Limnol. Oceanogr., 54(6), 2009, 2072–2080

The effects of elevated carbon dioxide concentrations on the metamorphosis, size, and survival of larval hard clams (*Mercenaria mercenaria*), bay scallops (*Argopecten irradians*), and Eastern oysters (*Crassostrea virginica*)

Stephanie C. Talmage* and Christopher J. Gobler

Stony Brook University, School of Marine and Atmospheric Sciences, Stony Brook, New York

Abstract

We present experiments that examined the metamorphosis, growth, and survivorship of larvae from three species of commercially and ecologically valuable shellfish (*Mercenaria mercenaria*, *Argopecten irradians*, and *Crassostrea virginica*) at the levels of CO_2 projected to occur during the 21st century and beyond. Under CO_2 concentrations estimated to occur later this century (~66 Pa, 650 ppm), *M. mercenaria* and *A. irradians* larvae exhibited dramatic declines (>50%) in survivorship as well as significantly delayed metamorphosis and significantly smaller sizes. Although *C. virginica* larvae also experienced lowered growth and delayed metamorphosis at ~66 Pa CO_2, their survival was only diminished at ~152 Pa CO_2. The extreme sensitivity of larval stages of shellfish to enhanced levels of CO_2 indicates that current and future increases in pelagic CO_2 concentrations may deplete or alter the composition of shellfish populations in coastal ecosystems.

The combustion of fossil fuels and the resultant increase in atmospheric CO_2 during the past century has had a multitude of effects on this planet, including acidification of the world's oceans. The oceans have absorbed nearly half of the anthropogenically produced CO_2 during the past century (Sabine et al. 2004), altering its inorganic carbon chemistry and pH. Model simulations indicate that combustion of the world's fossil fuel supply in the coming centuries could result in a fivefold increase in atmospheric CO_2 levels to nearly 203 Pa (2000 ppm) and a decrease in surface ocean pH by 0.77 units (Caldeira and Wickett 2003). This decline in the pH of surface waters will concurrently reduce carbonate ion (CO_3^{2-}) concentrations and the degree of calcium carbonate ($CaCO_3$) saturation (Ω) in surface waters, with potentially negative consequences for $CaCO_3$-synthesizing marine organisms.

Although levels of CO_2 in marine environments will continue to rise during this century, organisms in some coastal zones are already exposed to high levels of CO_2. Many estuaries are 'net heterotrophic' as a result of terrestrial, riverine, and wetland supplements of allochthonous carbon (Gattuso et al. 1998; Ram et al. 2003; Koch and Gobler 2009), which can lead to waters that are supersaturated with CO_2. Moreover, in temperate coastal zones, many bivalve mollusks, such as Eastern oysters (*Crassostrea virginica* [Gmelin 1791]), bay scallops (*Argopecten irradians* [Lamarck 1819), and hard clams or northern quahogs (*Mercenaria mercenaria* [Linnaeus 1758]) spawn during summer (Kennedy and Krantz 1982; Bricelj et al. 1987; Kraeuter and Castagna 2001), when the net heterotrophic nature of these systems is maximal (Blight et al. 1995; Ram et al. 2003; Thomas et al. 2004). Additionally, coastal upwelling and riverine discharge can result in coastal waters with CO_2 levels exceeding 101 Pa and with subsaturating levels of CO_3^{2-} (Feely et al. 2008; Salisbury et al. 2008). Hence, it is likely that $CaCO_3$-synthesizing organisms in coastal zones are often challenged with high levels of CO_2 and low levels of pH and carbonate ion.

Calcite and aragonite are the primary biogenic forms of calcium carbonate in ocean animals. Studies to date have documented shell dissolution in pteropods (aragonite) as well as in calcifying coccolithophores (calcite) with increasing CO_2 and decreases in $CaCO_3$ saturation (Riebesell et al. 2000; Orr et al. 2005). Decreases in CO_3^{2-} concentrations can reduce the ability of reef-building corals to produce $CaCO_3$ skeletons (Kleypas et al. 2006). Sediments undersaturated with respect to aragonite can cause enhanced mortality of juvenile clams (*M. mercenaria*; Green et al. 2004), and settlement of *Mya arenaria* has been shown to increase when sediment saturation states are increased by buffering from crushed *M. arenaria* shells, indicating that juvenile settlement is influenced by CO_2 levels (Green et al. 2009). Moreover, elevated CO_2 can cause decreased calcification rates in mussels (*Mytilus edulis*) and oysters (*Crassostrea gigas*; Gazeau et al. 2007) and decreased growth and metabolic rates in mussels (Berge et al. 2006). The earliest developmental stages of calcifying marine organisms also may be highly sensitive to increased CO_2. Experimentally enhanced CO_2 has been shown to decrease the development rate of Pacific oyster larvae (*C. gigas*; Kurihara et al. 2007) and to have negative effects on the early development of sea urchins (*Hemicentrotus pulcherrirnus*, *Echinometra mathaei*; Kurihara 2008). *C. virginica* larvae have displayed smaller shell area when exposed to the CO_2 levels predicted for the end of the 21st century (Miller et al. 2009). To date, no study has examined the effects of the CO_2 levels predicted to occur this century on the metamorphosis, growth, and survival of larval shellfish.

This study investigated the effects of increasing CO_2 levels on the growth and survival of the larvae of three species of $CaCO_3$-synthesizing bivalves: the hard clam (*M. mercenaria*), the Eastern oyster (*C. virginica*), and the bay

* Corresponding author: stalmage@ic.sunysb.edu

scallop (*A. irradians*). These shellfish are important economic resources and ecosystem engineers in shallow coastal waters (Newell 2004). Larvae represent an important life stage for shellfish populations, as reductions in the growth and survival of larvae have the potential to translate into declines in adult populations (Gosselin and Qian 1997; Schneider et al. 2003; Arnold 2008). We conducted a series of experiments to determine how CO_2 levels estimated to occur this century (~41–76 Pa) and through the year 2250 (~152 Pa) (Caldeira and Wickett 2003; Zeebe et al. 2008) would affect larval survival, growth, and metamorphosis into juvenile stages.

Methods

Carbon dioxide treatments and measurements—A gas proportionator system (Cole Parmer® Flowmeter system, multitube frame) was used to deliver CO_2 gas to seawater treatments at multiple rates. The gas proportionator mixed appropriate flow rates of 5% carbon dioxide gas and pressurized air (~39 Pa CO_2) to yield the concentrations of carbon dioxide desired for experiments at a net flow rate that resulted in a total volume of gas (350 ± 5 mL min^{-1}), which turned over the volume of experimental beakers >100 times daily. For experiments, the CO_2 gas mixtures from the proportionator system were continuously delivered to the bottom of four replicated, polypropylene 1-liter beakers containing 0.2-μm filtered seawater from eastern Shinnecock Bay, New York. Control containers were continuously bubbled with air at the same rate as the treatment containers with elevated levels of CO_2. With continuous bubbling, all treatment beakers were saturated with respect to oxygen (~8 mg L^{-1}). To quantify precise CO_2 levels attained in experimental beakers, seawater in beakers was bubbled for 24 h and then analyzed before (no larvae or phytoplankton in seawater) and immediately after (larvae removed, phytoplankton present) each experiment using an EGM-4 Environmental Gas Analyzer® (PP Systems) system that quantifies total dissolved inorganic carbon levels after separating the gas phase from seawater using a Liqui-Cel® Membrane (Membrana). Levels of CO_2 were subsequently calculated based on measured levels of total inorganic carbon, pH (total scale; mol kg $seawater^{-1}$), temperature (~24°C), salinity (~28), and first and second dissociation constants of carbonic acid in seawater, according to Roy et al. (1993), using the program CO2SYS (http://cdiac.ornl.gov/ftp/co2sys/). All beakers were also monitored daily for pH level using an Oakton® or Thermo Scientific Orion Star Series™ Benchtop pH meter (±0.01 and 0.001, respectively) to provide further assurance that constant CO_2 levels were administered throughout the experiment. For comparison of the levels of CO_2 administered during experiments to those found today in coastal zones, bi-weekly measurements of total inorganic carbon, pH, temperature, and salinity were made in eastern Shinnecock Bay (40.87°N, 72.45°W) during the typical shellfish spawning period (June through September; Kennedy and Krantz 1982; Bricelj et al. 1987; Kraeuter and Castagna 2001). Water samples were collected at 12:00 (±1 h) on each sampling day from 0.5 m below the water column using a Van Dorn bottle. Water was immediately transferred without bubbling to 300-mL borosilicate bottles and measured for total inorganic carbon, as described above. Levels of CO_2 were estimated as described above using CO2SYS.

Experimental design—Experiments were conducted using three species of bivalves: *M. mercenaria*, *C. virginica*, and *A. irradians*. For each experiment, three levels of carbon dioxide were administered: a high level (~152 Pa CO_2), predicted for the year 2250; a moderate level (~66 Pa CO_2), predicted for the year 2100 (Caldeira and Wickett 2003; Zeebe et al. 2008); and ambient air (~39 Pa CO_2). An additional experiment was conducted in which the range of CO_2 levels predicted during this century (~41, 51, 61, and 71 Pa; Joos et al. 1999) were administered to *M. mercenaria* larvae. *A. irradians*, *C. virginica*, and *M. mercenaria* larvae were obtained from the East Hampton Shellfish Hatchery (East Hampton, New York) 24 h after fertilization.

As a food source, a culture of *Isochrysis galbana* (Tahitian strain, T-Iso) was maintained in exponential phase growth using standard culture conditions and was added at a density of 2×10^7 cells daily to each experimental beaker (2×10^4 mL^{-1}). This algal species administered at this density and at this rate is known to produce high growth rates and survivorship of shellfish larvae through metamorphosis (Carriker 2001; Cragg 2006; Padilla et al. 2006). To promote the high survivorship, none of the containers that were in contact with larvae were exposed to chemicals or detergents (Padilla et al. 2006). To discourage the growth of bacteria during experiments, an antibiotic solution (Sigma-Aldrich No. 4083; 5000 units of Penicillin, 5 mg of Streptomycin, and 10 mg of Neomycin per milliliter of solution) was added to each beaker at 1% of its original concentration at the beginning of each experiment and at the time of each water change (approximately two times weekly). This antibiotic mixture at this concentration has been shown to have no negative effects on the growth and survivorship of shellfish larvae (Padilla et al. 2006).

For each experiment, approximately 100 larvae were distributed to each experimental beaker. Each treatment began with approximately 900 mL to allow beaker volume for the algal culture to be added daily as a food source. Twice weekly during the experiments larvae were gently poured onto a 64-μm mesh, and the condition (live or dead) and developmental stage of each larvae (veligers, pediveligers, and metamorphosed) were determined visually under a dissecting microscope; every individual larvae was counted at every water change. Within a 15-min period, larvae from each beaker (n = 4 per treatment) were removed, counted, observed, and transferred into a new beaker with new filtered seawater, food, and antibiotics. All beakers were submerged in a water bath maintained at 24°C and recorded every 6 min throughout experiments using in situ loggers (Onset©). This temperature (24°C) generally yields high growth rates for *A. irradians*, *C. virginica*, and *M. mercenaria* larvae (Carriker 2001; Matthiessen 2001; Cragg 2006). Percent survivorship of all larvae was determined at each of the bi-weekly water

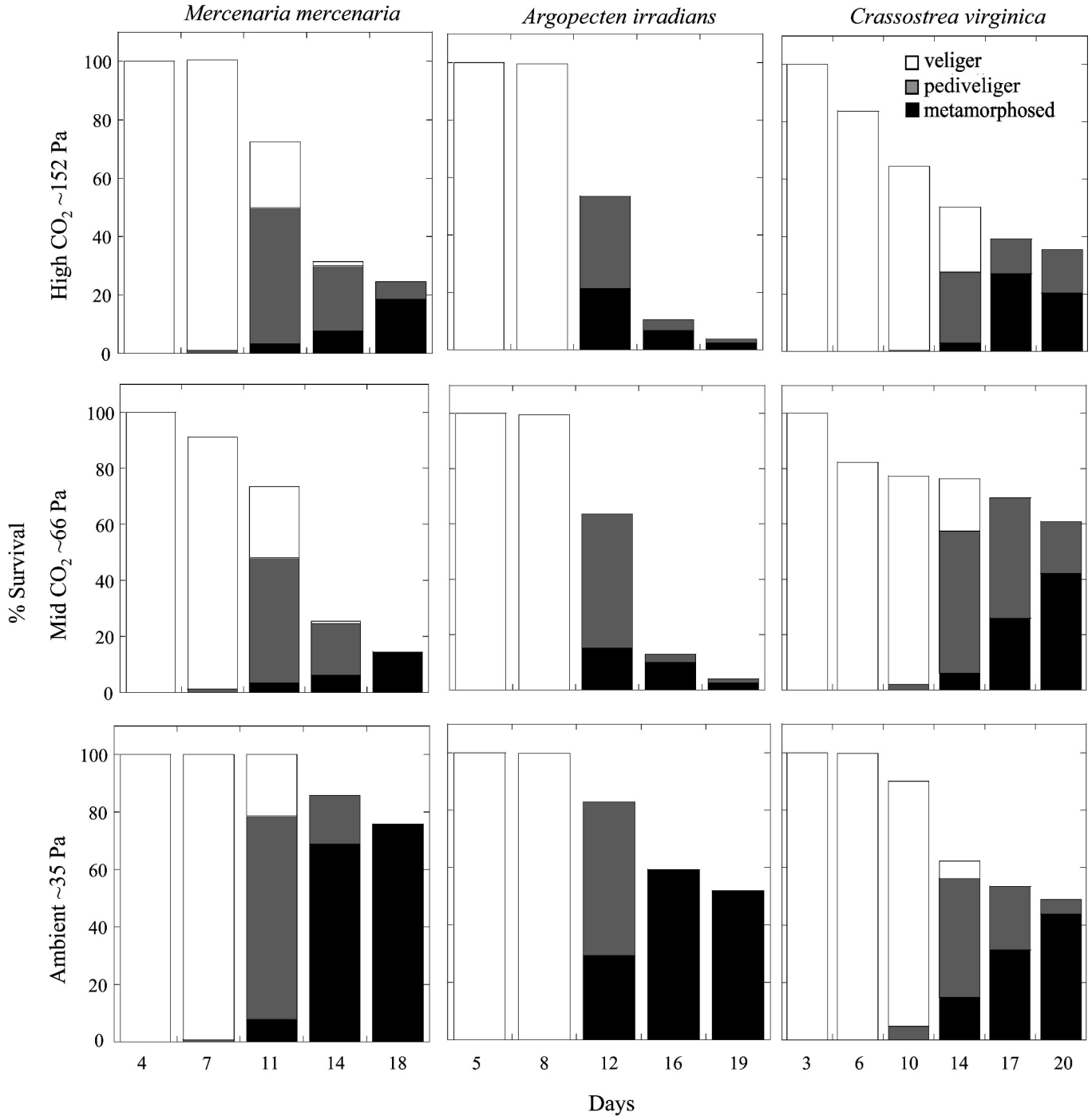

Fig. 1. Survival and development of *Mercenaria mercenaria*, *Argopecten irradians*, and *Crassostrea virginica* larvae under three levels of CO_2, approximately 35, 66, and 152 Pa (Table 1). Counts of all larvae at each stage (veliger, pediveliger, and metamorphosed) were made on the days indicated. The relative standard deviation of replicated vessels per treatment for all time points and experiments was 9% ($n = 4$ per treatment).

changes when the numbers of larvae in each stage of veligers, pediveligers, and metamorphosed juveniles were quantified. Experiments were terminated when at least 50% of the remaining larvae had metamorphosed. At this time, digital photos of 15 randomly selected larvae were obtained from under a dissecting microscope, and lengths were determined using Image J® software. Statistical differences among replicate beakers were examined using *Q*-tests ($p < 0.1$) although none were present in any experiment for all parameters quantified. To statistically assess the effect of CO_2 treatments on larval survival, a goodness-of-fit test or *G*-Test was calculated for each experimental treatment, since this is robust for analysis of differences in percent survivorship among treatments (Sokal and Rohlf 1995).

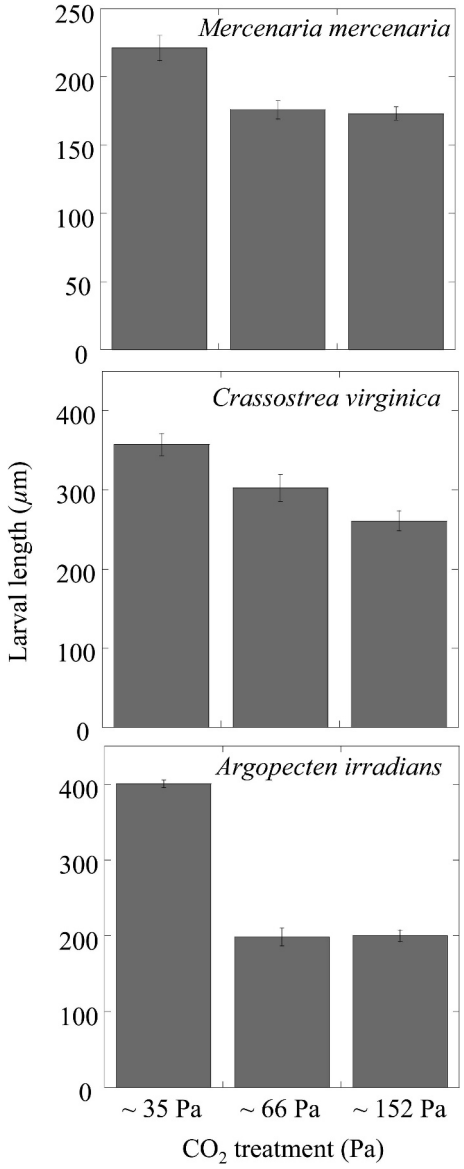

Fig. 2. Mean length of larval shellfish (±1 standard error) at three levels of CO_2 ($n = 15$ per treatment). Measurements were made on day 18 (*Mercenaria mercenaria*), day 20 (*Crassostrea virginica*), and day 19 (*Argopecten irradians*). The precise CO_2 levels administered appear in Table 1.

One-way ANOVAs and post-hoc Tukey multiple comparison tests were performed to examine the differences among larval size at each CO_2 level.

Results

Enriched levels of dissolved CO_2 had a pronounced negative effect on the survival of all three species of shellfish larvae. *M. mercenaria* larvae were greatly affected by ~66 Pa and ~152 Pa CO_2 and the resultant decreases of pH, CO_3^{2-} availability, and $CaCO_3$ saturation state (Fig. 1; Table 1). There was a significant decrease in survivorship of larvae with increased CO_2 compared to survivorship of larvae receiving ambient levels (*G*-test, $p < 0.001$; Fig. 1). Fewer than 20% of *Mercenaria* larvae survived to fully metamorphose into juvenile clams in the ~66 Pa and 152 Pa CO_2 treatments, compared to 76% ± 4% in the ambient, control treatment (Fig. 1). Larvae within elevated CO_2 treatments also experienced delays in metamorphosis. For example, although all surviving clam larvae had fully metamorphosed after 18 d under ambient conditions, 18% ± 6% had still not done so in the highest CO_2 treatment (Fig. 1). The mean length of 18-d-old *M. mercenaria* larvae decreased significantly from 220 ± 9.5 μm at 35 Pa CO_2 to 170 ± 6.7 μm and 170 ± 4.8 μm at 66 and 152 Pa CO_2, respectively (ANOVA, $p < 0.001$; Tukey, $p < 0.01$; Fig. 2).

Since the levels of CO_2 predicted in the world's oceans later this century (~66 Pa) caused significant increases in mortality and delays in development times for *M. mercenaria* compared to current CO_2 levels, an experiment examining the range of CO_2 predicted through this century was conducted (~41, 51, 61, and 71 Pa; Table 2). There were significant differences in larval survival between all of the CO_2 treatments (~71, 61, 51, and 41 Pa; *G*-test, $p < 0.001$). *M. mercenaria* larval survivorship declined from 86% ± 6% to 66% ± 2% with a 10-Pa rise in CO_2 (~41 to 51 Pa; Fig. 3), a change predicted to occur early in the 21st century (Caldeira and Wickett 2003). Survivorship of *Mercenaria* larvae through the experiment continued to decrease to 63% ± 3% and 20% ± 6% of individuals at ~61 and 71 Pa CO_2, respectively (Fig. 3).

A. irradians (bay scallop) larvae were also extremely sensitive to higher CO_2 concentrations. Only 3% ± 1% and 2% ± 0.5% of *Argopecten* larvae survived to metamorphosis at ~62-Pa and 170-Pa CO_2 levels, respectively (a 7.9% d^{-1} population mortality rate), while 52% survived in the ambient treatment (Fig. 1; Table 1), a highly significant difference (*G*-test, $p < 0.001$). High CO_2 levels also slowed development rates of *Argopecten* larvae. After 16 d, only 54% and 76% of scallop larvae had metamorphosed to the juvenile stage at 170 and 62 Pa CO_2, whereas 100% of the surviving individuals under ambient conditions (~36 Pa) had metamorphosed at this time (Fig. 1). At day 19, the lengths of *A. irradians* larvae grown under high CO_2 were half the size (200 ± 12 μm) of individuals grown under ambient CO_2 conditions (400 ± 4.6 μm at ~35 Pa; Tukey, $p < 0.0001$; Fig. 2).

In contrast to *Mercenaria* and *Argopecten*, *C. virginica* (Eastern oyster) larvae responded differently to the enrichment of CO_2 in seawater. The rate of oyster larvae metamorphosis was significantly delayed by exposure to enrichment of CO_2 (*G*-test, $p < 0.001$). After 2 weeks, one third of oyster larvae exposed to current CO_2 conditions had fully metamorphosed, while only 6% ± 2% and 3% ± 1% had done so at ~66 and 152 Pa CO_2, respectively (Fig. 1). Similarly, after 3 weeks, metamorphosis had occurred in 89% ± 9%, 69% ± 12%, and 58% ± 12% of surviving individuals at ~35, 66, and 152 Pa CO_2, respectively, differences that were statistically significant (*G*-test, $p < 0.001$; Fig. 1). Furthermore, *C. virginica* larvae reared under higher CO_2 (64 and 150 Pa) achieved lengths (300 ± 17 μm and 260 ± 12 μm, respectively) that were significantly smaller than those grown at ambient CO_2 (360 ± 14 μm at 35 Pa; Tukey, $p < 0.05$; Fig. 2). However, in

Table 1. Temperature, pH, carbonate chemistry, alkalinity, salinity, and percent mortality of larvae per day (±1 standard deviation [SD]) during the three-level carbon dioxide experiments with *Mercenaria mercenaria*, *Crassostrea virginica*, and *Argopecten irradians* larvae.

Parameter	High CO_2	Mid CO_2	Ambient
Mercenaria mercenaria			
Temperature (°C)	24 ± 0.52	24 ± 0.52	24 ± 0.52
pH	7.49 ± 0.021	7.84 ± 0.042	8.02 ± 0.021
pCO_2 (Pa)	150 ± 18	64 ± 8	36 ± 4
$\Omega_{calcite}$	1.33 ± 0.121	2.98 ± 0.623	3.68 ± 0.222
$\Omega_{aragonite}$	0.85 ± 0.12	1.92 ± 0.421	2.37 ± 0.124
Total DIC (μmol L^{-1})*	1850 ± 159	1850 ± 249	1580 ± 109
CO_3^{2-} (μmol L^{-1})	54 ± 8.4	121 ± 3.4	150 ± 5.2
Alkalinity (TA)*	1888 ± 20.1	2002 ± 21.4	1791.8 ± 79.2
Salinity	28.0 ± 1.0	28.0 ± 1.0	28.0 ± 1.0
% mortality d^{-1}	−6.3	−6.7	−1.8
Crassostrea virginica			
Temperature (°C)	24 ± 0.51	24 ± 0.52	24 ± 0.52
pH	7.50 ± 0.012	7.85 ± 0.031	8.07 ± 0.012
pCO_2 (Pa)	150 ± 5	63 ± 5	36 ± 2
$\Omega_{calcite}$	1.43 ± 0.121	2.97 ± 0.233	4.52 ± 0.241
$\Omega_{aragonite}$	0.92 ± 0.032	1.91 ± 0.111	2.91 ± 0.123
Total DIC (μmol L^{-1})	1920 ± 41.3	1840 ± 18.2	1770 ± 67.2
CO_3^{2-} (μmol L^{-1})	58 ± 7.2	121 ± 3.5	180 ± 25.1
Alkalinity (TA)*	1961 ± 39.2	1996 ± 54.4	2014 ± 12.3
Salinity	28.0 ± 1.0	28.0 ± 1.0	28.0 ± 1.0
% mortality d^{-1}	−3.9	−1.9	−3.5
Argopecten irradians			
Temperature (°C)	24 ± 0.53	24 ± 0.53	24 ± 0.53
pH	7.48 ± 0.061	7.83 ± 0.032	8.08 ± 0.034
pCO_2 (Pa)	163 ± 27	69 ± 5	36 ± 3
$\Omega_{calcite}$	1.41 ± 0.123	2.9 ± 0.24	4.8 ± 0.42
$\Omega_{aragonite}$	0.91 ± 0.12	1.87 ± 0.121	3.06 ± 0.341
Total DIC (μmol L^{-1})	1980 ± 54.3	1900 ± 15.2	1830 ± 66.1
CO_3^{2-} (μmol L^{-1})	57 ± 6.2	118 ± 3.4	194 ± 26.2
Alkalinity (TA)*	2011 ± 24.3	2047 ± 30.2	2087 ± 51.0
Salinity	28.0 ± 1.0	28.0 ± 1.0	28.0 ± 1.0
% mortality d^{-1}	−7.9	−7.8	−3.8

* DIC, dissolved inorganic carbon; TA, total alkalinity.

contrast to these differences in size and the time required for larval metamorphosis, there was no difference in survivorship between exposure to 35 and 66 Pa CO_2 (61% ± 16%), although the highest levels of CO_2 (~152 Pa) yielded significantly lower survival for oyster larvae (35% ± 13%; *G*-test, $p < 0.001$; Fig. 1).

Discussion

This study has demonstrated that the levels of CO_2 projected to occur in the world's oceans this century and beyond are capable of significantly decreasing the size, rates of metamorphosis, and survivorship of larvae from three species of commercially and ecologically valuable shellfish (*M. mercenaria*, *A. irradians*, and *C. virginica*). The ability of calcifying organisms to synthesize $CaCO_3$ shells is strongly influenced by CO_3^{2-} concentrations and Ω values. Our highest CO_2 treatments (152–162 Pa) yielded $\Omega_{aragonite}$ values <1.0 (Table 1), indicating that dissolution of aragonite would be predicted and synthesis of aragonite would not be favorable. Since the three shellfish larvae studied have shells that are partly composed of aragonite (Stenzel 1964; Carriker 1996), the enhanced mortality and delayed development of these larvae in these treatments (Fig. 1) may result from their inability to adequately synthesize shell material. However, calcification rates in coccolithophores, foraminifera, and corals have been shown to decrease with reduced CO_3^{2-} concentration, even when $\Omega > 1$ (Riebesell et al. 2000; Kleypas et al. 2006), likely in part because biotic aragonite is less crystalline than nonbiogenic aragonite (Weiss et al. 2002). Additionally, the presence of amorphous calcium carbonate, which is substantially more soluble than aragonite (Brecevic and Nielsen 1989), in the shells of larval mollusks (Weiss et al. 2002) likely exacerbates their sensitivity to declining CO_3^{2-} availability. Consistent with this hypothesis, even decreases in aragonite Ω in the saturating range caused by increasing CO_2 adversely affected the ability of shellfish larvae to survive and metamorphose (Fig. 1; Table 1). In the future, such effects will be exacerbated within higher latitudes with

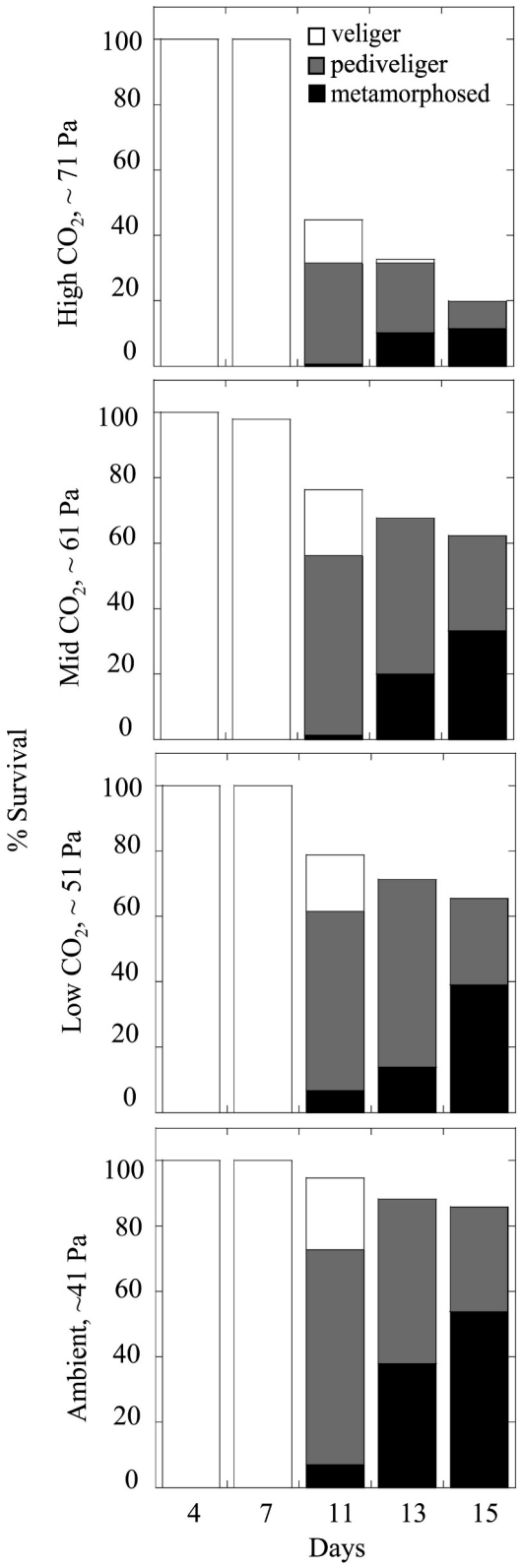

Fig. 3. Survival and development of *Mercenaria mercenaria* larvae under four levels of CO_2, approximately 41, 51, 61, and 71 Pa (Table 2). Counts of all larvae at each stage (veliger, pediveliger, and metamorphosed) were made on the days indicated. The relative standard deviation of replicated vessels per treatment for all time points was 7% ($n = 4$ per treatment).

colder waters and lower states of $CaCO_3$ saturation (Joos et al. 1999).

It is probable that the negative effects of elevated CO_2 on shellfish larvae will extend beyond those observed during our experiments. For example, since pelagic predation pressure on bivalve larvae is high (Andre and Rosenberg 1991), the delayed metamorphosis of shellfish larvae caused by enhanced CO_2 (Fig. 1) would likely translate into prolonged predation and, hence, would further decrease survival of larvae in the field. Similarly, the up to 50% decrease in the size of larvae under high CO_2 (Fig. 2) could translate into higher mortality rates for these individuals once they are settled (Marshall et al. 2003). Since recently settled bivalves are highly prone to dissolution and shell loss (Green et al. 2004), even surviving larvae may not fully develop in some coastal zones. Finally, it is plausible that rapid evolution of shellfish and other calcifying species will lead to the proliferation of strains that are more resistant to ocean acidification, perhaps circumventing some of the effects described here. However, the current rates of atmospheric CO_2 increases are 100 times faster than any recorded in the past 1 million years, rapidly changing the ocean chemistry to levels not experienced in hundreds of millions of years (Sabine et al. 2004), indicating that this evolutionary challenge is without precedent for many extant calcifying species.

The negative effects of rising levels of CO_2 on shellfish larvae in our study are consistent with results of prior studies of calcifying organisms in the ocean, such as coral reefs, coccolithophores, and even juvenile stages of shellfish (Riebesell et al. 2000; Green et al. 2004; Kleypas et al. 2006). Among planktonic species of coccolithophores, some strains have shown decreased levels of calcification with increased levels of CO_2 (Riebesell et al. 2000), while others have displayed an increase in calcification and abundance under such conditions (Iglesias-Rodriguez et al. 2008). Similarly, the levels of CO_2 predicted later this century may be more detrimental to some species (*M. mercenaria*, *A. irradians*) than to others (*C. virginica*). It has been previously noted that *C. virginica* eggs and larvae are more tolerant of low pH than are *M. mercenaria* larvae (Calabrese and Davis 1966), a finding that is consistent with our demonstration that oyster larvae survival was higher than that of larval hard clams at the CO_2 levels of ~66 Pa (Fig. 1).

Larvae survival can greatly affect future adult shellfish population densities (Gosselin and Qian 1997; Schneider et al. 2003; Arnold 2008). All three shellfish in this study are important ecological and commercial species along the Atlantic coast, with annual U.S. landings of these species being worth hundreds of millions of dollars; their ecosystem services far exceed this value (Costanza et al. 1997). However, in recent decades wild populations of shellfish have been under increasing pressure from overfishing, loss of habitat, hypoxia, and eutrophication, and their populations have experienced precipitous declines (Lotze et al. 2006).

Globally, shellfish restoration efforts, such as habitat restoration, transplantation, and reduction of fishing pressure, are being implemented to enhance stocks of these

Table 2. Temperature, pH, carbonate chemistry, alkalinity, salinity, and percent mortality of larvae per day (±1 standard deviation [SD]) during the four-level carbon dioxide experiments with *Mercenaria mercenaria* larvae.

Parameter	High CO_2	Mid O_2	Low CO_2	Ambient
Temperature (°C)	24 ± 0.52	24 ± 0.51	24 ± 0.52	24 ± 0.52
pH	7.79 ± 0.012	7.88 ± 0.021	7.97 ± 0.022	8.02 ± 0.012
pCO_2 (Pa)	73 ± 2	60 ± 4	49 ± 2	41 ± 2
$\Omega_{calcite}$	2.57 ± 0.123	3.15 ± 0.101	4.0 ± 0.222	4.14 ± 0.133
$\Omega_{aragonite}$	1.66 ± 0.042	2.03 ± 0.102	2.58 ± 0.122	2.67 ± 0.111
Total DIC (μmol L^{-1})*	1830 ± 12.2	1850 ± 8.43	1910 ± 61.2	1800 ± 16.0
CO_3^{2-} (μmol L^{-1})	105 ± 10.1	129 ± 6.2	163 ± 4.6	169 ± 1.1
Alkalinity (TA)*	1961 ± 18.2	2020 ± 34.2	2126 ± 34.2	2036.3 ± 4.6
Salinity	28 ± 1.0	28 ± 1.0	28 ± 1.0	28 ± 1.0
% mortality d^{-1}	−8.2	−3.8	−3.5	−1.4

* DIC, dissolved inorganic carbon; TA, total alkalinity.

species (Arnold et al. 2002). In the future, these efforts will need to account for the differential ability of shellfish larvae to persist in a high-CO_2 environment, with some populations, such as the Eastern oyster, perhaps having a greater success in the future than other species, such as hard clams or bay scallops.

While the high levels of CO_2 used during our experiments are predicted to occur within the open oceans during the coming century, they can already be found in some coastal zones. Upwelling of deeper ocean water and riverine discharge into coastal regions can both expose pelagic shellfish larvae to CO_2 levels exceeding 101 Pa and subsaturating levels of carbonate ion (Feely et al. 2008; Salisbury et al. 2008). Furthermore, many coastal regions are often 'net heterotrophic' as a result of terrestrial, riverine, and wetland supplements of allochthonous carbon (Gattuso et al. 1998; Thomas et al. 2004; Koch and Gobler 2009), leading to supersaturated CO_2 and lower pH. The degree of net heterotrophy in coastal zones is generally maximal during summer, when spring bloom productivity is degraded (Blight et al. 1995; Ram et al. 2003) and shellfish larvae are spawned in temperate estuaries (Kennedy and Krantz 1982; Bricelj et al. 1987; Kraeuter and Castagna 2001). Moreover, as anthropogenic nutrient loading rates rise, the concomitant increase in the intensity of algal blooms and subsequent heterotrophic degradation of bloom-derived organic matter may result in larger declines in pH and increases in CO_2. Consistent with these ideas, our cursory examination of CO_2 concentrations in a New York estuary during summer months (June–September) revealed that levels were frequently above 51 Pa and averaged ~66 Pa (Table 3). Hence, the three species of bivalve mollusks presented here, which are native to temperate estuaries in North America, such as Shinnecock Bay, New York, are likely to produce larvae under the elevated CO_2 conditions found in this and other similar systems. Therefore, the elevated levels of CO_2 in coastal zones coupled with the 10-Pa rise in ocean CO_2 levels that occurred during the 20th century (Caldeira and Wickett 2003) may have already diminished shellfish populations and partly contributed to their global decline (Lotze et al. 2006).

Acknowledgments

We are grateful for the logistical assistance and our supply of larvae from the East Hampton Shellfish Hatchery. We thank Michael H. Doall, Ying Zhong Tang, and Matt Harke for advice and laboratory assistance during this project. Constructive reviews came from Josef D. Ackerman, Mark A. Green, and two anonymous reviewers. We thank them for their critical comments, which greatly improved this manuscript. This research was supported by the New Tamarind Foundation.

Table 3. Measurements of temperature, salinity, pH, total inorganic carbon, carbon dioxide, $\Omega_{calcite}$, and $\Omega_{aragonite}$ in eastern Shinnecock Bay, New York, during summer 2007.

Date	Temperature (°C)	Salinity	pH	Total CO_2 (μmol L^{-1})	pCO_2 (Pa)	$\Omega_{calcite}$	$\Omega_{aragonite}$
09 Jun	17.4	26.2	7.937	1523	41	2.20	1.38
15 Jun	21.2	28.2	7.989	1506	46	2.94	1.88
07 Jul	21.9	27.3	7.656	1756	96	1.67	1.06
20 Jul	24.4	27.6	7.848	1695	59	2.74	1.76
27 Jul	26.1	24.6	7.777	1253	53	1.74	1.11
10 Aug	25.1	28.4	7.778	1926	79	2.78	1.80
17 Aug	26.2	27.5	8.019	1814	41	4.51	2.92
24 Aug	25.1	28.9	8.015	1892	43	4.59	2.98
07 Sep	21.4	30.0	7.691	1642	81	1.74	1.12
28 Sep	19.7	27.9	7.563	1826	122	1.30	.83

References

Andre, C., and R. Rosenberg. 1991. Adult-larval interactions in the suspension feeding bivalves *Cerastoderma edule* and *Mya arenaria*. Mar. Ecol. Prog. Ser. **71:** 227–234.

Arnold, W. S. 2008. Application of larval release for restocking and stock enhancement of coastal marine bivalve populations. Rev. Fish. Sci. **16:** 65–71.

———, D. C. Marelli, M. Parker, P. Hoffman, M. Frischer, and J. Scarpa. 2002. Enhancing hard clam (*Mercenaria* spp.) population density in the Indian River Lagoon, Florida: A comparison of strategies to maintain the commercial fishery. J. Shellfish Res. **21:** 659–672.

Berge, J. A., B. Bjerkeng, O. Pettersen, M. T. Schaanning, and S. Oxnevad. 2006. Effects of increased sea water concentrations of CO_2 on growth of the bivalve *Mytilus edulis* L. Chemosphere **62:** 681–687.

Blight, S. P., T. L. Bentley, D. Lefevre, C. Robinson, R. Rodrigues, J. Rowlands, and P. J. L. Williams. 1995. Phasing of autotrophic and heterotrophic plankton metabolism in a temperate coastal ecosystem. Mar. Ecol. Prog. Ser. **128:** 61–75.

Brecevic, L., and A. E. Nielsen. 1989. Solubility of amorphous calcium-carbonate. J. Crystal Growth **98:** 504–510.

Bricelj, V. M., J. Epp, and R. E. Malouf. 1987. Intraspecific variation in reproductive and somatic growth cycles of bay scallops *Argopecten-irradians*. Mar. Ecol. Prog. Ser. **36:** 123–137.

Calabrese, A., and H. C. Davis. 1966. The pH tolerance of embryos and larvae of *Mercenaria mercenaria* and *Crassostrea virginica*. Biol. Bull. **131:** 427–436.

Caldeira, K., and M. E. Wickett. 2003. Anthropogenic carbon and ocean pH. Nature **425:** 365.

Carriker, M. R. 1996. The shell and ligament, p. 75–168. *In* V. S. Kennedy, R. I. E. Newwell and A. E. Eble [eds.], The Eastern oyster: *Crassostrea virginica*. Maryland Sea Grant College, Univ. of Maryland System.

———. 2001. Embryogenesis and organogenesis of veligers and early juveniles, p. 77–115. *In* J. N. Kraeuter and M. Castagna [eds.], Biology of the hard clam. Elsevier Science.

Costanza, R., and others. 1997. The value of the world's ecosystem services and natural capital. Nature **387:** 253–260.

Cragg, S. M. 2006. Development, physiology, behaviour, and ecology of scallop larvae, p. 45–122. *In* S. E. Shumway and G. J. Parsons [eds.], Scallops: Biology, ecology, and aquaculture. Elsevier.

Feely, R. A., C. L. Sabine, J. M. Hernandez-Ayon, D. Ianson, and B. Hales. 2008. Evidence for upwelling of corrosive "acidified" water onto the continental shelf. Science **320:** 1490–1492.

Gattuso, J. P., M. Frankignoulle, and R. Wollast. 1998. Carbon and carbonate metabolism in coastal aquatic ecosystems. Annu. Rev. Ecol. Syst. **29:** 405–434.

Gazeau, F., C. Quiblier, J. M. Jansen, J. P. Gattuso, J. J. Middelburg, and C. H. R. Heip. 2007. Impact of elevated CO_2 on shellfish calcification. Geophys. Res. Lett. **34:** LO7603, doi:10.1029/2006GL028554.

Gosselin, L. A., and P. Y. Qian. 1997. Juvenile mortality in benthic marine invertebrates. Mar. Ecol. Prog. Ser. **146:** 265–282.

Green, M. A., M. E. Jones, C. L. Boudreau, R. L. Moore, and B. A. Westman. 2004. Dissolution mortality of juvenile bivalves in coastal marine deposits. Limnol. Oceanogr. **49:** 727–734.

———, G. G. Waldbusser, S. L. Reilly, K. Emerson, and S. O'Donnell. 2009. Death by dissolution: Sediment saturation state as a mortality factor for juvenile bivalves. Limnol. Oceanogr. **54:** 1037–1047.

Iglesias-Rodriguez, M. D., and others. 2008. Phytoplankton calcification in a high CO_2 world. Science **320:** 336–340.

Joos, F., G. K. Plattner, T. F. Stocker, O. Marchal, and A. Schmittner. 1999. Global warming and marine carbon cycle feedbacks an future atmospheric CO_2. Science **284:** 464–467.

Kennedy, V. S., and L. B. Krantz. 1982. Comparative gametogenic and spawning patterns of the oyster *Crassostrea virginica* in central Chesapeake Bay, USA. J. Shellfish Res. **2:** 133–140.

Kleypas, J. A., R. A. Feely, V. J. Fabry, C. Langdon, C. L. Sabine, and L. L. Robbins. 2006. Impacts of ocean acidification on coral reefs and other marine calcifiers. Impacts of ocean acidification on coral reefs and other marine calcifiers: A guide for future research, report of a workshop held 18–20 April 2005, St. Petersburg, Florida, sponsored by NSF, NOAA, and U.S. Geological Survey: Contribution No. 2897. Available online at http://www.healthyreefs.org/pdf/communicati.pdf

Koch, F., and C. J. Gobler. 2009. The effects of tidal export from salt marsh ditches on estuarine water quality and plankton communities. Estuar. Coasts **32:** 261–275.

Kraeuter, J. N., and M. Castagna. 2001. Biology of the hard clam. Elsevier.

Kurihara, H. 2008. Effects of CO_2-driven ocean acidification on the early developmental stages of invertebrates. Mar. Ecol. Prog. Ser. **373:** 275–284.

———, S. Kato, and A. Ishimatsu. 2007. Effects of increased seawater pCO_2 on early development of the oyster *Crassostrea gigas*. Aquat. Biol. **1:** 91–98.

Lotze, H. K., and others. 2006. Depletion, degradation, and recovery potential of estuaries and coastal seas. Science **312:** 1806–1809.

Marshall, D. J., T. F. Bolton, and M. J. Keough. 2003. Offspring size affects the post-metamorphic performance of a colonial marine invertebrate. Ecology **84:** 3131–3137.

Matthiessen, G. C. 2001. Development in culture techniques, p. 47–74. *In* G. C. Matthiessen [ed.], Oyster culture. Blackwell Science.

Miller, A. W., A. C. Reynolds, C. Sobrino, and G. F. Riedel. 2009. Shellfish face uncertain future in high CO_2 world: Influence of acidification on oyster larvae and growth in estuaries. Plos ONE **4:** 1–8.

Newell, R. I. E. 2004. Ecosystem influences of natural and cultivated populations of suspension-feeding bivalve molluscs: A review. J. Shellfish Res. **23:** 51–61.

Orr, J. C., and others. 2005. Anthropogenic ocean acidification over the twenty-first century and its impact on calcifying organisms. Nature **437:** 681–686.

Padilla, D. K., M. H. Doall, C. J. Gobler, A. Hartson, and K. O'Boyle. 2006. Brown tide alga, *Aureococcus anophagefferens*, can affect growth but not survivorship of *Mercenaria mercenaria* larvae. Harmful Algae **5:** 736–748.

Ram, A. S. P., S. Nair, and D. Chandramohan. 2003. Seasonal shift in net ecosystem production in a tropical estuary. Limnol. Oceanogr. **48:** 1601–1607.

Riebesell, U., I. Zondervan, B. Rost, P. D. Tortell, R. E. Zeebe, and F. M. M. Morel. 2000. Reduced calcification of marine plankton in response to increased atmospheric CO_2. Nature **407:** 364–367.

Roy, R. N., L. N. Roy, K. M. Vogel, C. Porter-Moore, T. Pearson, C. E. Good, F. J. Millero, and D. M. Campbell. 1993. The dissociation constants of carbonic acid in seawater at salinities 5 to 45 and temperatures 0 to 45°C. Marine Chemistry **44:** 249–267.

Sabine, C. L., and others. 2004. The oceanic sink for anthropogenic CO_2. Science **305:** 367–371.

Salisbury, J., M. Green, C. Hunt, and J. Campbell. 2008. Coastal acidification by rivers: A new threat to shellfish? Eos Trans. Am. Geophys. Union **89:** 513.

Schneider, D. W., J. A. Stoeckel, C. R. Rehmann, K. D. Blodgett, R. E. Sparks, and D. K. Padilla. 2003. A developmental bottleneck in dispersing larvae: Implications for spatial population dynamics. Ecol. Lett. **6:** 352–360.

Sokal, R. R., and F. J. Rohlf. 1995. Biometry: The principles and practice of statistics in biological research, 3rd ed. W. H. Freeman and Company.

Stenzel, H. B. 1964. Oysters: Composition of the larval shell. Science **145:** 155–156.

Thomas, H., Y. Bozec, K. Elkalay, and H. J. W. de Baar. 2004. Enhanced open ocean storage of CO_2 from shelf sea pumping. Science **304:** 1005–1008.

Weiss, I. M., N. Tuross, L. Addadi, and S. Weiner. 2002. Mollusc larval shell formation: Amorphous calcium carbonate is a precursor phase for aragonite. J. Exp. Zool. **293:** 478–491.

Zeebe, R. E., J. C. Zachos, K. Caldeira, and T. Tyrrell. 2008. Carbon emissions and acidification. Science **321:** 51–52.

Associate editor: Josef D. Ackerman

Received: 13 February 2009
Accepted: 30 June 2009
Amended: 07 July 2009