



Effects of past, present, and future ocean carbon dioxide concentrations on the growth and survival of larval shellfish

Stephanie C. Talmage and Christopher J. Gobler¹

School of Marine and Atmospheric Sciences, Stony Brook University, Southampton, NY 11968

Edited by David M. Karl, University of Hawaii, Honolulu, HI, and approved August 31, 2010 (received for review December 3, 2009)

The combustion of fossil fuels has enriched levels of CO₂ in the world's oceans and decreased ocean pH. Although the continuation of these processes may alter the growth, survival, and diversity of marine organisms that synthesize CaCO₃ shells, the effects of ocean acidification since the dawn of the industrial revolution are not clear. Here we present experiments that examined the effects of the ocean's past, present, and future (21st and 22nd centuries) CO₂ concentrations on the growth, survival, and condition of larvae of two species of commercially and ecologically valuable bivalve shellfish (*Mercenaria mercenaria* and *Argopecten irradians*). Larvae grown under near preindustrial CO₂ concentrations (250 ppm) displayed significantly faster growth and metamorphosis as well as higher survival and lipid accumulation rates compared with individuals reared under modern day CO₂ levels. Bivalves grown under near preindustrial CO₂ levels displayed thicker, more robust shells than individuals grown at present CO₂ concentrations, whereas bivalves exposed to CO₂ levels expected later this century had shells that were malformed and eroded. These results suggest that the ocean acidification that has occurred during the past two centuries may be inhibiting the development and survival of larval shellfish and contributing to global declines of some bivalve populations.

bivalve larvae | climate change | ocean acidification

More than 8 Pg of carbon dioxide (CO₂) is released annually into our planet's atmosphere via the combustion of fossil fuels (1). About one-third of anthropogenically derived CO₂ has entered the world's oceans during the past two centuries (2) and atmospheric and surface ocean CO₂ levels are expected to reach ~750 ppm by 2100 (3, 4). CO₂ entering the ocean decreases the availability of carbonate ions (CO₃²⁻) and reduces ocean pH, a process known as ocean acidification. These changes in ocean chemistry may have dire consequences for ocean animals that produce hard parts made from calcium carbonate (CaCO₃). The experimental enrichment of CO₂ to levels expected in the coming century has been shown to dramatically alter the growth, survival, and morphology of numerous calcifying organisms including coccolithophores, coral reefs, crustose coralline algae, echinoderms, foraminifera, and pteropods (5–7). Many shellfish also produce calcareous shells, and juvenile and adult clams, mussels, and oysters have been shown to be adversely affected by elevated CO₂ (8–12). The earliest life history stages of shellfish, larvae, have been shown to be especially vulnerable to high CO₂, displaying large declines in survival and delays in metamorphosis at levels predicted to occur later this century, suggesting recruitment of these populations may be adversely impacted by ocean acidification (12–14).

Although it is clear that calcifying ocean animals such as shellfish are sensitive to the increases in CO₂ projected for the future, the extent to which the rise in CO₂ that has occurred since the dawn of the industrial revolution has impacted these populations is poorly understood. Here we present experiments that examined the effects of past (250 ppm), present (390 ppm), and future (>400 ppm) CO₂ concentrations on larvae of two species of shellfish: the Northern quahog or hard clam, *Mercenaria mercenaria*, and the bay scallop, *Argopecten irradians*. These bivalves are ecologically and

commercially valuable resources: US mollusk harvests are \$750 million annually (15), with ecosystem services far exceeding that value (16, 17). For experiments, CO₂ was delivered via a gas proportionator system and CO₂ levels in seawater were determined by quantifying dissolved inorganic carbon and pH during experiments using an EGM-4 Environmental Gas Analyzer (PP Systems) and the program CO2SYS (<http://cdiac.ornl.gov/ftp/co2sys/>). Dissolved inorganic carbon was measured with a methodological precision of ±3.6% and full recovery (102 ± 3%) of Dr. Andrew Dickson's (Scripps Institution of Oceanography, University of California at San Diego, La Jolla, CA) certified reference material for total inorganic carbon in seawater [Batch 102 = 2,013 μmol dissolved inorganic carbon (DIC) kg seawater⁻¹] was obtained with our analytical procedures. Static delivery of CO₂ at rates that turned over experimental vessels several times an hour resulted in constant pH levels during experiments [$<0.5\%$ relative standard deviation (RSD) within treatments based on multiple daily measurements]. The rates of larval growth, development, and survivorship and lipid content of larvae were monitored through metamorphosis. Differences in the sizes and shells of larvae and early juvenile stage individuals were documented by cross-sectioning individuals and observing them with an SEM.

Results and Discussion

Larvae grown under near preindustrial levels of CO₂ (250 ppm) displayed the highest rates of metamorphosis, growth, and survival. After 36 d of development, 40% of *M. mercenaria* grown under ~250 ppm CO₂ had survived, whereas only 20% survived at modern day CO₂ levels (~390 ppm), and only 6% survived at ~1,500 ppm CO₂ ($P < 0.001$; Fig. 1). *A. irradians* displayed similar patterns, with 74% of individuals surviving 38 d under ~250 ppm CO₂, 43% surviving at ~390 ppm, and only 5.4% remaining at ~1,500 ppm CO₂ ($P < 0.001$; Fig. 1). Larvae grown under the lowest CO₂ levels displayed remarkably faster rates of metamorphosis compared with individuals grown under present day CO₂. For example, after 14 d of development, 51% of *M. mercenaria* larvae had fully metamorphosed at ~250 ppm CO₂, whereas <7% had done so under higher levels of CO₂ ($P < 0.001$; Fig. 1). After 12 d of development, *A. irradians* larvae displayed a somewhat similar trend because 87% of the larvae had metamorphosed at ~250 ppm CO₂, whereas 68% had done so at ~390 ppm CO₂ ($P < 0.001$; Fig. 1). The mean diameters attained by both species of larvae also were strongly affected by CO₂. *M. mercenaria* and *A. irradians* larvae grown under 250 ppm CO₂ (523 ± 38 and 531 ± 51 μm) were significantly larger than those grown under present day (282 ± 5 and 449 ± 35 μm) and higher (210 ± 9 and 311 ± 26 μm at ~1,500 ppm) levels of CO₂ ($P < 0.001$; Fig. 2). These trends in the size of individuals were obvious during the examination of individuals under SEM (Figs. 3 and 4).

Author contributions: S.C.T. and C.J.G. designed research; S.C.T. performed research; S.C.T. and C.J.G. analyzed data; and S.C.T. and C.J.G. wrote the paper.

The authors declare no conflict of interest.

This article is a PNAS Direct Submission.

¹To whom correspondence should be addressed. E-mail: Christopher.Gobler@stonybrook.edu.

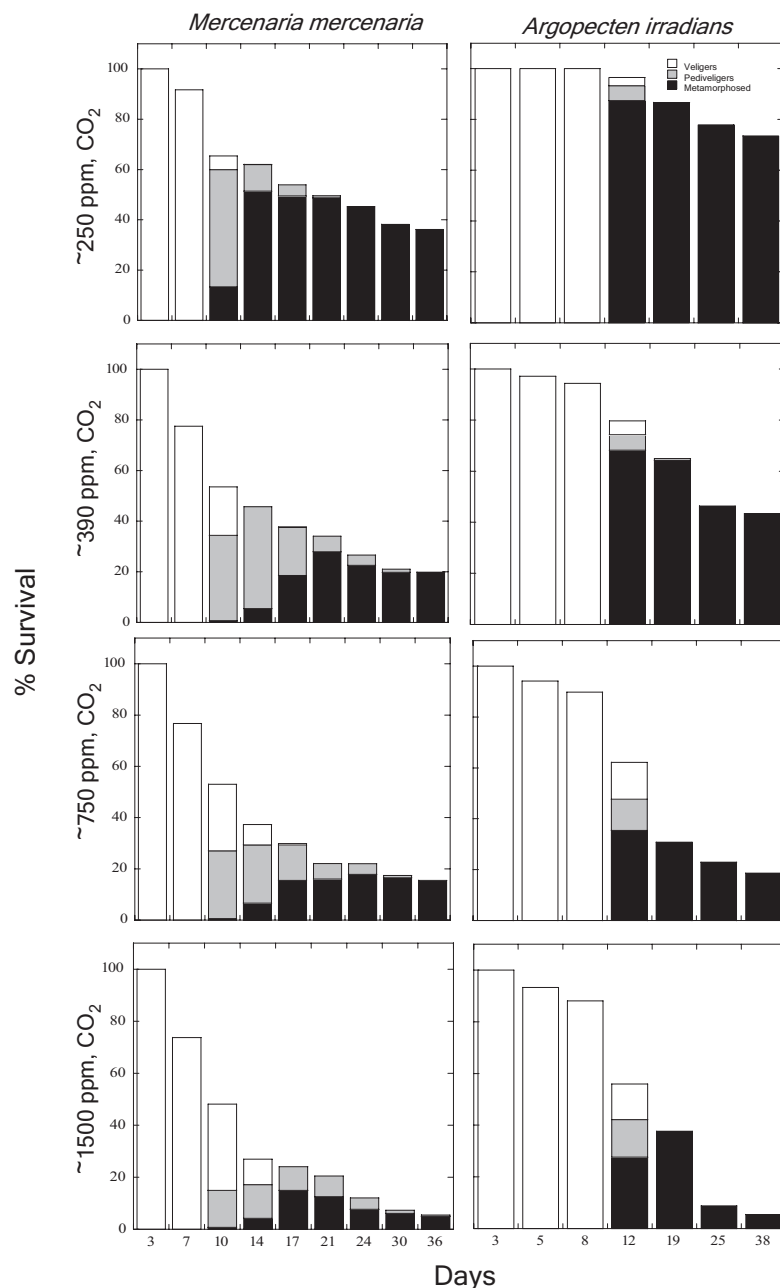


Fig. 1. Development and survival of *M. mercenaria* and *A. irradians* larvae. Percent survival and developmental stage (veliger, pediveliger, and metamorphosed) of larvae grown under four levels of CO₂, ~250, 390, 750, and 1,500 ppm (Table 1). The relative SD of larval survival among replicated vessels per treatment for all times points and experiments was 4% ($n = 4$ per treatment).

Levels of CO₂ strongly influenced the early formation of *M. mercenaria* and *A. irradians* shells. For example, after 17 d of development *M. mercenaria* shells were $17 \pm 2 \mu\text{m}$ thick under ~250 ppm CO₂, $6.7 \pm 2 \mu\text{m}$ at ~390 ppm CO₂, and $3.8 \pm 1 \mu\text{m}$ at ~750 ppm and ~1,500 ppm CO₂ ($P < 0.001$; Figs. 2 and 3). *A. irradians* shells also decreased in thickness with increasing CO₂ being 20 ± 3 , 12 ± 1 , 11 ± 1 , and $6.3 \pm 1 \mu\text{m}$ thick under ~250, 390, 750, and 1,500 ppm CO₂ ($P < 0.001$, Fig. 2), respectively. Beyond impacting shell thickness, elevated levels of CO₂ severely altered the development of the hinge structure of early stage bivalves. As CO₂ levels increased from ~250 to ~1,500 ppm, there were dramatic declines in the size, integrity, and connectedness of the hinge (Fig. 3). Although the *M. mercenaria* hinge displayed a “tongue and groove” pattern under low CO₂ (250 and 390 ppm), under higher CO₂

concentrations the hinge and associated hinge teeth became increasingly separated and detached. Given that the bivalve hinge facilitates opening and closing of shells, allowing for intake of food and the excretion of waste (18), the compromised hinges observed under elevated CO₂ may hinder the ability of individuals to obtain and process suspended particles for nutrition. This hypothesis is consistent with changes in lipid stores of larval shellfish exposed to differing CO₂ concentrations. For both species, with each increasing level of CO₂, the lipid content (as estimated by an index) decreased significantly ($P < 0.001$; Fig. 2). Increasing CO₂ concentrations also caused marked changes in the morphology of the outer edge of juvenile shells (Figs. 3 and 4). With increasing levels of CO₂, this region of the shell became increasingly riddled with holes, pockmarks, and crevices, observations consistent with other

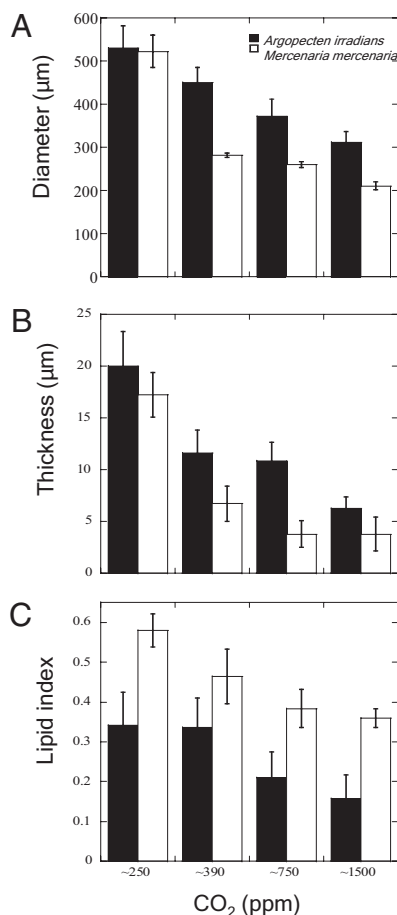


Fig. 2. Diameters, shell thickness, and lipid index of bivalve larvae grown under a range of CO₂ concentrations. Data are from four levels of CO₂, ~250, 390, 750, and 1,500 ppm. (A) Diameters of *M. mercenaria* (day 24) and *A. irradians* (day 20). (B) Thickness of *M. mercenaria* (day 36) and *A. irradians* (day 52) shells at midpoint between the hinge and valve edge of the upper and lower shell of cross sectioned individuals. (C) Lipid index (lipid area/total area) for *M. mercenaria* (day 24) and *A. irradians* (day 20). Error bars represent SD of replicated vessels per treatment ($n = 4$ per treatment).

juvenile and larval shellfish reared under high CO₂ (10, 14), suggesting CaCO₃ shells were malforming and/or dissolving under more acidic conditions. Altered shell morphology was also obvious in juvenile scallops that had distinct ridges, characteristic of later stages of development, under preindustrial CO₂, whereas individuals reared under higher CO₂ conditions lacked ridges, a sign of slower development (Fig. 4 and 19).

Shell integrity is one of the most important lines of defense for larval and juvenile bivalve shellfish, because shells provide physical support for soft and delicate internal organs (20) and protection from benthic and pelagic predators and suspended particles (21, 22). As such, the thinner, frailer shells displayed by early life history bivalves reared under modern day and elevated CO₂ would likely make individuals more vulnerable to predation and/or other environmental stressors. Similarly, within an ecosystem setting, larvae that accumulate fewer lipids (Fig. 2) are generally slower to metamorphose (19) and are more likely to perish once settled (23). Finally, individuals with extended metamorphosis times (Fig. 1) and that are smaller (Fig. 2) would be susceptible to greater rates of predation and natural mortality (23, 24). Hence, within an ecosystem setting, mortality rates of early life history bivalves that develop under modern day and higher CO₂ levels would be expected to be even greater than the rates observed during our experiments. Given

that bivalves in coastal areas naturally experience extremely high mortality rates in the transition from larvae to benthic juveniles (9), increases in mortality due to elevated CO₂ could have profound effects on estuarine bivalve populations (5).

Our findings regarding the effects of future CO₂ levels on larval shellfish are consistent with recent investigations of ocean acidification demonstrating that calcifying organisms will experience declines in survival and growth, as well as malformed CaCO₃ shells and hard parts (25). However, our examination of the development of larval shellfish at levels of CO₂ present before the industrialization of the planet provides important insight regarding the potential effects ocean acidification has had on calcifying organisms during the past two hundred years. Consistent with our findings, larval oysters (*Crassostrea virginica*) have displayed slightly larger shell area when grown under preindustrial CO₂ levels compared with modern levels (26).

During the ~24 million years before the industrial revolution, atmospheric CO₂ levels are estimated to have been relatively static, likely fluctuating in a narrow range significantly below the concentrations present today (27, 28). Moreover, periods of higher CO₂ before this era may not have been accompanied by lower pH and carbonate ion concentrations because the oceans may have buffered the more gradual changes in CO₂ that have occurred through geological history (3, 29). The evolution of calcification in ocean animals is unknown, and the multiple forms of CaCO₃ synthesized by modern day calcifiers (calcite, aragonite, amorphous CaCO₃, and high magnesium CaCO₃) differ widely in their vulnerabilities to dissolution under lower pH (30). Although the precise evolutionary tracks of modern bivalves remain somewhat uncertain (31), fossil evidence suggests that 906 of the 958 living genera of bivalve mollusks, including the species presented here, have a record that began in the mid- to late Cenozoic with the greatest continuous increase in genera between ~15 and ~25 Mya (32), a period of estimated lower CO₂ levels compared with today (27, 28). Together with our results, this suggests that ocean acidification since the industrial revolution may have applied selection pressure on modern marine bivalves and may continue to do so in the future.

The shallow marine environments that many marine bivalves occupy can harbor dynamic levels of pH and CO₂ (33, 34) and the precise degree of phenotypic plasticity of survival among bivalve larvae in the face of higher CO₂ has not been established. Adaptation and evolution could promote the proliferation of bivalve strains that are more resistant to the increases in ocean CO₂ expected in the coming century and some calcifying organisms may even benefit from higher CO₂ levels (25, 35). Importantly, however, the current rates of increase in atmospheric CO₂ are significantly faster than any recorded in tens of millions of years (27, 28), suggesting this evolutionary challenge may be without precedent for extant calcifying species.

A comparison of our two study species may provide insight into future evolutionary pressure of ocean acidification on marine calcifiers. Globally, *M. mercenaria* has a larger, more diverse geographic distribution (36) than *A. irradians* (37), an attribute that generally provides resistance to evolutionary pressures (38) such as increasing CO₂ levels. In addition, predicted extinction rates are higher for the marine mollusk family Pectinidae, which includes *A. irradians*, than the Veneridae family, which includes *M. mercenaria* (39). This information, combined with the more dramatic declines in survival displayed by *A. irradians* under higher CO₂ levels compared with *M. mercenaria* (Fig. 1), suggests *A. irradians* may face a greater evolutionary challenge in adapting to future increases in CO₂ concentrations.

Precipitous declines in wild populations of bivalves during the 20th century have been attributed to overfishing, loss of habitat, hypoxia, and harmful algal blooms (40, 41). Our results suggest that ocean acidification is another process that may have contributed to the declines of these populations in the recent past

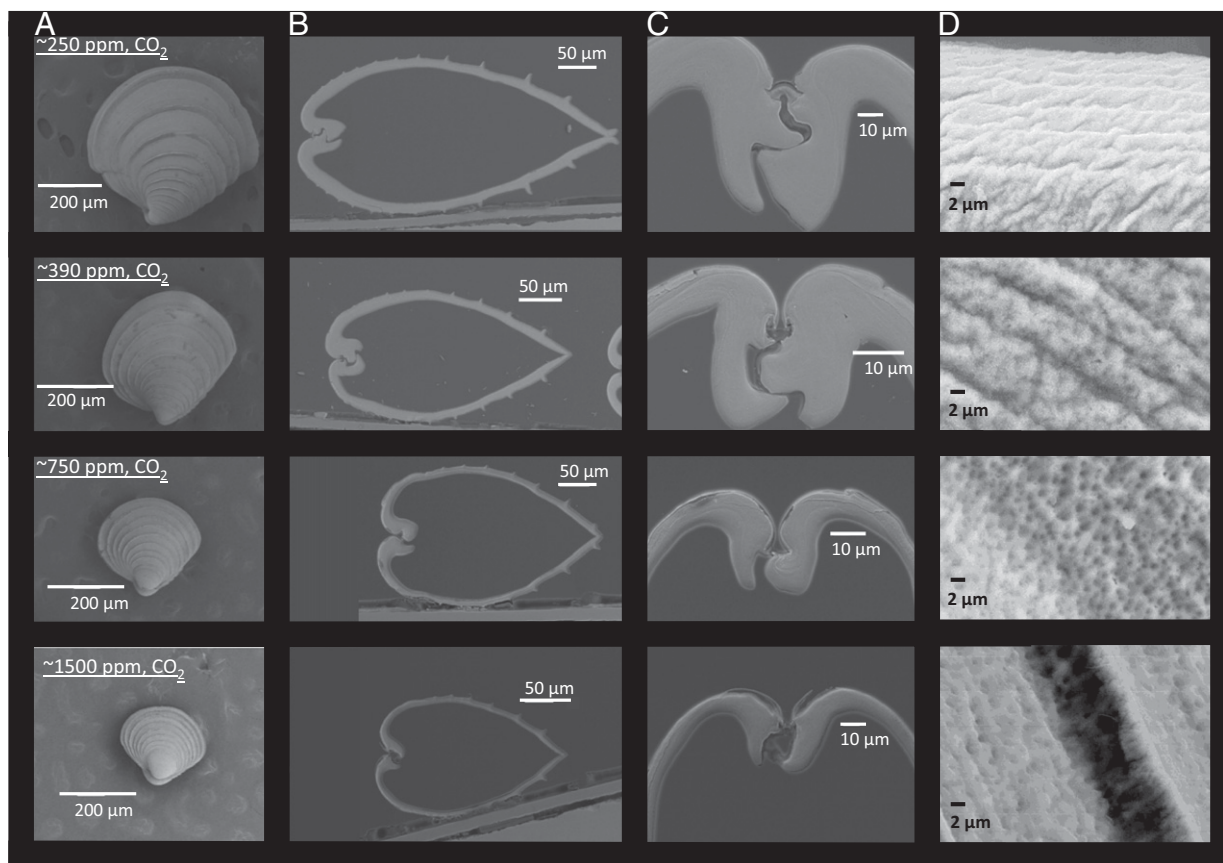


Fig. 3. SEM images of *M. mercenaria* larvae grown under a range of CO_2 concentrations. SEM images of 36-d-old *M. mercenaria* grown under different levels of CO_2 , ~250, 390, 750, and 1,500 ppm (Table 1). (A) Images of individual larvae under each CO_2 level. (B) Hinge to valve cross sections of individuals under each CO_2 level. (C) The hinge of individuals under each CO_2 level. (D) A magnification of the outermost shell of individuals under each CO_2 level.

and could further impact bivalve population densities and diversity in the future. Looking forward, marine organisms will be threatened by aspects of climate change beyond elevated CO_2 , including higher temperatures. Given that the rise in ocean temperatures projected for the coming century (4) is within a range that could also hinder the growth and survival of bivalve larvae (19, 42), future studies should consider the impact of higher CO_2 in conjunction with temperature changes in line with such projections.

Materials and Methods

CO_2 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, low carbon dioxide gas, and pressurized air (~390 ppm CO_2) to yield the concentrations of carbon dioxide desired for experiments at a net flow rate ($350 \pm 5 \text{ mL min}^{-1}$) that turned over the volume of plexiglass covered experimental beakers >400 times daily. Experiments were repeated with tanked gas premixed at each specific CO_2 level and nearly identical seawater chemistry and larval responses were obtained. For experiments, the CO_2 gas mixtures from the proportionator system were continuously delivered to the bottom of four replicated, polypropylene 1-L beakers containing 0.2 μm filtered seawater from eastern Shinnecock Bay, NY. With continuous bubbling, all treatment beakers remained 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 analyzed at the start (immediately before the addition of larvae and phytoplankton) and at the end (larvae removed, phytoplankton present) of 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). This instrument provided a methodological precision $\pm 3.6\%$

for replicated measurements of total dissolved inorganic carbon and provided full recovery ($102 \pm 3\%$) of Dr. Andrew Dickson's (Scripps Institution of Oceanography, University of California at San Diego, La Jolla, CA) certified reference material for total inorganic carbon in seawater (batch 102 = 2,013 $\mu\text{mol DIC kg seawater}^{-1}$). Levels of CO_2 were subsequently calculated based on measured levels of total inorganic carbon, pH (total scale; $\text{mol kg seawater}^{-1}$), temperature (~24 °C), salinity (~28 ppt), and first and second dissociation constants of carbonic acid in seawater according to Roy et al. (43) using the program CO2SYS (<http://cdiac.ornl.gov/ftp/co2sys/>). Multiple daily measurements of pH (calibrated prior each use with NIST traceable standards, ± 0.002 , Orion Star Series Benchtop pH meter; Thermo Scientific) indicated experiment beakers maintained a constant pH level throughout all experiments (<0.5% RSD within treatments).

Experimental Design. The drafted recommendations of the "best practices" for small microcosm experiments set forth by European Project on Ocean Acidification (EPOCA) were followed for this project. For example, aeration of seawater was used to reach a target pCO_2 level, the ideal mechanism to manipulate seawater carbon chemistry (43). Experiments were conducted using two species of bivalves: *M. mercenaria* and *A. irradians*. For each experiment, four levels of carbon dioxide were administered: a high level (~1,500 ppm CO_2), predicted for the year 2250; a moderate level (~750 ppm CO_2), predicted for the year 2100 (3, 44); ambient air (~390 ppm CO_2); and a near preindustrial level (~250 ppm CO_2 ; 25, 26 and Table 1). *A. irradians* and *M. mercenaria* larvae were obtained from locally obtained broodstock spawned at the East Hampton Shellfish Hatchery.

A culture of *Isochrysis galbana* (Tahitian strain, T-Iso) was maintained in exponential phase growth using standard culture conditions and added at a density of 2×10^7 cells daily to each experimental beaker ($2 \times 10^4 \text{ mL}^{-1}$) as a food source. 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 (19, 42, 45). To promote the high survivorship, containers that were in contact with larvae were never exposed to chemicals

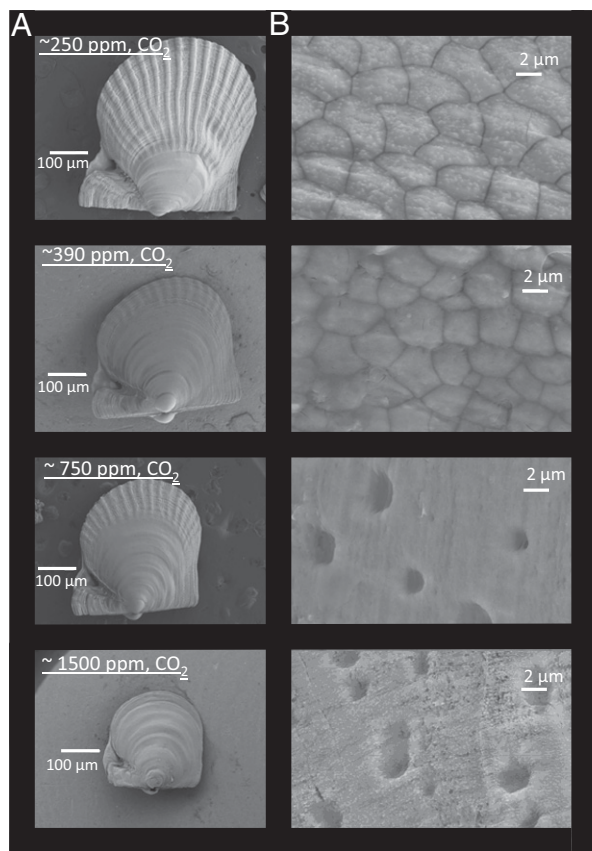


Fig. 4. SEM images of 52-d-old *A. irradians* grown under different levels of CO₂: ~250, 390, 750, and 1,500 ppm, (Table 1). (A) Image of a full individual larvae under each CO₂ level. (B) A magnification of the outermost shell of individuals under each CO₂ level.

or detergents (45). To discourage the growth of bacteria during experiments, an antibiotic solution (5,000 units of penicillin, 5 mg of streptomycin, and 10 mg of neomycin per mL of solution, No.4083; Sigma-Aldrich) was added to each beaker at 1% its original concentration at the beginning of

each experiment and during 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 (45). For each experiment, ~200 larvae were distributed to each experimental beaker, achieving an environmentally realistic abundance of larvae (42). Each treatment began with ~900 mL to allow enough beaker volume for the algal culture to be added daily as a food source. Twice weekly during experiments, larvae were gently poured onto a 64- μ m mesh, and the condition (live or dead) and developmental stage of each larvae (veligers, pediveligers, and metamorphosed) was determined visually under a dissecting microscope; every individual larvae was counted at every water change. 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 within a 15 min period. Throughout experiments, all beakers were submerged in a water bath maintained at 24 °C via the use of commercially available heaters and chillers. This temperature generally yields high growth rates for *A. irradians* and *M. mercenaria* larvae (19, 42). Percent survivorship of all larvae was determined at each of the biweekly water changes when the numbers of larvae in each stage of veligers, pediveligers, and metamorphosed juveniles were quantified. Experiments were terminated after all surviving larvae in all treatments had metamorphosed. To statistically evaluate the effect of CO₂ treatments on larval survival, goodness of fit tests (G Tests) were performed (46).

SEM. To document differences in the size and structure of larval and early juvenile shellfish exposed to differing levels of CO₂, randomly chosen individuals ($n = 4$ per treatment) were mounted for SEM in two distinct ways. Firstly, to image the outside of shells, individuals were attached at 45° relative to a level surface to a conductive substrate using carbon, double-sided tape and were subsequently coated with ~12 nm of gold using an Edwards 150B rotary pump. To image the thickness and internal dimensions, cross-sections of shellfish were prepared. Individuals were mounted on glass microscope slides using UV-curing adhesive coating (Locite 4304) and were impregnated with low-viscosity epoxy (Stuers' Specifix-20) under vacuum outgassing, a step that did not alter the original shape or size of individuals. After curing, the epoxy mount was progressively ground and polished to the centerline (hinge to shell edge) of the shellfish using silicon carbide sandpapers, followed by successively finer diamond polishing grits (15, 6, and 3 μ m), 0.05 μ m aluminum oxide suspension, and finally with colloidal silica. All individuals were cross-sectioned at the same location (hinge to shell edge) across the shell. This mount was then attached to a conductive substrate using carbon double-sided tape and coated with ~4 nm of gold. SEM images were collected on both types of samples with a Leo (Zeiss) Model #1550 electron microscope using a high voltage of 20 KV and a Robinson backscatter detector. All components of individual bivalve shells displayed in Figs. 3 and 4 were probed using advanced EDAX/EDA microanalysis in the LEO

Table 1. Temperature, pH, carbonate chemistry, alkalinity, and salinity (\pm SD) during the four-level CO₂ experiments with *M. mercenaria*, and *A. irradians* larvae

Parameter	Near preindustrial CO ₂	Ambient, present day CO ₂	Year 2100 CO ₂	Year 2200 CO ₂
<i>M. mercenaria</i>				
Temperature (°C)	24 \pm 0.52	24 \pm 0.52	24 \pm 0.52	24 \pm 0.52
pH	8.171 \pm 0.022	8.052 \pm 0.036	7.801 \pm 0.004	7.532 \pm 0.021
pCO ₂ (ppm)	247.1 \pm 6.231	380.0 \pm 33.02	742.3 \pm 9.111	1516 \pm 31.21
Ω_{calcite}	5.31 \pm 0.47	4.53 \pm 0.41	2.82 \pm 0.05	1.67 \pm 0.05
$\Omega_{\text{aragonite}}$	3.42 \pm 0.30	2.92 \pm 0.26	1.82 \pm 0.03	1.08 \pm 0.03
Total DIC (μ mol L ⁻¹)	1646 \pm 94.21	1831 \pm 52.34	1947 \pm 21.33	2108 \pm 18.06
CO ₃ ²⁻ (μ mol L ⁻¹)	208.0 \pm 20.22	178.0 \pm 16.03	111.0 \pm 1.806	66.0 \pm 1.904
Alkalinity (TA)	1938 \pm 117.3	2070 \pm 66.42	2080 \pm 22.63	2127 \pm 49.71
Salinity	28.0 \pm 1.0	28.0 \pm 1.0	28.0 \pm 1.0	28.0 \pm 1.0
<i>A. irradians</i>				
Temperature (°C)	24 \pm 0.51	24 \pm 0.52	24 \pm 0.52	24 \pm 0.52
pH	8.170 \pm 0.026	8.041 \pm 0.044	7.801 \pm 0.005	7.530 \pm 0.011
pCO ₂ (ppm)	244.1 \pm 4.006	386.5 \pm 40.04	738.9 \pm 9.941	1529 \pm 35.05
Ω_{calcite}	5.18 \pm 0.06	4.55 \pm 0.47	2.81 \pm 0.06	1.66 \pm 0.05
$\Omega_{\text{aragonite}}$	3.34 \pm 0.35	2.94 \pm 0.30	1.81 \pm 0.04	1.07 \pm 0.03
Total DIC (μ mol L ⁻¹)	1613 \pm 53.54	1850 \pm 30.98	1941 \pm 25.54	2101 \pm 9.221
CO ₃ ²⁻ (μ mol L ⁻¹)	202.0 \pm 23.42	180.0 \pm 18.44	111.0 \pm 2.341	66.02 \pm 1.911
Alkalinity (TA)	1899 \pm 35.24	2090 \pm 50.01	2075 \pm 26.84	2146 \pm 11.21
Salinity	28.0 \pm 1.0	28.0 \pm 1.0	28.0 \pm 1.0	28.0 \pm 1.0

(Zeiss) Model #1550 electron microscope and were confirmed to contain almost exclusively C, O, and Ca.

Size and Lipid Analysis. To estimate the relative lipid content of larvae, Nile Red stain was used to bind to neutral lipids and fluoresce under an FITC filter on an epifluorescent microscope (23, 47). A Nile Red stock solution was made of 1.25 mg of Nile Red crystals in 100 mL of acetone. Randomly selected larvae ($n = 15$) from each treatment were stained with a 1:9 dilution of the stock solution and 0.2 μm filtered seawater. Larvae were exposed to the stain for ~ 1.5 h, rinsed with filtered seawater, and digitally photographed with a Roper Scientific Photometrics Coolsnap ES camera under an epifluorescent microscope. Digital images of each larva were analyzed for the

area of lipid accumulation and the diameter and the area of individuals using ImageJ. A lipid index was estimated by dividing the area of the larvae containing the fluorescing lipids by the total larval area, thereby allowing for direct comparisons among treatments. One-way ANOVAs and posthoc Tukey multiple comparison tests were performed to examine the differences among larval lipid indexes, shell length, and thickness, at each CO_2 level.

ACKNOWLEDGMENTS. We are grateful for our supply of larvae from the East Hampton Shellfish Hatchery. We thank Jim Quinn for SEM assistance and James Waldvogel for cross sectioning assistance during this project. Constructive reviews came from two anonymous reviewers. This research was supported by the New Tamarind Foundation.

- Le Quere C, et al. (2009) Trends in the sources and sinks of carbon dioxide. *Nat Geosci* 2:831–836.
- Sabine CL, et al. (2004) The oceanic sink for anthropogenic CO_2 . *Science* 305:367–371.
- Caldeira K, Wickett ME (2003) Oceanography: Anthropogenic carbon and ocean pH. *Nature* 425:365.
- I.P.C.C. (2007) Intergovernmental Panel on Climate Change. Summary for Policy-makers. *The Physical Sciences Basis. Working Group I Contribution to the Fourth Assessment Report of the IPCC*, eds Solomon, et al. (Cambridge University Press, Cambridge).
- Guinotte JM, Fabry VJ (2008) Ocean acidification and its potential effects on marine ecosystems. *Ann N Y Acad Sci* 1134:320–342.
- Kleypas JA, et al. (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* (NOAA / Pacific Marine Environmental Laboratory, Seattle). Contribution 2897.
- Riebesell U, et al. (2000) Reduced calcification of marine plankton in response to increased atmospheric CO_2 . *Nature* 407:364–367.
- Gazeau F, et al. (2007) Impact of elevated CO_2 on shellfish calcification. *Geophys Res Lett* 34:L07603:1–5.
- Green MA, Jones ME, Boudreau CL, Moore RL, Westman BA (2004) Dissolution mortality of juvenile bivalves in coastal marine deposits. *Limnol Oceanogr* 49:727–734.
- Green MA, Waldbusser GG, Reilly SL, Emerson K, O'Donnell S (2009) Death by dissolution: Sediment saturation state as a mortality factor for juvenile bivalves. *Limnol Oceanogr* 54:1037–1047.
- Kurihara H, Asai T, Kato S, Ishimatsu A (2008) Effects of elevated pCO_2 on early development in the mussel *Mytilus galloprovincialis*. *Aquat Biol* 4:225–233.
- Kurihara H, Kato S, Ishimatsu A (2007) Effects of increased seawater pCO_2 on early development of the oyster *Crassostrea gigas*. *Aquat Biol* 1:91–98.
- Talmage SC, Gobler CJ (2009) 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*). *Limnol Oceanogr* 54:2072–2080.
- Watson SA, Southgate PC, Tyler PA, Peck LS (2009) Early larval development of the Sydney rock oyster *Saccostrea glomerata* under near-future predictions of CO_2 driven ocean acidification. *J Shellfish Res* 28:431–437.
- Cooley SR, Doney SC (2009) Anticipating ocean acidification's economic consequences for commercial fisheries. *Environ Res Lett* 4:1–8.
- Costanza R, et al. (1997) The value of the world's ecosystem services and natural capital. *Nature* 387:253–260.
- Cooley SR, Kite-Powell HL, Doney SC (2009) Ocean acidification's potential to alter global marine ecosystem services. *Oceanography (Wash DC)* 22:172–180.
- Eble AE (2001) *Anatomy and Histology of Mercenaria mercenaria*. *Biology of the Hard Clam*, eds Kraeuter JN, Castagna M (Elsevier, Amsterdam), pp 117–216.
- Cragg SM (2006) Development, Physiology, Behaviour, and Ecology of Scallop Larvae. *Scallops: Biology, Ecology, and Aquaculture*, eds Shumway SE, Parsons GJ (Elsevier, Amsterdam), pp 45–122.
- Carriker MR (1986) Influence of suspended particles on biology of oyster larvae in estuaries. *Am Malacol Bull Special Ed* 3:41–49.
- Carriker MR (1996) The shell and ligament. *The Eastern Oyster: Crassostrea virginica*, eds Kennedy VS, Newwell RIE, Eble AE (Maryland Sea Grant College, University of Maryland System, College Park, MD), pp 75–168.
- Purcell JE, Cresswell FP, Cargo DG, Kennedy VS (1991) Differential ingestion and digestion of bivalve larvae by the scyphozoan *Chrysaora quinquecirrha* and the ctenophore *Mnemiopsis leidyi*. *Biol Bull* 180:103–111.
- Phillips NE (2002) Effects of nutrition-mediated larval condition on juvenile performance in a marine mussel. *Ecology* 83:2562–2574.
- Tamburri MN, Zimmer-Faust RK (1996) Suspension feeding: Basic mechanisms controlling recognition and ingestion of larvae. *Limnol Oceanogr* 41:1188–1197.
- Doney SC, Fabry VJ, Feely RA, Kleypas JA (2009) Ocean acidification: The other CO_2 problem. *Annu Rev Mater Sci* 1:169–192.
- Miller AW, Reynolds AC, Sobrino C, Riedel GF (2009) Shellfish face uncertain future in high CO_2 world: Influence of acidification on oyster larvae and growth in estuaries. *Plos ONE* 4:e5661.
- Pagani M, Zachos JC, Freeman KH, Tipple B, Bohaty S (2005) Marked decline in atmospheric carbon dioxide concentrations during the Paleogene. *Science* 309:600–603.
- Pearson PN, Palmer MR (2000) Atmospheric carbon dioxide concentrations over the past 60 million years. *Nature* 406:695–699.
- Caldeira K, Berner R, Sundquist ET, Pearson PN, Palmer MR (1999) Seawater pH and atmospheric carbon dioxide. *Science* 286:2043a.
- Mann S (2001) *Biomineralization: Principles and Concepts in Bioinorganic Materials Chemistry* (Oxford University Press, New York).
- Harper EM, Taylor JD (2000) *The Evolutionary Biology of the Bivalvia*, ed JAC, (Geological Society, London, Special Publications).
- Jablonski D, Roy K, Valentine JW, Price RM, Anderson PS (2003) The impact of the pull of the recent on the history of marine diversity. *Science* 300:1133–1135.
- Feely RA, Sabine CL, Hernandez-Ayon JM, Ianson D, Hales B (2008) Evidence for upwelling of corrosive "acidified" water onto the continental shelf. *Science* 320:1490–1492.
- Salisbury J, Green M, Hunt C, Campbell J (2008) Coastal acidification by rivers: A new threat to shellfish? *Eos Trans AGU* 89:513.
- Ries JB, Cohen AL, McCorkle DC (2009) Marine calcifiers exhibit mixed responses to CO_2 induced ocean acidification. *Geology* 37:1131–1134.
- Harte ME (2001) Systematics and Taxonomy. *Biology of the Hard Clam*, eds Kraeuter JN, Castagna M (Elsevier, Amsterdam), pp 3–43.
- Brand AR (2006) Scallop ecology: Distributions and behaviour. *Scallops: Biology, Ecology, and Aquaculture*, eds Shumway SE, Parsons GJ (Elsevier, Amsterdam), pp 651–713.
- Jablonski D (1987) Heritability at the species level: Analysis of geographic ranges of cretaceous mollusks. *Science* 238:360–363.
- Roy K, Hunt G, Jablonski D (2009) Phylogenetic conservatism of extinctions in marine bivalves. *Science* 325:733–737.
- Gobler CJ, Lonsdale DJ, Boyer GL (2005) A review of the causes, effects, and potential management of harmful brown tide blooms caused by *Aureococcus anophagefferens* (Hargraves et Sieburth). *Estuaries* 28:726–749.
- Jackson JBC, et al. (2001) Historical overfishing and the recent collapse of coastal ecosystems. *Science* 293:629–637.
- Carriker MR (2001) Embryogenesis and Organogenesis of Veligers and Early Juveniles. *Biology of the Hard Clam*, eds Kraeuter JN, Castagna M (Elsevier, Amsterdam), pp 77–115.
- Roy RN, et al. (1993) The dissociation constants of carbonic acid in seawater at salinities 5 to 45 and temperatures 0 to 45 °C. *Mar Chem* 44:249–267.
- Riebesell U, Fabry VJ, Hansson L, Gattuso JP (2010) *Guide to Best Practices for Ocean Acidification Research and Data Reporting* (Publications Office of the European Union, Luxembourg).
- Zeebe RE, Zachos JC, Caldeira K, Tyrrell T (2008) Oceans. Carbon emissions and acidification. *Science* 321:51–52.
- Padilla DK, Doall MH, Gobler CJ, Hartson A, O'Boyle K (2006) Brown tide alga, *Aureococcus anophagefferens*, can affect growth but not survivorship of *Mercenaria mercenaria* larvae. *Harmful Algae* 5:736–748.
- Sokal RR, Rohlf FJ (1995) *Biometry: The Principles and Practice of Statistics in Biological Research* (W.H. Freeman and Company, New York), 3rd Ed.
- Castell LL, Mann R (1994) Optimal staining of lipids in bivalve larvae with Nile Red. *Aquaculture* 119:89–100.