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Carbonate Mineral Saturation State as the Recruitment Cue for Settling Bivalves in Marine Muds

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Abstract After a pelagic larval phase, infaunal bivalves undergo metamorphosis and transition to the underlying sediments to begin the benthic stage of their life history, where they explore and then either accept or reject sediments. Although the settlement cues used by juvenile infaunal bivalves are poorly understood, here we provide evidence that carbonate saturation state is a significant chemical cue in both direct observation laboratory studies and field manipulations. In the laboratory, plantigrade-stage *Mercenaria mercenaria* (200 μm shell height) showed a significant positive relationship between percent burrowed and $\Omega_{\mathrm{aragonite}}$, with an increasing probability of settlement with increasing saturation state. In the field, we increased bivalve recruitment by a factor of three in a 30-day field study by raising the pH (~0.3) and saturation state of surface sediments by buffering sediments with crushed shell ($CaCO_3$). The susceptibility of infaunal bivalves to dissolution mortality and the tight coupling of other sedimentary biogeochemical processes with carbonate dynamics suggest that mineral thermodynamics may be an overarching cue new settlers are responding to.

Keywords Ocean acidification · Calcium carbonate · Dissolution · Juvenile bivalves · Recruitment cues

Introduction

A significant proportion of biogenic $CaCO_3$ produced shoreward of the shelf-break dissolves in both terrigenous and carbonate sediments (McNichol et al. 1988; Rude and Aller 1991; Green and Aller 2001). It is now widely accepted that the decomposition of sedimentary organic matter is responsible for lowering saturation state promoting extensive dissolution of calcite and aragonite (Aller 1982; Walter and Burton 1990; Green and Aller 1998, 2001). Calcium carbonate undersaturation may vary temporally and spatially in nearshore regions; generally, however, the greatest undersaturation will occur at the sediment–water interface (SWI) where newly settled, shallow-burrowing juvenile bivalves congregate (Zwarts and Wanink 1989). The effects of saturation state at the SWI on settlement patterns of marine bivalves are unknown. Newly metamorphosed juvenile bivalves have been shown to respond to various sediment chemical cues (Woodin 1998; Engstrom and Marinelli 2005) and may reject undersaturated sediment, if aragonite undersaturation is a negative settlement cue.

Carbonate mineral saturation state and dissolution is largely dependent on availability of acids and the associated titration of $CO_3{}^{2-}$ according to:

$$H^+ + CaCO_3 \rightarrow HCO_3^- + Ca^{2+} \tag{1}$$

A lowering of $[CO_3{}^{2-}]$ will alter the calcium carbonate saturation state (Ω), often expressed as:

$$\Omega = \left[CO_3{}^{2-}\right]\left[Ca^{2+}\right] \Big/ K'_{\mathrm{sp}} \tag{2}$$

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where, K'_{sp} is the stoichiometric solubility product and is dependent on temperature, salinity, pressure, and the carbonate mineral phase: calcite, aragonite, high-mg calcite, and low-mg calcite. Because $[Ca^{2+}]$ is typically high in seawater (10 mmol L^{-1} at salinity 35), the deviation of seawater from carbonate mineral equilibrium results almost entirely from changes in concentrations of CO_3^{2-}.

Unlike the relatively straightforward thermodynamic controls on CO_3^{2-} concentrations below the chemical lysocline in the deep sea (aerobic CO_2 production and CO_3^{2-} titration), the processes responsible for lowering CO_3^{2-} in coastal regions are more diverse. For example, high rates of organic matter remineralization in coastal marine deposits result in production of CO_2 at or near the sediment water, where aerobic microbial respiration occurs (Rasmussen and Jorgensen 1992). This CO_2 production results in lower pH, in the same way increasing atmospheric CO_2 results in ocean acidification (Feely et al. 2004). In addition, the interplay of seasonal temperature change, spatial heterogeneity, aerobic respiration at the SWI, proton production during oxidation of reduced metabolites (e.g., NH_4^+, Mn^{2+}, Fe^{2+}, H_2S), and additional transport-related controls such as particle reworking and bioirrigation all contribute to variable CO_3^{2-} in coastal sediments (Aller 1982; Green 2004a, b). Undersaturated surface sediments with respect to aragonite have been documented in many coastal regions along the east coast of the USA and are presumably found worldwide in regions with similar sediment types (Aller 1982; McNichol et al. 1988; Walter and Burton 1990; Green and Aller 2001). The production of acids in the upper millimeters of sediment results in this region being the zone most corrosive to calcium carbonate, paradoxically the same depth that newly settled bivalves must reside (Zwarts and Wanink 1989).

Recently, Green et al. (2009) found that corrosive conditions cause mortality in *Mercenaria mercenaria* when sediments are undersaturated and overlying water saturated with respect to aragonite, with the greatest mortality occurring in the smallest sizes. Dissolution mortality increased over 10 $\%\text{day}^{-1}$ for 0.2-mm individuals with a drop in $\Omega_{\text{aragonite}}$ from 1.6 to 0.6. Supporting the dissolution mortality hypothesis, Waldbusser et al. (2010) found that the smallest post-larval sizes of *M. mercenaria* were unable to net calcify in undersaturated (wrt aragonite) conditions, whereas larger sizes were able to deposit new shell even in waters where $\Omega<1$. Likewise, recent work showed that terrigenous sediment deposition lowered porewater pH, and this corresponded to loss of infaunal bivalves (Cummings et al. 2009). These experimental studies highlight the potential role sediment calcium carbonate thermodynamics may play in infaunal bivalve population success.

It has been clearly demonstrated that settling bivalves can determine whether habitat is favorable and either settle or reject sediment and continue searching for a more suitable habitat (e.g., Emerson and Grant 1991; Norkko et al. 2001; Lunquist et al. 2004; Valanko et al. 2010). The post-larval settlement process begins with what appears to be active site selection driven in part by evaluation of geochemical properties of the sediment porewater at the sediment–water interface (Pawlik 1992; Marinelli and Woodin 2002, 2004; Engstrom and Marinelli 2005). The myriad factors affecting settling larvae in marine sediments are well documented and are reviewed elsewhere (e.g., Rodriguez et al. 1993). Settlement and ultimately recruitment to adult populations are driven, at least in part, by this acceptance or rejection of sediment during this initial site selection process (Butman et al. 1988; Woodin 1995; Hunt et al. 2007; Valanko et al. 2010) and occur during a short window of opportunity (Engstrom and Marinelli 2005). However, after this initial selection period, juveniles can still relocate using a passive secondary site selection process (Norkko et al. 2001; Hunt et al. 2007). Secondary site selection occurs largely through physical processes such as bed load transport; however, biological processes (e.g., depth of burrowing) will dictate the extent to which physical processes (e.g., tidal currents) are capable of acting on an individual (Hunt et al. 2007).

Once burrowed, settled juvenile bivalves are then confined to the upper millimeters of sediment until the development of siphons allows for deeper burrowing (Zwarts and Wanink 1989; Carriker 2001). As noted, it is these upper few millimeters of sediment that are usually most corrosive to the mineral shell (Aller 1982; McNichol et al. 1988; Walter and Burton 1990; Green and Aller 2001). Here, we test the role of sediment saturation state ($\Omega_{\text{aragonite}}$) at the SWI as an environmental cue that influences behavioral responses of newly metamorphosed clams.

Methods

Surface Sediment Saturation State in a Coastal Maine Mudflat Surface sediment saturation state with respect to aragonite was calculated in a South Portland, Maine, mudflat during early June 2009. Sampling occurred during low tide approximately 30 min after air exposure and lasted approximately 2 h. pH was measured using an Accument AB15 pH meter equipped with a Corning rugged combination electrode by slowly inserting the electrode directly into the sediment using a modified micro-manipulator which allowed pH to be measured in the top 2 mm of the deposit. Eighty random measurements were made within a 2×5-m plot. Immediately following pH measurements, a butyrate core liner (5 cm ID) was gently inserted into the sediment and removed, leaving a mark in the sediment within which surface sediment and pore water was sampled. Using a stainless steel spatula, the top 2 mm of sediment was removed and placed into a 60-cm^3 syringe that had been plugged at the base of the syringe with a 30-μm nylon mesh and fitted at the end with a 0.45-μm Acrodisc filter. Pore water was squeeze filtered into washed 20-mL scintillation vials, placed on ice, and returned to the lab. Total alkalinity was determined on a 2-mL aliquot of pore water using titration with 0.01 N HCl after Edmond (1970). pHs, total alkalinity, and Cl^- concentrations (Labconco Digital Chloridometer) were used to calculate carbonate ion (CO_3^{2-}) concentration and pore water saturation state with respect to aragonite ($\Omega_{aragonite}$) using the CO_2SYS program, the first and second dissociation constants of carbonic acid in seawater (Mehrbach et al. 1973 refit by Dickson and Millero 1987), and the surface sediment temperature (20 °C).

Sediment Rejection/Acceptance Experiments In the laboratory, we developed an experimental system to regulate sediment $\Omega_{aragonite}$ at the sediment surface where new bivalve settlers explore and either burrow or reject the deposit (described in detail in Green et al. 2009). Briefly, the laboratory system separated a thin layer of sediment (1 mm) and a CO_2 gas chamber by a gas-permeable membrane (Teflon). CO_2 passing through the gas chamber diffused across the gas-permeable membrane and lowered surficial sediment pH in a fashion analogous to CO_2 production during aerobic carbon remineralization in coastal deposits (Rasmussen and Jørgensen 1992). Small changes in CO_2 flow rates permitted many pH values, and therefore $\Omega_{aragonite}$ values, and were adjusted to replicate the range of saturation states documented in our field estimates of $\Omega_{aragonite}$ described previously. Previous measurements on this experimental setup have shown that the increase in CO_2 in sediments did not significantly alter other possible covariates such as hydrogen sulfide and oxygen (Green et al. 2009); therefore, we assume that organism responses are primarily due to changes in carbonate thermodynamics.

Sediment acceptability was measured by the number of ten, 200-μm (shell height), plantigrade-stage *M. mercenaria* which burrowed within 5 min after being deposited on the sediment surface of experimental sediments. *M. mercenaria* was purchased from Research Aquaculture Hatchery in Tequesta, Florida. Individuals were shipped overnight from the hatchery the day following settlement. Experiments were conducted on the same day of arrival of bivalves in Maine so that the relative age of bivalves was approximately 36 h after becoming competent to settle. *M. mercenaria* were deposited on the sediment surface and then burrowing monitored over a 5-min window. After this period, we assumed that bivalves which had not burrowed had rejected the sediment. The burrowing response of *M. mercenaria* has been found to be rapid in acceptable sediments (Woodin 1998; Marinelli and Woodin 2002, 2004). We therefore hypothesized that, should favorable carbonate thermodynamics (higher pH, CO_3^{2-}, and $\Omega_{aragonite}$) be a positive recruitment cue, individuals would burrow rapidly to avoid predation or advection to other potentially less favorable sites (Woodin et al. 1995; Richards and Huxman 1999; Hunt el al. 2007; Bowen and Hunt 2009).

Immediately following the 5-min observation period and enumeration of the number of burrowers, pH was measured by direct electrode insertion into individual sediment plugs (triplicate measurements per sediment plug). Total alkalinity was measured in pore water collected by removing sediment from plugs, centrifuging at 1,500 rpm for 5 min in microcentrifuge tubes and filtering through 0.45-μm mini-filters. Low porewater recovery meant that total alkalinity titration (Edmond 1970) was performed on 0.5 mL of sample, diluted to 2.0 mL with distilled water (relative standard deviation, ~3 %). Surface sediment saturation state (aragonite) in each plug was then estimated using the method described above using the experimental temperature (21 °C) and salinity (30).

Sediment Buffering Experiment A field-based experiment in which we buffered intertidal mudflat sediments with ground clam shell and measured settlement of *Mya arenaria* was performed. The experiment was performed in a small, sheltered intertidal mudflat off Portland Harbor in Portland, Maine. We hypothesized that if muds undersaturated with respect to calcium carbonate are buffered to increase pH and saturation state, then a greater number of settling bivalves will accept buffered muds relative to control-unbuffered muds in adjacent field locations.

Two plots (1×5 m) were established adjacent to one another, one to contain buffered cores and one to contain control cores. These plots were immediately adjacent to the area used to measure the saturation state distributions, approximately 3 week earlier, and as described previously. Within each plot, fifty-six 10-cm-diameter experimental cores were established in rows of four, with each row of four serving as a replicate set of cores from the buffered and control plots. Core liners were inserted into the sediment until the top edge was flush with the SWI and the top 2 cm of sediments was removed, homogenized, and either buffered or replaced without buffering. In the buffered plot, the sediment was weighed, and enough crushed biogenic $CaCO_3$ (*M. arenaria* shell, 1-mm particle size) was added to yield a final $CaCO_3$ concentration of 8 % by weight (original $CaCO_3$ was ~1 % by weight). In the disturbance control cores, the same procedure was followed, except no biogenic $CaCO_3$ was added to the 2-cm sediment volume prior to its replacement in the sediment core.

As designed, the treatment replication within plots, rather than across plots, could lead to other across-plot factors resulting in the differences in settlement we describe subsequently. However, analysis of sediment conditions in both plots prior to the setup of the experiment showed that the between-plot sediment conditions were similar with respect to organic content, $CaCO_3$ content, surface pore water porosity (0–1 cm), tidal exposure time, initial pH distribution, and visual properties of the sediment surface. We acknowledge that we cannot completely rule out other cofactors we did not account for and therefore assume that these were minor relative to the large signal we created with our experimental treatment.

Following the onset of settling *M. arenaria* in an adjacent monitoring plot, we began sampling the control-unbuffered and experimental-buffered cores. Three cores from each treatment plot (one core from each of three of the four rows) were collected approximately every 3 days for 14 sampling periods to enumerate the number of *M. arenaria*. The surface 1 cm of sediment was removed and preserved in 70 % buffered EtOH. For enumeration, mud was sieved through a 62-μm sieve and numbers of *M. arenaria* counted. In addition, one core from each treatment was collected at each sampling interval and used to determine porewater $\Omega_{\text{aragonite}}$ in the surface 0.25 cm of the deposit. Saturation state was calculated using a method similar to the calculations of saturation state distributions in the mudflat described above.

Data Analysis Sediment acceptance experiments were analyzed using a logistic regression (Sokal and Rolf 1995) to determine the proportional response of burrowing to aragonite saturation state. The probability of burrowing must fall between a value of 1.0 and 0.0, and therefore, the functional response to describe this behavior should be constrained, thereby limiting utility of a standard linear regression. The form of the logistic model is:

$$1 - P = \frac{1}{1 + e^{z}} \tag{3}$$

where P is the probability of burrowing, between 0 and 1, and Z is a linear equation defined as:

$$Z = m\Omega_{\text{aragonite}} - b \tag{4}$$

where Ω is the saturation state of aragonite, m is the slope, and b the intercept. The test of significance of the overall model was determined by the likelihood ratio (chi-square), testing whether the parameterized model fits better than an empty model. The model parameters of slope and intercept were also tested for significance by maximum likelihood (Wald chi-square). The adjusted odds ratio was also computed, to determine the increase in probability with incremental increases in the independent variable ($\Omega_{\text{aragonite}}$).

Statistical differences in the field settlement data were determined by calculating the rates of settlement for each experimental plot (n=3 per treatment) over the peak settlement period (35 days) and analyzing with a simple analysis of variance (ANOVA). First, the slope of the linear regression of total clams per core on time was determined for each of the three buffered and control plots; then, an ANOVA was conducted to determine significant differences at α=0.05. Assumptions of ANOVA were checked by examination and analyses of residuals. Statistical analyses were conducted using SAS v9.1.

Results

Surface Sediment Saturation State in a Coastal Maine Mudflat Measurements of saturation state in intertidal sediments in South Portland Harbor, Maine, illustrate the spatial variability common in these regions (Fig. 1). Average $\Omega_{\text{aragonite}}$ was 0.9± 0.5 with a median value of 0.75. Similar surface sediment saturation states have been documented in other coastal temperate regions and may be indicative of the thermodynamic hardships faced by transitioning marine bivalves (e.g., Green et al. 2009). Approximately 40 % of aragonite measurements in South Portland Harbor, Maine, show $\Omega_{\text{aragonite}} \leq 0.6$ suggesting dissolution may be an issue to many bivalves in these muds when alternative muds are not available. Scanning electron micrographs of juvenile *M. mercenaria* reared in sediments with $\Omega_{\text{aragonite}}$=0.6 values show obvious signs of shell dissolution after 7 days in these muds (see Green et al. 2009).

Sediment Rejection/Acceptance Experiments A significant positive relationship between percent burrowed and $\Omega_{\text{aragonite}}$ was found (Fig. 2) by fitting a logistic regression to this binary response data with $\Omega_{\text{aragonite}}$ as the predictor variable. Increasing the saturation state of sediments was found to significantly increase the probability of settlement ($\Omega_{\text{aragonite}}$=5.95±0.43, Wald chi-square=195.26, P<0.0001). Nearly all clams burrowed as saturation state approached 1.0. The adjusted odds ratio, determined from the logistic regression, predicted an

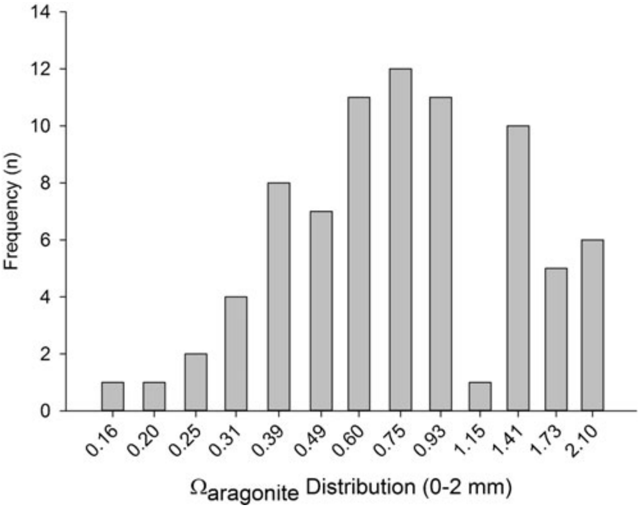

Fig. 1 Frequency distribution plot of surface sediment saturation state ($\Omega_{\text{aragonite}}$, 0–0.5 cm) in South Portland, Maine

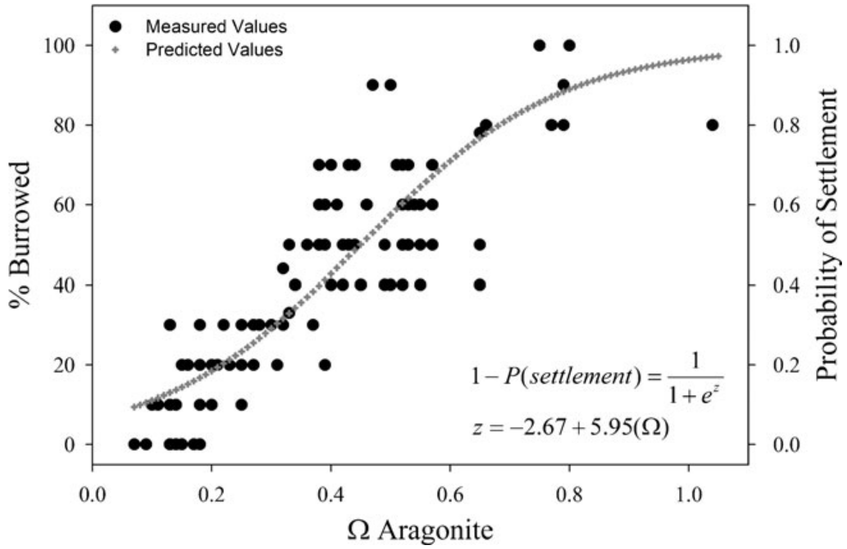

Fig. 2 Measured and model predicted values of percent burrowing juveniles of the hard clam *M. mercenaria* when placed on sediments with variable aragonite. Increasing the saturation state of sediments was found to significantly increase the probability of settlement ($\Omega=5.9534\pm0.4260$, Wald chi-square=195.2609, $P<0.0001$). Nearly all clams burrowed as saturation state approached 1.0. The adjusted odds ratio, determined from the logistic regression, predicted an increase in probability of settlement of 1.81 with each 0.1 increase in saturation state

increase in probability of settlement of 1.81 with each 0.1 increase in saturation state. In other words, with each 0.1 increase in saturation state (within our measurement range), a hard clam plantigrade is almost twice as likely to burrow and settle within the 5-min window.

Sediment Buffering Experiment Our biogenic $CaCO_3$ treatment significantly raised Ω from an average of 0.68±0.07 in disturbance controls to an average of 1.30±0.10 in buffered cores ($t_{24}=16.66$, $P<0.0001$) and we presume was the settlement cue settling bivalves used to select the more buffered experimental sediments relative to controls (Fig. 3). Buffering in situ sediments with biogenic calcium carbonate significantly increased recruitment by just over a factor of two ($F_{1,4}=84.69$, $P=0.0008$). The raw counts of total *M. arenaria* (live and dead) illustrate approximately double the number of *M. arenaria*, relative to unbuffered sediments (Fig. 4) at the end of the experiment. In addition, there is a distinct difference in the ratios of live to dead *M. arenaria* in the experimental plots relative to controls (Fig. 5). Slopes of live vs. dead are 1.25±0.26 and 2.2±0.3 in control and experimental plots, respectively, and are both significantly different from zero ($P<0.0001$). Although previous attempts to experimentally buffer sediment resulted in only a slight increase in $\Omega_{aragonite}$ (Green et al. 2009), the current experimental approach using smaller shell particles (1 mm in diameter) was more successful over the month-long experimental period and makes sense from a kinetic perspective as the greater surface area relative to volume will increase dissolution rate and provide greater buffer (Morse 1983). The dramatic increase in settlement should provide a more robust population base to weather a suite of other post-settlement mortality factors which have been reported (Richards et al. 1999).

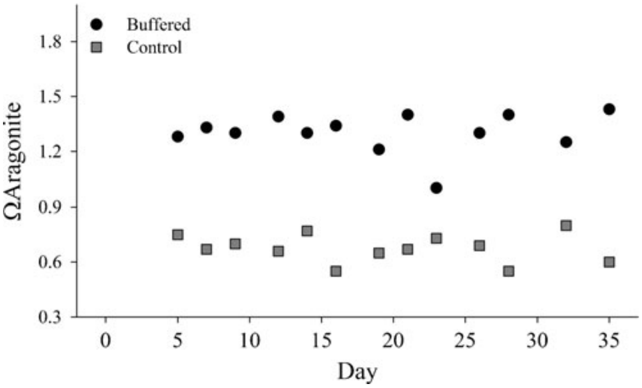

Fig. 3 Biogenic $CaCO_3$ treatment significantly raised Ωaragonite from 0.68 in disturbance controls to 1.30 in buffered cores ($t_{24}=16.66$, $P<0.0001$). We presume saturation state is the settlement cue settling bivalves used to select the more buffered experimental sediments relative to controls

Discussion

Bivalve larvae and their juvenile benthic counterparts represent critical life stages for shellfish populations. It has been widely accepted that local bivalve abundance is controlled by factors related to early life history of the larval stage, survival, and habitat selection during settlement (Gosselin and Qian 1997; Hunt and Scheilbling 1997). Significant relocation may also occur following the primary settlement of larval bivalves (Emerson and Grant 1991; Norkko et al. 2001) further influencing the final population numbers and spatial extent of a community. Understanding factors that control where bivalves ultimately burrow and reside is important as even small reductions in numbers of larval/juvenile settlers can translate into large declines in adult populations. In earlier work, Green et al. (2009) and Waldbusser et al. (2010) both highlight the size/stage dependence of corrosive sediments on early settlers, with larger juveniles able to overcome dissolution pressure. If in fact death by dissolution is a significant mortality factor

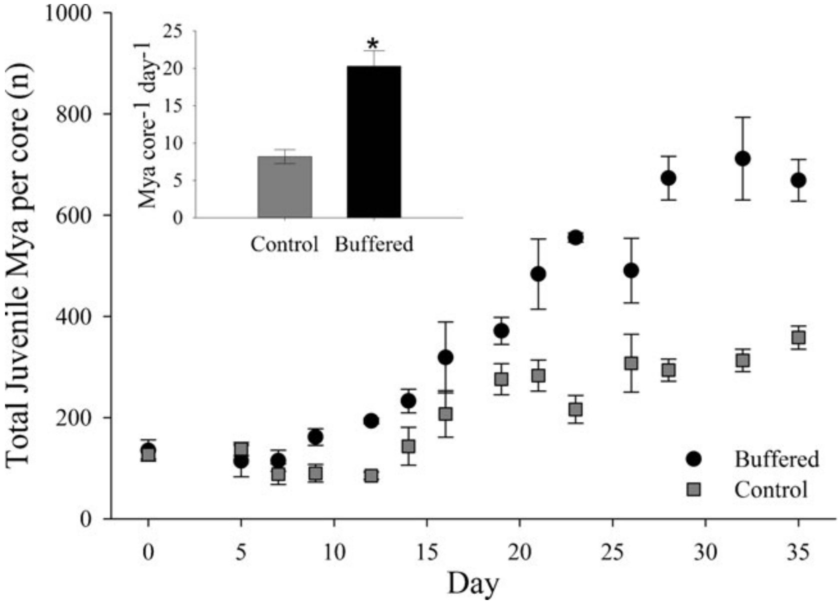

Fig. 4 Plot of total (live and dead) *M. arenaria* as a function of time in both buffered and unbuffered sediment cores. Buffering in situ sediments with biogenic calcium carbonate significantly increased recruitment by just over a factor of two ($F_{1,4}$=84.69, P=0.0008) over the 30+ day course of the experiment

in coastal marine sediments for settling bivalve larvae, then it may be argued that settlers should be able to evaluate carbonate chemical conditions at the sediment–water interface to ultimately increase recruitment to the population.

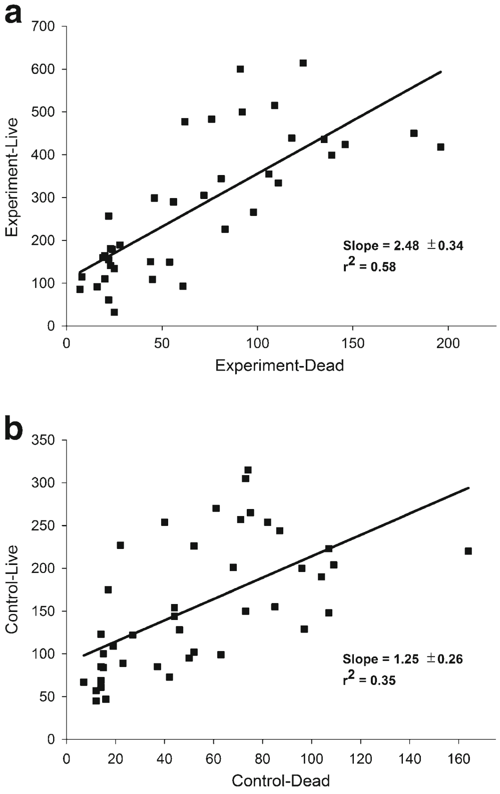

Fig. 5 **a** Live vs. dead *M. arenaria* in control-unbuffered and **b** experimental-buffered plots. The slope of live vs. dead in the experimental and control plots are 2.5±0.3 and 1.25±0.26, respectively. On average, there are approximately 2× the numbers of live *M. arenaria* juveniles in sediments buffered with $CaCO_3$

M. arenaria have approximately a 14-day larval stage and 30-day peak spawning period in these northern waters (Ayers 1956). As such, differences in numbers of *M. arenaria* during successive sampling points could have contained both individuals captured following their initial settlement period as well as other earlier settlers captured following secondary dispersal episodes. Because we lack size measurements of sampled *M. arenaria*, we cannot differentiate the relative age of sampled bivalves. In this study, we are not able to differentiate between changes in *M. arenaria* settlement patterns which resulted from selection processes during the primary settlement period compared with secondary dispersal generally associated with physical processes such as tidal currents and transport as bedload (Roegner et al. 1995; Hunt et al. 2007). Both processes have the potential to alter population dynamics (Armonies 1996). We observed in some preliminary experiments that if post-settlement juveniles burrowed into experimental sediment cores that had no gas flow, they would usually emerge from the sediment once CO_2 flow was initiated (pers. obs.). These observations coupled with the distinct abundance patterns in buffered vs. unbuffered sediments that occur as the experiment progresses highlight that settlers seem capable of continued evaluation of sediment carbonate conditions, following their initial settlement behavior. Evaluation of sediment and then emergence behavior would increase their susceptibility to passive transport and suggests an active secondary transport (e.g., Norkko et al. 2001).

The clear differences in settlement between the sediments undersaturated and saturated with respect to $CaCO_3$ (Figs. 2 and 4) suggest that passive transport was small or nonexistent and not capable of erasing the patterns established during initial recruitment. Alternatively, post-settlement passive transport may also have an active site selection associated with it. The study area is a sheltered embayment (43° 38′ 21.11″N/70° 14′ 57.93″W), and during the experimental period, there were no storm or wind events. The study site is dominated by fine-grain muds, and no obvious scour patterns of these fine grains were ever noted, suggesting that passive transport by scouring was not possible. Current velocities were not measured, so we have no way to compare the movement of water in this region to critical shear velocities known to cause bedload transport of *M. arenaria* (Roegner et al. 1995). Previous work by Moller (1986) found that initial settlement patterns of *M. arenaria* dominated the final recruitment patterns relative to post-settlement dispersal and may be the case at our study location. However, we acknowledge the relative importance of post-settlement transport vs. larval settlement as the coupled factors which can influence final abundance (e.g., Roegner et al. 1995; Hunt et al. 2007). Our findings of a behavioral response of settling clam larvae to sediment carbonate chemistry are an important contribution towards understanding the complex milieu of factors affecting early life history of calcified infauna and highlights the geochemical importance of shell material in these habitats.

Elucidating the exact cue settlers are responding to when transitioning from the water column to the benthos is difficult due to the complexity of possible factors settling organisms are likely to face (Cummings et al. 2009; Pawlik 1992; Rodriquez et al. 1993). We believe that many of the negative response geochemical cues already discussed in the literature, and which have been used to explain sediment rejection by juveniles, may be explained in terms of pH and carbonate mineral saturation state. For example, settling juveniles of *M. mercenaria* are known to reject sediments recently disturbed by removal of the upper several millimeters of the deposit and will accept them again within several hours (Woodin et al. 1995). In organic-rich deposits of many coastal regions, removal of the very surface layer of sediment (top several millimeters) will expose reduced metabolites and/or electron-rich mineral phases (e.g., NH_4^+, Mn^{2+}, Fe^{2+}, FeS) to oxidation, resulting in proton production, a lower pH, and a transient depressed carbonate saturation state. Sediment reworking has been shown to similarly expose reduced metabolites to O_2, lowering pH and saturation state, and promoting dissolution in nearshore terrigenous muds (Aller 1982).

It is clear that reduced compounds in sediments may be toxic to fauna in high concentrations; however, infaunal bivalves pump overlying waters through their mantle cavity so that only their exterior shell is directly exposed to the geochemical conditions of the ambient pore water. Post-larval settlers have to remain right below the SWI due to the lack of fully formed siphons (Zwarts and Wanink 1989; Carriker 2001). This region typically has the lowest pH of the deposit in coastal deposits (Mackin and Swider 1989), where subsurface pH minima occurs in the zone where O_2 is still present and where the coupled contribution of aerobic CO_2 production and oxidation of reduced metabolites migrating upward along their diffusive gradients converge. Infaunal bivalves cannot effectively control the environment surrounding their shell, although the exterior surface of the shell has a periostracum that provides some degree of mechanical and likely geochemical protection. Earlier studies have clearly demonstrated juvenile shell dissolution under corrosive conditions (Green et al. 2004a, b, 2009); however, this threshold is poorly constrained and dependent on developmental state (Waldbusser et al. 2010). In short, bivalves will generally have access to more saturated overlying water that would be in contact with internal tissue and organs via siphoning, but reduced and corrosive porewater would remain in contact with exterior shell.

Bivalve larvae have shells usually composed of aragonite (e.g., Fritz 2001), while juvenile and adult biocalcification results in calcite, aragonite, or a mix, depending on species. Infaunal clams, however, are generally composed of aragonite throughout their life history. Additionally, some pelagic larvae appear to contain amorphous calcium carbonate (ACC) (Weiss et al. 2002), although recent work challenges this finding (Kudo et al. 2010). ACC is substantially more soluble than aragonite (Brecevic 1989); Green et al. (2009) showed preferential dissolution of the early larval shell in *M. mercenaria* juveniles in conditions undersaturated with respect to aragonite. It seems important then that juvenile bivalves must grow quickly following settlement to reach sizes capable of dealing with conditions undersaturated with respect to aragonite. Additionally, bivalves must have life history strategies to cope with geochemically corrosive conditions. For example, hydrothermal vent clams manage to sustain a population at highly corrosive hydrothermal vent communities (Schöne and Giere 2005), but the strategies for doing so are not completely understood and probably relate to taking advantage of transient favorable conditions. In this study, we show the importance of carbonate saturation state in determining initial settlement responses of infaunal marine bivalves with a clear correlation between level of undersaturation and sediment rejection. The fact that larvae and early juvenile bivalves dissolve when exposed to carbonate mineral undersaturation (Green et al. 2004a, b, 2009; Talmage and Gobler 2010; Waldbusser et al. 2010) indicates that carbonate thermodynamics is an important indicator of sediment conditions and which is evaluated during settlement.

A curious finding of our laboratory experiments was the relatively high proportion of burrowing juveniles in conditions that are corrosive to calcium carbonate aragonite (Fig. 2). From previous survival experiments, very early juvenile hard clams show significant mortality at these aragonite saturation states (Green et al. 2009). This incongruity between the settlement behavior experiments presented here and previous findings suggests that dissolution pressure is one of several selection factors for juvenile hard clams. It is well known that the transition to the benthos is an energetically demanding metamorphosis (Gallager et al. 1986); presumably, settling bivalves must weigh settling into more corrosive sediments against delaying metamorphosis and growth. The susceptibility of larval and juvenile bivalves appears to be strongly linked to energetic status (Talmage and Gobler 2010) and has been documented in corals (Holcomb et al. 2012). This is one of several trade-offs that occur during early life history, and although we lack the data to test the effects of energetic status on behavioral responses to sediment carbonate chemistry, this is an area of important future research. Likewise, a “decision” to not burrow must be weighed against the predation pressures that exist in remaining on the sediment surface. This “choice” between dissolution or predation pressure represents another important area of future work.

We hypothesized that by adding small amounts of biogenic $CaCO_3$ to intertidal mud, the pH and saturation states can be raised relative to mud not augmented with $CaCO_3$ and that enhanced site selection and settlement would occur for transitioning bivalves. Indeed, our results show that when juveniles settle, there is then significant enhancement of the numbers of individuals in buffered mud relative to adjacent unbuffered sediments. The authors do acknowledge, however, that the treatment replication in our experimental design (within plots, rather than across plots), could lead to other across-plot factors resulting in the differences in settlement. However, as mentioned, analysis of sediment conditions in both plots prior to the setup of the experiment showed that the between-plot sediment conditions were similar with respect to organic content, $CaCO_3$ content, surface pore water porosity (0–1 cm), tidal exposure time, initial pH distribution, and visual properties of the sediment surface. Acknowledging that we cannot completely rule out other cofactors we did not account for, we assume that these were minor relative to the large signal created with $CaCO_3$ treatment and that our results stem largely from between-plot differences in carbonate saturation state. Although the ecological benefits of shell for clam settlement have been well documented (Carriker 1961; Gutierrez et al. 2003; Powell and Klinck 2007), our buffering experiments in the field (in which $CaCO_3$ was used) are matched by laboratory experiments in which only CO_2 is used to alter sediment aragonite saturation state. One potential flaw of this study is the use of two different species in the laboratory experiment (*M. mercenaria*) compared with the natural set during the buffering experiment (*M. arenaria*). This discrepancy was unavoidable and resulted from the time constraints of running the lab experiment relative to the availability of pediveliger species from hatcheries. Future work could certainly replicate these experiments with similar species. However, this consistency in positive responses of settling of two species of bivalves to enhanced saturation state by two different manipulations suggests to us that this is a strong geochemical effect and may be a cue used for settling individuals in many bivalve species. We note, however, that this geochemical effect is not exclusive of other documented positive ecological effects, such as refuge from predation that shell material provides (e.g., Carriker 1961; Gutierrez et al. 2003).

Our results highlight the potential importance of shell material to coastal and estuarine ecosystems, especially where dense bivalve populations were or are currently found. Nearshore habitats where some infaunal bivalves are found are often subject to increased sediment input and eutrophication due to human watershed impacts, both presumably increasing organic material at the sediment–water interface and thus increasing dissolution pressure on newly settled bivalves. The buffering of intertidal mud by returning shell material may be a valuable management tool (Kelly et al. 2011) for increasing recruitment of native bivalve populations to certain areas or to increase survivorship of juveniles when reseeding efforts are employed. Increased economic value of enhanced recruitment to certain regions, coupled with the ecosystem services a robust clam population provides, could make sediment buffering a profoundly important best management practice not currently employed.

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