampling occasions, it was difficult to characterize this pond. As a result, we excluded it from further analyses. At the reference site in Panama, pH remained above 7.62 (Table 1).

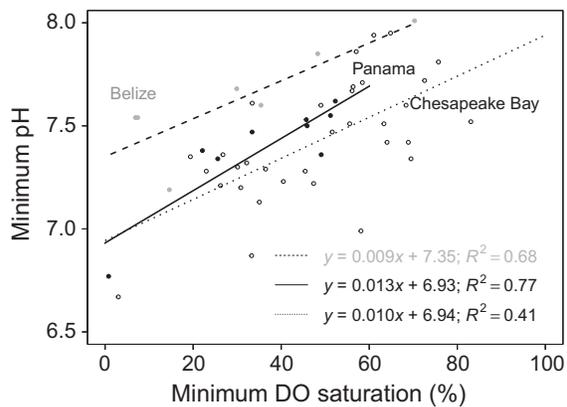


Fig. 4. Minimum dissolved oxygen saturation (%) and minimum pH for replicate ponds in Belize (gray, dashed line) and Panama (black, solid line). For comparison to tropical study sites, data from brackish and saltwater sites in the Chesapeake Bay continuous water quality monitoring program (Maryland Department of Natural Resources 2017) are also plotted (dotted line).

Interestingly, the relationship between minimum pH and minimum DO saturation in Belize and Panama was similar to the same relationship in brackish and saltwater sites in the Chesapeake Bay ($P < 0.0001$, $R^2 = 0.41$, Fig. 4). Minimum DO saturation and region were both significant predictors in linear regression, and a model that included region explained significantly more variation than one without (ANOVA, $F_{45,47} = 9.44$, $P = 0.0003$). Yet, regression coefficients across all regions (Belize = 0.009, Panama = 0.013, Chesapeake Bay = 0.010) and intercepts (Belize = 7.35, Panama = 6.93, Chesapeake Bay = 6.94) were quite similar.

Water temperature also exhibited a diel cycle in ponds. Two shallow ponds in Belize, Boa Flats and Aandarra Flats, had particularly large daily temperature ranges, spanning more than 11°C. These shallow ponds reached maximum temperatures of 37.2°C and 40.2°C, respectively. In Panama, diel changes in temperature in ponds were less extreme, up to 5.6°C and reaching a maximum temperature of 32.6°C (Table 1).

Of seven correlations greater than ± 0.4 between physical and abiotic variables (Appendix S1: Table S3), three had significant linear relationships (Appendix S1: Table S4). We found that pond area and volume were significantly negatively correlated

with pH range and pond depth was significantly negatively correlated with temperature range (Appendix S1: Table S4).

Community composition of ponds

Benthic macroalgae.—Nearly all Panamanian ponds had abundant macroalgae (Fig. 5A) and exhibited a strong biotic gradient in algal cover. Aside from pond Popa I, which was virtually unvegetated, ponds had macroalgal cover on their more vegetated side ranging from 21% to 99% ($82.6\% \pm 28.2\%$, mean \pm SD). In five of the seven ponds with vegetation, the less vegetated side of the pond was nearly bare ($1.6\% \pm 3.1\%$ vegetated, mean \pm SD).

Caulerpa verticillata was the most common species of macroalgae; it was present in all surveyed ponds and represented up to 84% of the macroalgal cover (Fig. 5A). *Caulerpa sertularioides* and *Acetabularia* spp. were each present along transects in five of eight Panamanian ponds. In Shark Hole C and Shark Hole E, where macroalgal cover was high across the entire pond, algal mats were over 10 cm thick (Shark Hole C: 13.3 ± 7.4 cm; Shark Hole E: 10.1 ± 5.0 cm). In other ponds, mean algal mat thickness varied from 0.4 ± 1.5 cm (Shark Hole F) to 6.8 ± 6.5 cm (Loma Partida R). Areas devoid of algal mat often had a thick layer of flocculated sediment, with a mean depth that varied from 2.9 ± 3.9 cm in the predominantly vegetated pond Shark Hole E to 16.4 ± 9.8 cm in the predominantly bare pond Popa I. Other than the seagrass *Thalassia testudinum*, which we observed in some ponds near channels and which does calcify in some environments (Enríquez and Schubert 2014), calcifying macrophytes were rare (Fig. 5A).

Ten morphospecies of macroinvertebrates were found in our benthic surveys (Appendix S1: Table S1). Fan worms (Sabellidae) and burrows occupied by snapping shrimp (*Alpheus* or *Synalpheus* spp.) and gobies were common in ponds (Fig. 5B), reaching densities as high as 12.9 ± 2.3 m⁻² for fan worms (Shark Hole B) and 4.9 ± 1.5 m⁻² for burrows (Loma Partida R; Fig. 5B). Snapping shrimp and gobies typically remained partially hidden within burrows during our visual surveys, but were collected in seine net samples. *Melongena melongena* and *Cerithidium* spp. snails were also present in many ponds (Fig. 5B). We observed a number of

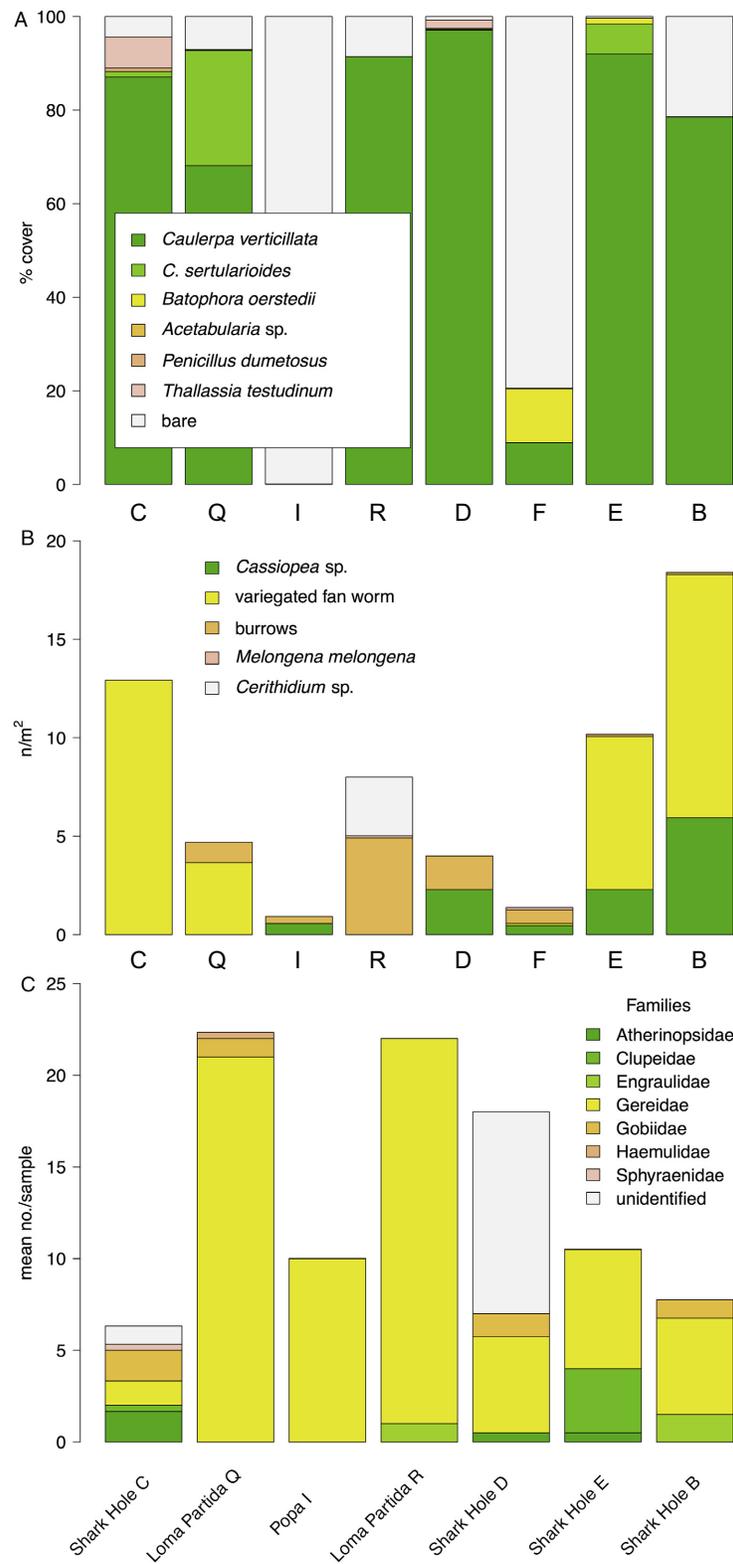


Fig. 5. Community composition of (A) algae, as percent cover, (B) benthic invertebrates, as no./m², and (C)

(Fig. 5. Continued)

fish, as mean no. per sample in Panamanian mangrove ponds. Sites are ordered by pH range, with range decreasing from left to right.

additional bivalve, sponge, and gastropod species in ponds, but not within our quadrats (a comprehensive list is in Appendix S1: Table S1). Echinoderms, other than the sea cucumber *Holothuria mexicana*, were absent except in channels leading to ponds.

Scyphozoans and ctenophores were conspicuous components of the faunal assemblage of the mangrove ponds. Average densities of the benthic scyphozoan, *Cassiopea* spp. (mostly *C. xamachana* with some *C. frondosa*), ranged from zero to 11.6 ± 20.6 individuals/m² across ponds (Fig. 5), and they were observed in all ponds in Panama. Eight additional species of large gelatinous zooplankton were observed in mangrove ponds (Fig. 2A, B; Appendix S1: Table S1).

Fish.—We caught 317 fish in 23 seine samples. Fish community composition was analyzed at the family level. Mojarras (family Gerreidae) were by far the most common fish in seine samples of pond fish communities, making up 21–100% of the catch in each pond, with 9.5 ± 10.5 individuals caught per seine sample (Fig. 5C). We observed six species or morphospecies of mojarra in ponds, the most common of which was *Eucinostomus gula* (Fig. 2E). Gobies (family Gobiidae) were also regularly observed; up to four different morphospecies were caught in four of seven sampled ponds (Fig. 5C). All fish individuals collected in nets were small, under 200 mm total length (35.4 ± 16.8 mm, mean \pm SD). However, larger individuals were qualitatively noted in the ponds while conducting benthic surveys.

Although 15 pairs of abiotic and biotic variables exhibited correlations greater than ± 0.4 (Appendix S1: Table S5), only one relationship emerged as significant in linear regression after accounting for multiple comparisons (Appendix S1: Table S6): Fish diversity was negatively correlated with pH range (Benjamini-Hochberg $P = 0.024$; Fig. 6; Appendix S1: Table S6). There were also trends in the data that suggested minimum pH was correlated with fish diversity (Benjamini-Hochberg, $P = 0.175$; Appendix S1: Fig. S1) and invertebrate abundance (Benjamini-Hochberg, $P = 0.175$; Appendix S1: Fig. S2). In NMDS of

benthic macrofaunal communities, gradients in abiotic variables were clearly defined, and minimum pH and minimum DO gradients aligned, suggesting similar or joint effects (Fig. 7). Models of minimum pH and minimum DO gradients independently explained approximately half the variation in NMDS values (GAM_{minpH}: adjusted $R^2 = 0.563$ and GAM_{minDO}: adjusted $R^2 = 0.477$).

Oyster disease assays.—*Perkinsus* sp. infections were detected in oyster populations in all sampled mangrove ponds. Prevalence of *Perkinsus* sp. infections in ponds ranged from 13 to 68% ($40\% \pm 22\%$, mean \pm SD) and was significantly higher outside than inside ponds (paired t -test, $t = 2.413$, $df = 9$, $P = 0.039$; Fig. 8A). Prevalence also varied between the two oyster species; *Perkinsus* prevalence and WP were higher in *I. alatus* than in *C. rhizophorae* (prevalence, $t = -9.031$, $df = 9$, $P < 0.0001$; Fig. 8A; WP, $t = -7.5532$, $df = 9$, $P < 0.0001$; Fig. 8C). In general, infections were low intensity; mean infection intensity of sampled populations was 0.96 ± 0.25 on the 5-point Mackin scale and did not differ by location within or outside of ponds ($t = 0.1659$, $df = 9$, $P = 0.87$), between species, or

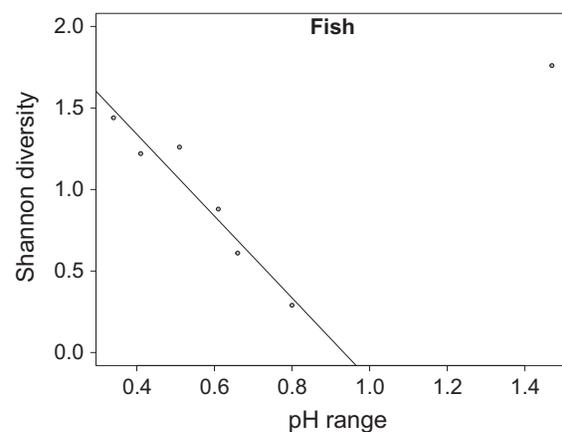


Fig. 6. The relationship between pH range and Shannon diversity index of sampled fish in mangrove ponds in Panama. There was a negative relationship between fish diversity and pH range ($F_{1,4} = 57.65$, $P < 0.05$, $R^2 = 0.92$) when outlier Shark Hole C (point at top right) was excluded.

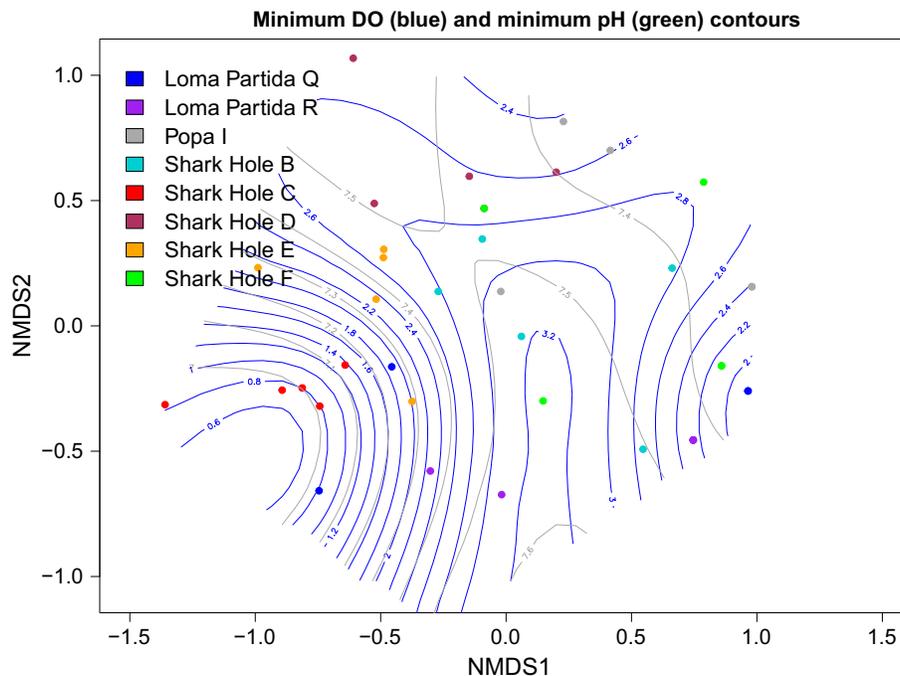


Fig. 7. Non-metric multidimensional scaling ordination of community data from macroalgae and benthic invertebrate surveys. Each point represents a single transect within a mangrove pond in Panama. Colors of the points represent the pond identity indicated in the legend. The contours on the plot show the fitted surface of minimum pH (gray) and minimum dissolved oxygen (blue).

from pond to pond (factorial ANOVA of species \times pond \times location: main effects of species $F_{1,244} = 3.155$, $P = 0.0769$ and pond, $F_{4,244} = 1.976$, $P = 0.0988$). Weighted prevalence indicated that low-level infections were common both inside and outside of ponds.

DISCUSSION

Physical and biological conditions that affect diel cycling in ponds

We found that many relatively undisturbed, oligotrophic mangrove ponds experience hypoxic and acidified nighttime conditions. About half of the 13 ponds that we sampled dropped below 2 mg/L DO at night, a level that is stressful for many marine animals (Diaz and Rosenberg 1995, Vaquer-Sunyer and Duarte 2008), while several of the ponds had relatively benign diel fluctuations more similar to reference sites (Fig. 3).

Given their shallow depth and warm temperatures, we predicted that mangrove ponds would exhibit high-magnitude diel cycles. Indeed,

relative to diel cycles described in other oligotrophic and even mildly eutrophic environments in the literature (Hofmann et al. 2011, Hendriks et al. 2015), the pH and DO range observed in several of the ponds was large, akin to the large ranges observed in a temperate salt marsh in eutrophic Long Island Sound, USA, where Baumann et al. (2015) found that the marsh was exporting acidified and hypoxic waters to the adjacent estuary during outgoing tides. The same is likely true of mangrove ponds. The average diel pH range across all of these Caribbean mangrove ponds was 0.6, or one to two times the magnitude of the 0.3–0.5 unit change predicted to occur in oceans over the next century (Caldeira and Wickett 2005). Our results suggest that diel cycling in DO and pH in mangrove ponds is a general phenomenon as we observed it in both mainland Panama sites and offshore islands in Belize. A similar occurrence was noted in Mangrove Bay, Bermuda, where Zablocki et al. (2011) observed a diel range in pH of 0.71 units in a mangrove pond.

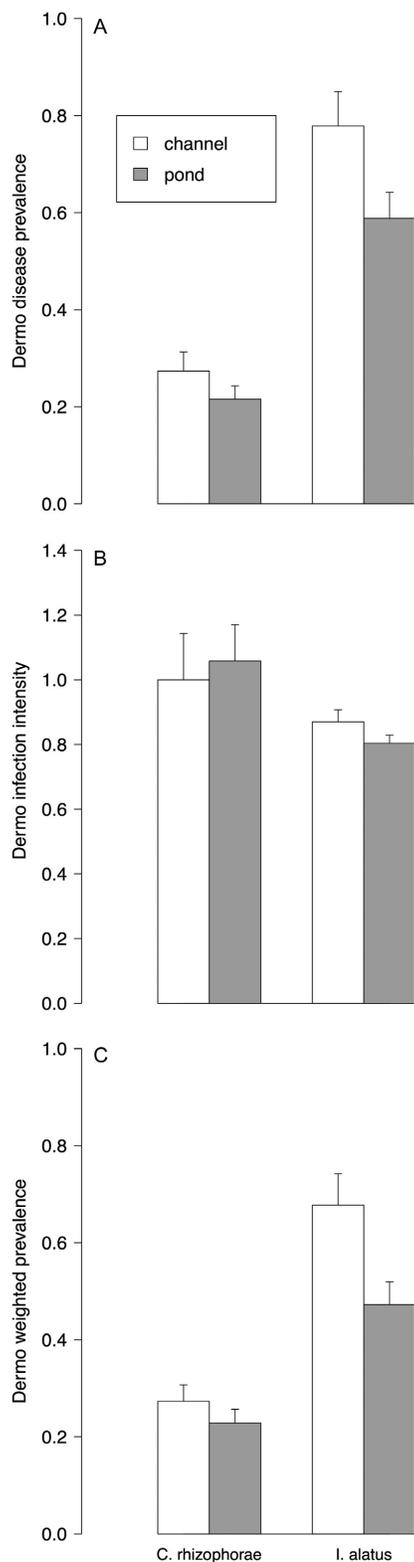


Fig. 8. *Perkinsus* sp. infections in two species of mangrove pond oyster, *Crassostrea rhizophorae* and *Isognomen alatus*. (A) Prevalence, (B) infection intensity, and (C) weighted prevalence in ponds and in channels just outside of ponds. Data are means \pm SE.

We expected that physical characteristics of ponds would affect the magnitude of the diel cycle. In eutrophic systems, intermittent high temperature and solar radiation promote higher-magnitude diel cycles because these conditions raise the metabolic activity and build-up of phytoplankton biomass (Tyler et al. 2009). Water exchange from channels, storms, tides, and mixing from wind, on the other hand, is known to disrupt or reduce diel cycles in eutrophic systems (Park et al. 1958, D'Avanzo and Kremer 1994, Shen et al. 2008, Tyler et al. 2009, Collins et al. 2013). In mangrove ponds, we found some, but not all, of these expected links between physical characteristics and diel cycles. Pond depth was associated with diel variation in pond temperature, and pond area and volume were associated with diel variation in pH. We did not find any significant predictors of diel variation in pond DO, likely due to a small sample size of ponds. Residence times, estimated coarsely in this study, were quite short, one to two tidal cycles for most ponds, and were not a good predictor of diel variation.

Because our water quality sampling periods were of short duration, we likely captured a conservative estimate of the true range and extremes in pH and DO at these study sites. Additional variation might result from seasonal changes, precipitation, tides, and storm events. Indeed, the wide variation in values detected in Shark Hole C may indicate a sporadic and brief deviation of water quality typical of all ponds. However, our ability to detect a signal of diel-cycling hypoxia and low pH in multiple ponds and study regions and the correspondence to variation within the biological community suggest that the water quality conditions that we sampled over short time periods were generally representative of relative differences among ponds and between ponds and open water.

Biologically, phytoplankton is often the most important primary producer in eutrophic systems where strong diel cycles have been observed (e.g.,

Brush and Nixon 2010, Howarth et al. 2014). Whereas chlorophyll levels in eutrophic systems frequently exceed 100 µg/L, chlorophyll levels were quite low in mangrove ponds. Macroalgae, another potential driver of diel cycling in eutrophic systems (e.g., D'Avanzo and Kremer 1994), were abundant in mangrove ponds and likely play a role in ecosystem metabolism, although no significant relationship was observed between the cover of macroalgae and the magnitude of the diel cycle. Given the depth and structure of the benthic sediments, microphytobenthos production and respiration may also play an important role in ecosystem metabolism in mangrove pond ecosystems and warrants further examination.

Biodiversity in mangrove ponds

We observed a total of 67 macroalgal, macroinvertebrate, and fish taxa in ponds during benthic, pelagic, and fish surveys and site visits (Appendix S1: Table S1). The true macrobiotic species diversity of mangrove ponds is much higher, as other organisms were present but sufficiently rare that they did not appear in our systematic surveys and most identifications were not to the species level. For example, abundant sabellid worms and alpheid shrimp in ponds include multiple, and possibly cryptic and undescribed, species. We also did not sample potentially diverse sub-habitats, such as fouling communities on mangrove prop roots. We did observe, however, that prop root communities in ponds were largely dominated by the mangrove oyster *I. alatus* and were notably less productive and less species-rich than prop root communities in channels and lagoons.

Many taxa persisted in all ponds, regardless of water quality. Common pond taxa such as *Cassiopea* spp., *Caulerpa* spp., sabellid worms, alpheid shrimp, and burrow-inhabiting gobies would be prime candidate species for investigating adaptations to coping with short-duration hypoxia and low pH (e.g., Wild and Naumann 2013). We predict that sessile and mobile fauna in mangrove ponds may respond differently to short-duration environmental stress, with less mobile species relying on a strategy of stress tolerance and many mobile species using a strategy of migration to evade conditions. It is unknown how much animal movement there is between ponds or from ponds to other mangrove sub-habitats (fringe, channel, platform), but such research would give

greater insight into the environmental filtering effect that defines pond community composition.

Finally, some major taxonomic groups were conspicuously absent from the pond communities. Notably, only a single individual echinoderm (sea cucumber *H. mexicana*) was sampled in our surveys. Seastars and brittle stars are a conspicuous component of mangrove prop root communities just outside of ponds, but were not observed within ponds. This could represent a particular sensitivity within tropical echinoderms to short-duration hypoxia or low pH. Of all taxonomic groups included in a meta-analysis of acidification impacts, echinoderms were most negatively affected in terms of survival (Kroeker et al. 2013), a finding that lends support to the hypothesis that echinoderms might be filtered out of mangrove pond ecosystems by diel-cycling pH. The absence of echinoderms might alternatively be attributed to unobserved low salinity events, unsuitable substrate, or other mechanisms. More work is needed to rigorously test these hypotheses.

Community response to diel-cycling hypoxia and pH

Our study is among the first to detect ecosystem-level effects of diel-cycling hypoxia and pH outside of heavily impacted eutrophic systems. Ponds with larger diel cycles, indicated by a higher range of pH, had lower fish diversity and tended to have fewer benthic macroinvertebrates. These differences may persist through time or they may be dynamic and coupled to variation in pond conditions, as more mobile fauna, such as fish, migrate to avoid exposure to hypoxia and acidified conditions and return in more favorable conditions.

We did not, however, find elevated levels of oyster *Perkinsus* infections in ponds, which we predicted would occur as an indicator of organismal stress due to short-duration hypoxia and low pH. Rather, prevalence tended to be slightly higher outside of ponds than within. Water quality outside of ponds likely more closely resembles well-flushed reference sites. Disease effects may have been masked by our collection from the intertidal, where oysters can exhibit higher prevalence of *Perkinsus* infections than in the subtidal (Malek and Breitbart 2016). Alternatively, the generally high prevalence but low intensity of *Perkinsus* infections in pond environments may reflect resistance to disease by species acclimated

to living in habitually stressful conditions (as has been observed in some corals, Castillo and Helmuth 2005, Oliver and Palumbi 2011).

Mangrove ponds as model ecosystems

The relationship between DO and pH dynamics that we observed in both regions (Fig. 4), and its similarity to that of eutrophic systems (Cai et al. 2011, Wallace et al. 2014, Baumann et al. 2015, Breitburg et al. 2015), suggests that these two variables may be universally correlated in marine ecosystems through the mechanistic link between ecosystem-level photosynthesis and metabolism. By examining their tandem effects on biological communities in mangrove pond ecosystems, we can isolate the effects of short-duration hypoxia and acidification from a third confounding variable, anthropogenic nutrient inputs, which can reverse or conceal the effects of hypoxia (Breitburg 2002).

Replicate ponds across the landscape that vary in the magnitude of the diel cycle provide a natural laboratory for investigating the joint effects of hypoxia and acidification on community assembly and ecosystem dynamics. The combination of laboratory studies of short-duration hypoxia or low pH (e.g., Burrell et al. 2015, Miller et al. 2016) to explore their independent effects could be coupled with observations in ponds that vary in the magnitude of their diel cycle in a particularly powerful approach for understanding the biological and ecological impacts of hypoxia and acidification. In as much as diel-cycling hypoxia and acidification have become typical of populated coastal areas (Cai et al. 2011, Duarte et al. 2013, Wallace et al. 2014) and may act as a selection pressure there, mangrove ponds offer a model system for exploring the effects of similar selection pressures without the confounding effects of eutrophication.

CONCLUSION

Mangrove ponds are an important and conspicuous part of mangrove forests, and we have documented that these ecosystems exhibit prominent diel cycles in temperature, DO, and pH in two disparate Caribbean regions. Notably, both regions have limited evidence of eutrophication, and thus, abiotic conditions cannot be attributed to anthropogenic nutrient inputs, which drive productivity

and diel-cycling hypoxia and pH in eutrophic aquatic ecosystems. More likely, the cycling we observed is largely a natural phenomenon, and the community of organisms present in ponds are there due to environmental filtering and evolutionary selection for coping with large diel swings and low pH and DO. We found signals of environmental filtering of fish and benthic communities by the short-interval stresses characteristic of the mangrove pond environment. Mangrove ponds allow an opportunity for targeted studies of short duration, regularly occurring, hypoxia, and low pH stressors. These are two conditions that are increasing in frequency and occurrence due to widespread nutrient pollution and climate change in heavily impacted coastal ecosystems. Mangrove ponds can provide a tractable system for field study of hypoxia and acidification without the potentially confounding effects of nutrient enrichment that typically accompany diel-cycling hypoxia and pH.

ACKNOWLEDGMENTS

We thank Heather Soulen, Viktoria Fruhling, and Ronald Baker for help in the field. Kathryn Norman produced our map figure. This research was supported by a Smithsonian Marine Science Network Grant to Denise Breitburg, Ilka Feller, and Keryn Gedan.

LITERATURE CITED

- Altieri, A. H. 2008. Dead zones enhance key fisheries species by providing predation refuge. *Ecology* 89:2808–2818.
- Altieri, A. H., and K. B. Gedan. 2015. Climate change and dead zones. *Global Change Biology* 21:1395–1406.
- Andersson, A. J., et al. 2015. Understanding ocean acidification impacts on organismal to ecological scales. *Oceanography* 28:16–27.
- Baumann, H., R. B. Wallace, T. Tagliaferri, and C. J. Gobler. 2015. Large natural pH, CO₂ and O₂ fluctuations in a temperate tidal salt marsh on diel, seasonal, and interannual time scales. *Estuaries and Coasts* 38:220–231.
- Benjamini, Y., and Y. Hochberg. 1995. Controlling the false discovery rate: a practical and powerful approach to multiple testing. *Journal of the Royal Statistical Society, Series B* 57:289–2300.
- Brady, D. C., and T. E. Targett. 2010. Characterizing the escape response of juvenile summer flounder *Paralichthys dentatus* to diel-cycling hypoxia. *Journal of Fish Biology* 77:137–152.

- Breitburg, D. L. 1992. Episodic hypoxia in Chesapeake Bay: interacting effects of recruitment, behavior, and physical disturbance. *Ecological Monographs* 62:525–546.
- Breitburg, D. 2002. Effects of hypoxia, and the balance between hypoxia and enrichment, on coastal fishes and fisheries. *Estuaries* 25:767–781.
- Breitburg, D. L., J. W. Baxter, C. A. Hatfield, R. W. Howarth, C. G. Jones, G. M. Lovett, and C. Wigand. 1998. Understanding effects of multiple stressors: ideas and challenges. Pages 416–431 in M. L. Pace and P. M. Groffman, editors. *Successes, limitations, and frontiers in ecosystem science*. Springer, New York, New York, USA.
- Breitburg, D. L., et al. 2015. And on top of all that... coping with ocean acidification in the midst of many stressors by denise. *Oceanography* 28:48–61.
- Brush, M. J., and S. W. Nixon. 2010. Modeling the role of macroalgae in a shallow sub-estuary of Narragansett Bay, RI (USA). *Ecological Modelling* 221:1065–1079.
- Burrell, R. B., A. G. Keppel, V. M. Clark, and D. L. Breitburg. 2015. An automated monitoring and control system for flow-through co-cycling hypoxia and pH experiments. *Limnology and Oceanography: Methods* 14:168–185.
- Cai, W. J., et al. 2011. Acidification of subsurface coastal waters enhanced by eutrophication. *Nature Geoscience* 4:766–770.
- Caldeira, K., and M. E. Wickett. 2005. Ocean model predictions of chemistry changes from carbon dioxide emissions to the atmosphere and ocean. *Journal of Geophysical Research: Oceans* 110:C09S04.
- Castillo, K. D., and B. S. T. Helmuth. 2005. Influence of thermal history on the response of *Montastraea annularis* to short-term temperature exposure. *Marine Biology* 148:261–270.
- Collins, J. R., P. A. Raymond, W. F. Bohlen, and M. M. Howard-Strobel. 2013. Estimates of new and total productivity in central Long Island Sound from in situ measurements of nitrate and dissolved oxygen. *Estuaries and Coasts* 36:74–97.
- Crain, C. M., K. Kroeker, and B. S. Halpern. 2008. Interactive and cumulative effects of multiple human stressors in marine systems. *Ecology Letters* 11:1304–1315.
- Cubit, J. D., H. M. Caffey, R. C. Thompson, and D. M. Windsor. 1989. Meteorology and hydrography of a shoaling reef flat on the Caribbean coast of Panama. *Coral Reefs* 8:59–66.
- D'Avanzo, C., and J. N. Kremer. 1994. Diel oxygen dynamics and anoxic events in an eutrophic estuary of Waquoit Bay, Massachusetts. *Estuaries* 17:131–139.
- D'Croz, L., J. B. Del Rosario, and P. Gondola. 2005. The effect of fresh water runoff on the distribution of dissolved inorganic nutrients and plankton in the Bocas del Toro Archipelago, Caribbean Panama. *Caribbean Journal of Science* 41:414–429.
- Diaz, R. J., and R. Rosenberg. 1995. Marine benthic hypoxia: a review of its ecological effects and the behavioural responses of benthic macrofauna. *Oceanography and Marine Biology: An Annual Review* 33:245–303.
- Dickson, A., and F. Millero. 1987. A comparison of the equilibrium-constants for the dissociation of carbonic-acid in seawater media. *Deep-Sea Research Part A: Oceanographic Research Papers* 34:1733–1743.
- Dickson, A. G., C. L. Sabine, and J. R. Christian. 2007. Guide to best practices for ocean CO₂ measurements. (PICES) Special Publication 3, North Pacific Marine Science Organization, Sidney, British Columbia, Canada.
- Duarte, C. M., I. E. Hendriks, T. S. Moore, Y. S. Olsen, A. Steckbauer, L. Ramajo, J. Carstensen, J. A. Trotter, and M. McCulloch. 2013. Is ocean acidification an open-ocean syndrome? Understanding anthropogenic impacts on seawater pH. *Estuaries and Coasts* 36:221–236.
- Enríquez, S., and N. Schubert. 2014. Direct contribution of the seagrass *Thalassia testudinum* to lime mud production. *Nature Communications* 5:3835.
- Gambi, M. C., L. Musco, A. Giangrande, F. Badalamenti, F. Micheli, and K. J. Kroeker. 2016. Distribution and functional traits of polychaetes in a CO₂ vent system: winners and losers among closely related species. *Marine Ecology Progress Series* 550:121–134.
- Gobler, C. J., and H. Baumann. 2016. Hypoxia and acidification in ocean ecosystems: coupled dynamics and effects on marine life. *Biology Letters* 12:20150976.
- Hall-Spencer, J. M., R. Rodolfo-Metalpa, S. Martin, E. Ransome, M. Fine, S. M. Turner, S. J. Rowley, D. Tedesco, and M. C. Buia. 2008. Volcanic carbon dioxide vents show ecosystem effects of ocean acidification. *Nature* 454:96–99.
- Halpern, B. S., et al. 2008. A global map of human impact on marine ecosystems. *Science* 319:948–952.
- Harvell, C. D., et al. 1999. Emerging marine diseases: climate links and anthropogenic factors. *Science* 285:1505–1510.
- Helmuth, B. S., and G. E. Hofmann. 2001. Microhabitats, thermal heterogeneity, and patterns of physiological stress in the rocky intertidal zone. *Biological Bulletin* 201:374–384.
- Hendriks, I. E., C. M. Duarte, Y. S. Olsen, A. Steckbauer, L. Ramajo, T. S. Moore, J. A. Trotter, and

- M. McCulloch. 2015. Biological mechanisms supporting adaptation to ocean acidification in coastal ecosystems. *Estuarine, Coastal and Shelf Science* 152:A1–A8.
- Hofmann, G. E., et al. 2011. High-frequency dynamics of ocean pH: a multi-ecosystem comparison. *PLoS ONE* 6:e28983.
- Howarth, R. W., M. Hayn, R. M. Marino, N. Ganju, K. Foreman, K. McGlathery, A. E. Giblin, P. Berg, and J. D. Walker. 2014. Metabolism of a nitrogen-enriched coastal marine lagoon during the summertime. *Biogeochemistry* 118:1–20.
- Keppel, A. G., D. L. Breitburg, G. H. Wikfors, R. B. Burrell, and V. M. Clark. 2015. Effects of co-varying diel-cycling hypoxia and pH on disease susceptibility in the eastern oyster *Crassostrea virginica*. *Marine Ecology Progress Series* 538:169–183.
- Kjerfve, B., K. Rützler, and G. H. Kierspe. 1982. Tides at Carrie Bow Cay, Belize. *Smithsonian Contributions to the Marine Sciences* 12:47–52.
- Kroeker, K. J., R. L. Kordas, R. Crim, I. E. Hendriks, L. Ramajo, G. S. Singh, C. M. Duarte, and J. P. Gattuso. 2013. Impacts of ocean acidification on marine organisms: quantifying sensitivities and interaction with warming. *Global Change Biology* 19:1884–1896.
- Long, W. C., and R. D. Seitz. 2008. Trophic interactions under stress: Hypoxia enhances foraging in an estuarine food web. *Marine Ecology Progress Series* 362:59–68.
- Macintyre, I. G., K. Rützler, and I. C. Feller. 2004. The twin cays mangrove ecosystem, Belize: biodiversity, geological history and two decades of change. *Atoll Research Bulletin* Nos. 509–530.
- Mackin, J. G. 1962. Oyster disease caused by *Dermocystidium marinum* and other microorganisms in Louisiana. *Publications of the Institute of Marine Science* 7:132–229.
- Madigan, M. T., and D. O. Jung. 2009. An overview of purple bacteria: systematics, physiology, and habitats. Pages 1–15 in C. N. Hunter, F. Daldal, M. C. Thurnauer, and J. T. Beatty, editors. *The purple phototrophic bacteria*. Springer, Dordrecht, The Netherlands.
- Malek, J. C., and D. L. Breitburg. 2016. Effects of air-exposure gradients on spatial infection patterns of *Perkinsus marinus* in the eastern oyster *Crassostrea virginica*. *Diseases of Aquatic Organisms* 118:139–151.
- Maryland Department of Natural Resources. 2017. Eyes on the Bay. www.eyesonthebay.net
- Miller, S. H., D. L. Breitburg, R. B. Burrell, and A. G. Keppel. 2016. Acidification increases sensitivity to hypoxia in important forage fishes. *Marine Ecology Progress Series* 549:1–8.
- Oertel, G. F. 2001. Hypsographic, hydro-hypsographic and hydrological analysis of coastal bay environments, Great Machipongo Bay, Virginia. *Journal of Coastal Research* 17:775–783.
- Oksanen, J., F. G. Blanchet, R. Kindt, P. Legendre, P. R. Minchin, R. B. O'Hara, G. L. Simpson, P. Solymos, M. H. H. Stevens, and H. Wagner. 2015. *vegan: community ecology package*. R package version 2.0-10. 2013. <https://cran.r-project.org/web/packages/vegan/index.html>
- Oliver, T. A., and S. R. Palumbi. 2011. Do fluctuating temperature environments elevate coral thermal tolerance? *Coral Reefs* 30:429–440.
- Park, K., D. W. Hood, and H. T. Odum. 1958. Diurnal pH variation in Texas Bays, and its application to primary production estimation. *Contributions in Marine Science* 5:47–64.
- Pelletier, G. J., E. Lewis, and D. Wallace. 2007. CO2SYS. XLS: a calculator for the CO₂ system in seawater for Microsoft Excel/VBA. Washington State Department of Ecology, Olympia, Washington, USA.
- Pihl, L., S. P. Baden, R. J. Diaz, and L. C. Schaffner. 1992. Hypoxia-induced structural changes in the diet of bottom-feeding fish and Crustacea. *Marine Biology* 112:349–361.
- Pincebourde, S., E. Sanford, and B. Helmuth. 2008. Body temperature during low tide alters the feeding performance of a top intertidal predator. *Limnology and Oceanography* 53:1562–1573.
- Ray, S. 1952. A culture technique for the diagnosis of infections with *Dermocystidium marinum* Mackin, Owen, and Collier in oysters. *Science* 116:360–361.
- Ray, S. M. 1954. Studies on the occurrence of *Dermocystidium marinum* in young oysters. *Proceedings of the National Shellfish Association* 1953–44: 80–92.
- Rodriguez, W., and I. C. Feller. 2004. Mangrove landscape characterization and change in Twin Cays, Belize using aerial photography and IKONOS satellite data. *Atoll Research Bulletin* Number 513. National Museum of Natural History, Smithsonian Institution, Washington, D.C., USA.
- Rützler, K., I. Goodbody, M. C. Diaz, I. C. Feller, and I. G. Macintyre. 2004. The aquatic environment of twin Cays, Belize. *Atoll Research Bulletin* No. 512. National Museum of Natural History, Smithsonian Institution, Washington, D.C., USA.
- Shen, J., T. Wang, J. Herman, P. Mason, and G. L. Arnold. 2008. Hypoxia in a coastal embayment of the Chesapeake Bay: a model diagnostic study of oxygen dynamics. *Estuaries and Coasts* 31: 652–663.
- Spalding, M., M. Kainuma, and L. Collins. 2010. *World atlas of mangroves*. Earthscan, Washington, D.C., USA.

- Taylor, D. S., E. A. Reyier, C. C. McIvor, and W. P. Davis. 2007. An assessment of ichthyofaunal assemblages within the mangal of the Belize offshore cays. *Bulletin of Marine Science* 80:721–737.
- Tyler, R. M., D. C. Brady, and T. E. Targett. 2009. Temporal and spatial dynamics of diel-cycling hypoxia in estuarine tributaries. *Estuaries and Coasts* 32:123–145.
- Vaquer-Sunyer, R., and C. M. Duarte. 2008. Thresholds of hypoxia for marine biodiversity. *Proceedings of the National Academy of Sciences* 105:15452–15457.
- Wallace, R. B., H. Baumann, J. S. Grear, R. C. Aller, and C. J. Gobler. 2014. Coastal ocean acidification: the other eutrophication problem. *Estuarine, Coastal and Shelf Science* 148:1–13.
- Wild, C., and M. S. Naumann. 2013. Effect of active water movement on energy and nutrient acquisition in coral reef-associated benthic organisms. *Proceedings of the National Academy of Sciences* 110:8767–8768.
- Zablocki, J. A., A. J. Andersson, and N. R. Bates. 2011. Diel aquatic CO₂ system dynamics of a Bermudian mangrove environment. *Aquatic Geochemistry* 17:841–859.

SUPPORTING INFORMATION

Additional Supporting Information may be found online at: <http://onlinelibrary.wiley.com/doi/10.1002/ecs2.2053/full>