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Effects of ocean acidification on larval Atlantic surfclam (*Spisula solidissima*) from Long Island Sound in Connecticut

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Abstract—The Atlantic surfclam (*Spisula solidissima*) supports a $29.2-million fishery on the northeastern coast of the United States. Increasing global carbon dioxide (CO_2) in the atmosphere has resulted in a decrease in ocean pH, known as ocean acidification (OA), in Atlantic surfclam habitat. The effects of OA on larval Atlantic surfclam were investigated for 28 d by using 3 different levels of partial pressure of CO_2 (ρCO_2): low (344 µatm), medium (821 µatm), and high (1243 µatm). Samples were taken to examine growth, shell height, time to metamorphosis, survival, and lipid concentration. Larvae exposed to a medium ρCO_2 level had a hormetic response with significantly greater shell height and growth rates and a higher percentage that metamorphosed by day 28 than larvae exposed to the high- and low-level treatments. No significant difference in survival was observed between treatments. Although no significant difference was found in lipid concentration, Atlantic surfclam did have a similar hormetic response for concentrations of phospholipids, sterols, and triacylglycerols and for the ratio of sterols to phospholipids, indicating that larvae may have a homeoviscous adaptation to OA at medium ρCO_2 levels. Our results indicate that larval Atlantic surfclam have some tolerance to slightly elevated ρCO_2 concentrations but that, at high ρCO_2 levels, they may be susceptible to OA.

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The process of ocean acidification (OA) occurs when increased atmospheric carbon dioxide (CO_2) from anthropogenic activities (i.e., burning of fossil fuels and deforestation) is absorbed by ocean waters (Caldeira and Wickett, 2003; Raven et al.[1]; Doney et al., 2009). Consequently, concentrations of dissolved CO_2 and bicarbonate ions (HCO_3^-) rise, while concentration of carbonate ions (CO_3^{2-}), pH, and calcium carbonate saturation level (Ω) decline (Feely et al., 2004, 2010; Hönisch et al., 2012; Duarte et al., 2013). In recent years, the ocean's natural capacity for buffering, a process that normally occurs over a geologic timescale of 10,000–100,000 years, has been unable to keep pace with the rate of acidification (Hönisch et al., 2012; Zeebe, 2012; Zeebe et al., 2016), resulting in a global ocean pH drop of 0.1. This ongoing shift in the carbonate system and corresponding decline in pH and Ω may affect the growth and survival of marine organisms.

Representative concentration pathways (RCPs) are used to predict future CO_2 concentrations. By the year 2100, under the RCP 8.5 scenario (high emissions) used in climate modeling, CO_2 levels will be >1000 µatm, and under the RCP 6.0 scenario, CO_2 levels will be in the range of 720–1000 µatm (IPCC, 2014). Projected increases in CO_2 by 2100 are expected to result in a reduction of 0.2–0.4 in pH and in a 50% decline in aragonite saturation level ($\Omega_{aragonite}$), a measure often used in relation to Ω because aragonite is a common form of calcium carbonate in ocean waters (Feely et al., 2004, 2010; Hartin et al., 2016). It has been predicted that, by the year 2300, CO_2 levels will be >2000 µatm and will correspond to an additional drop of 0.4 in pH (Feely et al., 2004, 2010; Hartin et al., 2016).

In a recent climate vulnerability assessment of fish and invertebrates on the continental shelf of the northeastern

[1] Raven, J., K. Caldeira, H. Elderfield, O. Hoegh-Guldberg, P. Liss, U. Riebesell, J. Shepherd, C. Turley, and A. Watson. 2005. Ocean acidification due to increasing atmospheric carbon dioxide. R. Soc. Policy Doc. 12/05, 57 p. [Available from website.]

United States, shellfish species were categorized as highly susceptible to changing climate conditions (Hare et al., 2016). A conclusion of most of the research on effects of OA on marine mollusks has been that larval shellfish are more sensitive to OA than those in juvenile and adult stages (Gledhill et al., 2015; Siedlecki et al., 2021). In larval experiments with bay scallop (*Argopecten irradians*) (Talmage and Gobler, 2009; White et al., 2013), eastern oyster (*Crassostrea virginica*) (Miller et al., 2009; Talmage and Gobler, 2009; Gobler and Talmage, 2014), and northern quahog (*Mercenaria mercenaria*) (Green et al., 2004, 2009; Talmage and Gobler, 2009), reduced rates of survival and growth have been observed when larvae were exposed to acidification levels predicted to occur by 2100. The findings of these studies indicate that an $\Omega_{aragonite}$ of ~1.5 results in physiological responses in marine bivalve species. These studies focused on bivalve species that reside in estuarine water, where changes in temperature, salinity, and partial pressure of CO_2 (ρCO_2) can occur at daily rates (Feely et al., 2010; Dickinson et al., 2013; Duarte et al., 2013), and their results may not be applicable to coastal bivalve species.

A synthesis of available reports on ecological consequences of ocean and coastal acidification for species in the Northeast U.S. continental shelf large marine ecosystem has identified that to date little is known on how coastal bivalve species will respond to OA (Hare et al., 2016). Recently, juvenile Atlantic surfclam (*Spisula solidissima*) were found to have modified physiological processes at CO_2 levels of RCP 8.5 scenario during a 12-week exposure, with them being more sensitive than previously studied estuarine bivalves (i.e., oyster species and the blue mussel, *Mytilus edulis*) to increasing ρCO_2 levels (Pousse et al., 2020). The results of that study highlight the need to understand how larval Atlantic surfclam will respond to OA conditions.

Atlantic surfclam are planktonic larvae, transitioning to the pediveliger stage with a foot and "swimming-crawling" behavior (Fay et al., 1983; Cargnelli et al., 1999). Larval Atlantic surfclam are concentrated near the thermocline and are transported horizontally by currents (Zhang et al., 2015, 2016; Chen et al., 2019). Along the coast of the northeastern United States, surface waters of the Atlantic Ocean and Gulf of Maine are characterized by high variability, both spatially (with increased acidification northward) and seasonally (with the lowest values during winter and the highest values in summer) (Biao et al., 2004; Wang et al., 2013; Xu et al., 2017; Goldsmith et al., 2019; Friedland et al., 2020). Records of carbonate chemistry of the subsurface water column in the Gulf of Maine have included $\Omega_{aragonite}$ levels that were lowest in the spring, ranging from 0.9 at the seafloor to 2.2 at the surface, and highest during the summer, ranging from 1.4 at the bottom to 2.6 at the surface, with a seasonal range of 1.0–2.0 (Wanninkhof et al., 2015; Wang, 2016). Wang et al. (2017) observed a decrease of 0.01 in $\Omega_{aragonite}$ for every 1 µmol/kg increase in dissolved inorganic carbon (DIC), similar to what has been observed in the open ocean (Bates et al., 2014). Recently, an oceanographic glider, an autonomous underwater vehicle, was used off the coast of New Jersey to measure subsurface pH levels, which ranged from 7.91 to 8.20, and to measure $\Omega_{aragonite}$, which ranged from 1.5 to 2.2 with higher readings at the surface (Saba et al., 2019).

Although there is evidence of increasing ρCO_2 concentrations in waters where larval Atlantic surfclam are concentrated during development, data are sparse at the thermocline, and the processes and drivers of these changes are not understood fully (Boehme et al., 1998; Wang et al., 2017). The limited data that is available, combined with model projections for the entire region, indicate that some areas in the region where Atlantic surfclam concentrate will reach global levels of ρCO_2 predicted for 2100 as early as 2030–2050 (Ekstrom et al., 2015; Siedlecki et al., 2021).

Characterizing the effects of OA on Atlantic surfclam is complex in part because of the presence of 2 subspecies along the coast of the northeastern United States (Hare and Weinberg, 2005). Both subspecies are referred to as Atlantic surfclam; however, there are physiological differences between the 2 subspecies. The more northern subspecies, *S. s. solidissima*, is larger in size (length: 150–200 mm) and lives longer (25–30 years) than the more southern subspecies, *S. s. similis*, which ranges in length from 76 to 122 mm and lives for 4.0–5.5 years (Walker and O'Beirn, 1996; Weinberg and Helser, 1996). The subspecies differ in geographic range, with the northern subspecies of Atlantic surfclam found from the Gulf of Saint Lawrence in Canada south to Cape Hatteras in North Carolina and with the southern subspecies thought to be distributed primarily in shallow nearshore environments along the coast of Cape Hatteras and in waters of the Gulf of Mexico off the coast of the southeastern United States.

The Connecticut Bureau of Aquaculture identifies Atlantic surfclam in Long Island Sound (LIS) as *S. solidissima*, but results of DNA analysis indicate that *S. s. similis*, although it is considered the more southern subspecies, is located primarily off the coast of Massachusetts; there also is a confirmed population of the southern subspecies in waters of New York in LIS (Hare et al., 2010). Results of a recent survey conducted by the NOAA Northeast Fisheries Science Center indicate that the southern subspecies of Atlantic surfclam may be shifting northward because of increased water temperatures (NEFSC, 2017). The Mid-Atlantic Fishery Management Council in fiscal year 2019 solicited studies to examine whether there has been an expansion of the distribution of the southern subspecies of Atlantic surfclam into the habitat of the northern subspecies, recognizing there are potential implications for stock assessments if multiple species that are genetically different are managed together.

Until the geographical distribution of both subspecies is better understood, we will make no assumption of which subspecies was used in our experiment and will refer to the brood stock and larvae of Atlantic surfclam in our study as *S. solidissima*. This choice is consistent with current management of the commercial industry for Atlantic surfclam as a single fishery, with harvesting concentrated

in estuarine waters of LIS, nearshore coastal waters (0–5 km [0–3 mi]) of New Jersey and New York, and offshore waters along the Atlantic coast from New Jersey to Virginia. Highly valued, the Atlantic surfclam fishery generated more than $29.2 million in revenue during 2019 (National Marine Fisheries Service, commercial fisheries landings database, available from website).

Changes in environmental conditions of the habitat of Atlantic surfclam are comparable to changes in the open ocean, with seasonal variability in $\Omega_{aragonite}$ near or below the physiological threshold of 1.5 identified for other bivalves, yet there have been no studies examining how larval Atlantic surfclam respond to increasing ρCO_2 levels. On the basis of the RCP 6.0 and RCP 8.5 scenarios (IPCC, 2014) and current conditions of the habitat of Atlantic surfclam, our study investigated the effect of low, medium, and high levels of ρCO_2 (344, 821, and 1243 µatm) on growth, mortality, and metamorphosis of larval Atlantic surfclam. We hypothesized that larvae of Atlantic surfclam might have reduced rates of survival and growth and experience longer times to metamorphosis with increased ρCO_2 concentrations. We also hypothesized that allocation of energetic resources in larval Atlantic surfclam, measured by using lipid concentrations, might change in response to variations in carbonate chemistry.

Materials and methods

Spawning

Adult brood stock of Atlantic surfclam were collected from 2 locations in LIS. In 2014, 32 clam were caught on 4 November from Stratford, Connecticut, and 75 individuals were collected on 6 November from Norwalk, Connecticut, from on board a commercial shellfish boat. The brood stock acclimated for 169 d at the Milford Laboratory (41°12′43.82″N, 73°3′12.96″W) of the Northeast Fisheries Science Center in a flow-through seawater system (16°C [standard deviation 3]) delivering natural seston that was supplemented with a drip of cultured *Tetraselmis chui* (strain PLY429). Brood stock were conditioned for spawning following the protocol outlined in Goldberg (1989). On 22 April 2015, 30 scrubbed Atlantic surfclam from both collection sites were placed in a spawning table filled with 10-µm-filtered seawater. To induce spawning, seawater temperature was increased by 2°C every 30 min, to a maximum of 26°C. Upon release of gametes, male and female Atlantic surfclam were placed into separate glass dishes. Gametes were collected from 7 females and 5 males, and then pooled eggs were fertilized with pooled sperm in ambient seawater, yielding a total fertilized egg count of 81.6 million.

After fertilization, 30 embryos/mL were added to each of 9 clean 15-L buckets containing 10 L of 0.35-µm-filtered seawater that had been pumped directly from Milford Harbor and bubbled with CO_2 to reach target conditions (described in the "Experimental design" section). Larvae were fed after 24 h with cultured *Tisochrysis lutea* (isolator number: T-ISO) at an algal density of 50,000 cells/mL (Goldberg, 1989). Every Monday, Wednesday, and Friday, the larvae in buckets were screened, buckets were cleaned, and water in the buckets was replaced with preconditioned water for each ρCO_2 treatment level. Cleaning helped reduce ciliate levels that were present in all treatments. Larval experiments were conducted from 22 April through 20 May 2015.

Experimental design

Nine buckets were placed in a temperature-controlled water table (Table 1). The target ρCO_2 levels (~400, ~800, and ~1200 µatm) were chosen on the basis of RCP scenarios for future CO_2 concentrations in the region and the 3 levels identified in the guide to best practices for OA research and data reporting (Riebesell et al., 2011). Compressed air was passed through a PureGas[2] CO_2 absorber (Altec Air, Broomfield, CO) before being delivered to GFC mass flow controllers for air and carbon dioxide (Aalborg Instruments and Controls, Orangeburg, NY). The CO_2-stripped air was mixed with research-grade CO_2 to create and maintain 3 target ρCO_2 treatment levels and was distributed to 3 replicate buckets per treatment at a moderate aeration rate (12 L/min) that is routinely used in bivalve culture (Loosanoff and Davis, 1963; Bayne, 1965).

Biological sampling

Larvae samples for examining size, survival, metamorphosis, and lipids were taken on a volume basis weekly when buckets were cleaned. Briefly, larvae were screened on a nylon mesh sieve (40–150 µm) and concentrated in a 100-mL graduated cylinder. A subsample of 1 mL was removed from the graduated cylinder and preserved in a 1% solution of buffered formalin for measurement of shell heights (maximum length, from anterior to posterior, parallel to the hinge; number of samples [*n*]=50 larvae). When the experiment began on 22 April 2015, 4 samples were taken for initial measurements of size, and triplicate samples from each bucket were obtained and preserved weekly for size measurements and for determination of the percentages of all larvae in the sample that had died (percent mortality) and that had undergone metamorphosis by using an Olympus inverted microscope (Olumpus IX51, Olympus Corp., Tokyo, Japan) and the software ImageJ, vers. 1.49 (Rasband, 2015).

Lipid samples were obtained at the end of the experiment on day 28. Known volumes (0.3–0.5 mL) of concentrated Atlantic surfclam were collected and placed in glass tubes that had been prerinsed with methanol and chloroform. Samples were stored in 2 mL of chloroform, purged of air with nitrogen gas, and held at –80°C until analysis

[2] Mention of trade names or commercial companies is for identification purposes only and does not imply endorsement by the National Marine Fisheries Service, NOAA.

Table 1

Carbonate chemistry and environmental characteristics measured at low, medium, and high levels of partial pressure of carbon dioxide (ρCO_2) and used to investigate the effect of ρCO_2 levels on laboratory-reared larval Atlantic surfclam (*Spisula solidissima*) in 2015. The variables include mean measured pH (seawater scale, pH measured at 20°C), pH in situ (seawater scale, pH at the temperature of the experiment), temperature (°C), salinity, ρCO_2 (µatm), total dissolved inorganic carbon (DIC) (µmol/kg), and saturation levels of calcite ($\Omega_{calcite}$) and aragonite ($\Omega_{aragonite}$) for the duration of the experiment. Values are means with standard errors of the mean in parentheses. Asterisks (*) indicate values that were measured directly and not calculated by using the program CO2SYS. The adult brood stock used to rear individuals for this study were collected in 2014 from Long Island Sound near Stratford and Norwalk, Connecticut.

	Treatment level of ρCO_2		
Characteristic	Low	Medium	High
pH measured*	7.972 (0.003)	7.629 (0.003)	7.462 (0.003)
pH in situ	8.073 (0.003)	7.734 (0.003)	7.568 (0.003)
Temperature*	18.8 (0.1)	18.9 (0.1)	18.9 (0.1)
Salinity*	25.72 (0.08)	25.73 (0.08)	25.73 (0.08)
ρCO_2	343.9 (5.9)	820.8 (6.0)	1242.9 (5.9)
DIC*	1800.66 (2.35)	1903.74 (2.39)	1953.32 (2.37)
$\Omega_{calcite}$	3.46 (0.01)	1.74 (0.02)	1.22 (0.02)
$\Omega_{aragonite}$	2.18 (0.01)	1.09 (0.01)	0.77 (0.01)

was to begin. Samples were shipped overnight to the Memorial University of Newfoundland for lipid analysis, where they were extracted by following a modified Folch procedure (Parrish, 1999), and lipid classes were identified by using a Chromarod-Iatroscan system for thin-layer chromatography and flame ionization detection (Mark V, Iatron Laboratories, Tokyo, Japan).

Carbonate chemistry measurements

Seawater samples were collected from each bucket for carbonate chemistry measurements immediately before and after water changes (n=63). One sample per bucket was obtained by placing a tube fit with an airstone into the bottom of each bucket and filling 500-mL polypropylene collection bottles to overflow (1.5 times) by using a peristaltic pump. The airstone ensured that larvae and phytoplankton were not collected with the seawater samples. Samples were analyzed immediately for pH and DIC.

Total pH was determined colorimetrically by using m-Cresol purple indicator dye (Sigma-Aldrich, St. Louis, MO) (Dickson and Goyet[3]) with an Ocean Optics 2000+ UV spectrometer (Ocean Insight, Orlando, FL). All samples were analyzed for DIC without the addition of preservatives on a DIC analyzer (AS-C3, Apollo SciTech, LLC, Newark, DE). To ensure precision, replicate samples (n=5) of certified reference material also were analyzed, resulting in 1 standard deviation of 2.7 µmol/kg for total DIC replicates and of 0.0014 for total pH. The DIC instrument was part of a previous international, interlaboratory exercise for comparison of DIC in test seawater samples with low and high CO_2 levels; DIC during our study measured within 0.5% of assigned values (Bockmon and Dickson, 2015). Seawater temperature and salinity were measured daily by using a YSI Model 85 handheld oxygen, conductivity, salinity, and temperature system (YSI, Inc., Yellow Springs, OH). Dissolved inorganic carbon and pH values were entered into the program CO2SYS (Pierrot et al., 2006) to calculate ρCO_2 (in microatmospheres), calcite saturation level ($\Omega_{calcite}$), and $\Omega_{aragonite}$ by using the following constants: K'_1, K'_2 from Mehrbach et al. (1973) and refit by Dickson and Millero (1987), K hydrogen sulfate from Dickson (1990), and total boron from Uppström (1974).

Statistical analyses

The software Statgraphics Plus (vers 17.1.12, Statgraphics Technologies, The Plains, VA) was used for all statistical analyses. All data were checked for normality by using the Shapiro–Wilk test, with a significance level of 0.05, and for equal variance prior to statistical analysis.

[3] Dickson, A. G., and C. Goyet (eds.). 1994. Handbook of methods for the analysis of the various parameters of the carbon dioxide system in sea water, version 2. U.S. Dep. Energy, Oak Ridge Natl. Lab., Carbon Dioxide Inf. Anal. Cent. ORNL/CDIAC-74, 180 p. [Available from website.]

Linear growth during the larval stage (Powell et al., 2002; Pace et al., 2006) was estimated with this equation:

$$y = mx + b,$$

where y = length (in micrometers);
m = slope (in micrometers per day);
x = time (in days); and
b = intercept (initial length in micrometers).

For each bucket, mean rates of shell growth (in micrometers per day, with standard errors of the mean [SE]) were determined by the slope of shell length versus age. An analysis of covariance was used to compare the regression lines and to discern if they were statistically significant (P<0.05). If a significant difference was detected between slopes, further examination was done by using analysis of variance. Data for lipid concentration (in micrograms per gram of wet weight), for percent lipid (lipid concentration divided by total lipid concentration multiplied by100) and for percentages of all larvae in the sample that survived and that had successfully completed metamorphosis were collected at the end of the experiment. All data collected as percentages were transformed with arcsine (square root) prior to statistical analysis and prior to analysis of variance. Least square difference was used to determine different homogeneous groups. For all statistical analyses, degrees of freedom equaled 6.

Results

Seawater physicochemical variables

Carbonate chemistry and environmental variables in the 3 ρCO_2 treatments are shown in Table 1. Values for measured pH were 7.972 (SE 0.003), 7.629 (SE 0.003), and 7.462 (SE 0.003) for the treatments with low, medium, and high levels of ρCO_2, respectively. Values of DIC were 1800.66 µmol/kg (SE 2.35) for the low-ρCO_2 treatment, 1903.74 µmol/kg (SE 2.39) for the medium-ρCO_2 treatment, and 1953.32 µmol/kg (SE 2.37) for the high-ρCO_2 treatment. Other carbonate system characteristics (ρCO_2, $\Omega_{calcite}$, $\Omega_{aragonite}$, and pH in situ) were calculated at the in situ temperature and are reported in Table 1. The calculated levels of ρCO_2 were 343.9 µatm (SE 5.9), 820.8 µatm (SE 6.0), and 1242.9 µatm (SE 5.9) for the 3 treatments with different experimental conditions. There was a significant difference in ρCO_2 (F ratio=2945, P<0.01) between the low-, medium-, and high-ρCO_2 treatments. Mean seawater temperature (18.9°C [SE 0.1]) and salinity (25.73 [SE 0.08]) remained stable throughout the study period (Table 1).

Larval growth, survival, metamorphosis, and lipids

Significant differences were observed in the final shell height of Atlantic surfclam from fertilization to metamorphosis at the conclusion of the experiment, with greater shell height (in micrometers) for individuals in the medium-ρCO_2 treatment than for those in the treatments with low and high ρCO_2 levels (F ratio=6.986, P=0.03; Fig. 1). The specimens in the medium-ρCO_2 treatment had a final shell height that measured 13% larger than those in the the high- and low-ρCO_2 treatments, but there was no difference in final shell height between the individuals exposed to low and high ρCO_2 levels. Larvae in the treatment with a medium ρCO_2 level had a mean growth rate of 7.16 µm/d (SE 0.25) that was significantly greater (F ratio=5.84, P=0.04), 15% higher, than the mean rate observed for larvae in the low-ρCO_2 treatment, 8.26 µm/d (SE 0.25). Growth rates of larvae under the high-ρCO_2 treatment, with a mean rate of 7.25 µm/d (SE 0.25), were similar to those of larvae in the low-ρCO_2 treatment.

Although larval survival did not differ significantly (F ratio=3.23, P=0.17) between treatments, survival rates in the medium-ρCO_2 treatment (29% [SE 5]) were twice those observed in the treatments with high (17% [SE 5]) and low (11% [SE 5]) ρCO_2 levels. After 28 d, significantly more larvae (F ratio=12.89, P<0.01) in the medium-ρCO_2 treatment successfully completed metamorphosis (88% [SE 1]) than in the low- and high-ρCO_2 treatments, at 65% (SE 6) and 61% (SE 5), respectively. There was no

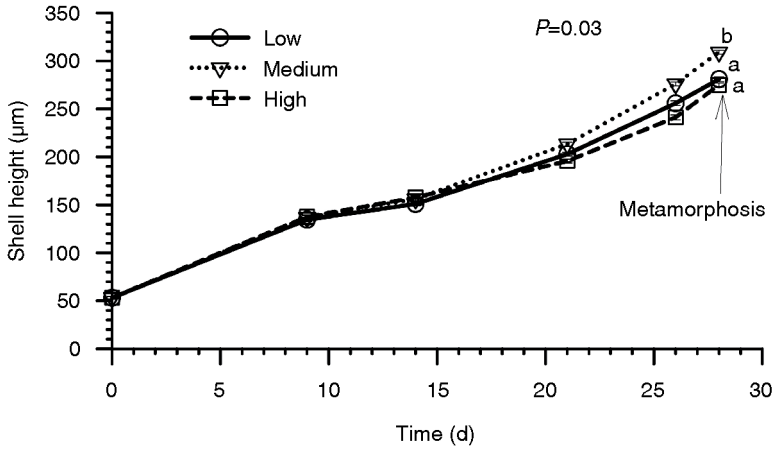

Figure 1

Mean shell height of laboratory-reared larval Atlantic surfclam (*Spisula solidissima*) over the 28 d of the experiment conducted in 2015 with 3 treatments that exposed larvae to low (circles, 344 µatm), medium (triangles, 821 µatm), and high (squares, 1243 µatm) levels of partial pressure of carbon dioxide (ρCO_2). Differences in the letters at the end of regression lines indicate significant differences in final shell height between treatments (P=0.03). The adult brood stock used to rear individuals for this study were collected in 2014 from Long Island Sound near Stratford and Norwalk, Connecticut.

significant difference in metamorphosis of Atlantic surfclam between the low- and high-ρCO_2 treatments.

Lipid concentration and percent lipid measured at the end of the experiment indicate variable responses to the tested OA conditions (Table 2). Phospholipids and triacylglycerols constituted a large portion of the total lipid concentration (58–69%) for all treatments, and there were no significant differences in these characteristics between treatments (F ratio=1.612, P=0.28). There was no significant difference in the sterol-to-phospholipid ratio (F ratio=0.77, P=0.77).

Discussion

Atlantic surfclam from LIS performed better at ρCO_2 levels predicted for the RCP 6.0 scenario than at current levels or levels predicted for the RCP 8.5 scenario, resulting in a hormetic (U-shaped) response for growth, larval shell height, and percentage of larvae that successfully completed metamorphosis. This response is atypical of estuarine bivalve larvae in OA experiments of previous studies; larvae in those studies had negative responses at ρCO_2 levels reported for the RCP 6.0 and RCP 8.5

Table 2

Final lipid concentrations (in micrograms per gram of wet weight) and percent lipid (lipid concentration divided by total lipid concentration multiplied by 100) for laboratory-reared larval Atlantic surfclam (*Spisula solidissima*) at the end of the experiment (after 28 d) conducted in 2015 with 3 treatments that exposed larvae to low (344 µatm), medium (821 µatm), and high (1243 µatm) levels of partial pressure of carbon dioxide (ρCO_2). Values with different superscript letters are significantly different (P<0.05). Standard errors of the mean are provided in parentheses. For all analyses, degrees of freedom equaled 6. The adult brood stock used to rear individuals for this study were collected in 2014 from waters of Connecticut in Long Island Sound. ND=not detected.

	Lipid concentration (µg/g) by treatment level of ρCO_2				
Lipid	Low	Medium	High	F ratio	P
Acetone mobile polar lipids	34.20 (18.44)	95.94 (18.44)	80.23 (18.44)	3.03	0.12
Alcohols	ND	ND	4.22 (2.43)	1.00	0.42
Diacylglycerols	ND	ND	ND	ND	ND
Ethyl esters	1.91 (1.10)	ND	ND	1.00	0.42
Ethyl ketones	15.07 (4.69)[a]	50.43 (4.69)[b]	37.63 (4.69)[a,b]	5.48	0.04
Free fatty acids	16.49 (4.90)	22.86 (4.90)	24.36 (4.98)	0.73	0.52
Glycerol ethers	ND	ND	ND	ND	ND
Hydrocarbons	3.42 (0.96)[a]	8.23 (1.27)[b]	5.43 (0.54)[a,b]	6.11	0.035
Methyl esters	ND	ND	ND	ND	ND
Methyl ketones	15.07 (8.16)	50.43 (8.16)	37.63 (8.16)	4.81	0.06
Phospholipids	117.43 (52.14)	219.68 (52.14)	199.07 (52.14)	1.08	0.40
Sterols	13.26 (5.04)	32.24 (5.04)	24.54 (5.04)	3.59	0.09
Steryl wsters/wax esters	ND	4.65 (2.68)	1.36 (2.68)	0.79	0.49
Triacylglycerols	105.82 (89.66)	342.17 (89.66)	220.12 (89.66)	1.74	0.25
Sterol–phospholipid ratio	0.14 (0.02)	0.15 (0.02)	0.13 (0.02)	0.77	<0.01
Total	316.12 (173.88)	806.35 (173.88)	613.01 (173.88)	2.02	0.21
	Percent lipid by treatment level of ρCO_2				
Lipid	Low	Medium	High	F ratio	P
Acetone mobile polar lipids	15.68 (2.85)	11.52 (2.85)	14.32 (2.85)	0.42	0.68
Alcohols	ND	ND	1.51 (0.87)	1.0	0.83
Diacylglycerols	ND	ND	ND	ND	ND
Ethyl esters	1.39 (0.80)	ND	ND	1.00	0.83
Ethyl ketones	3.59 (0.78)	3.96 (0.78)	2.46 (0.78)	1.12	0.39
Free fatty acids	7.04 (1.03)	2.82 (1.03)	4.37 (1.03)	4.52	0.06
Glycerol ethers	ND	ND	ND	ND	ND
Hydrocarbons	1.60 (0.46)	1.11 (0.46)	1.07 (0.46)	0.31	0.74
Methyl esters	ND	ND	ND	ND	ND
Methyl ketones	4.76 (0.55)	6.48 (0.55)	6.47 (0.55)	3.72	0.09
Phospholipids	35.35 (2.38)	28.11(2.38)	34.17 (2.38)	2.74	0.14
Sterols	4.81 (0.59)	4.08 (0.59)	4.42 (0.59)	0.37	0.71
Steryl esters/wax esters	ND	0.43 (0.26)	0.28 (0.26)	1.38	0.32
Triacylglycerols	22.79 (6.30)	41.50 (6.30)	30.95 (6.30)	1.57	0.28

scenarios (Miller et al., 2009; Talmage and Gobler, 2009; White et al., 2013; Waldbusser et al., 2015). In studies of other clam species, increasing ρCO_2 above current levels resulted in linear decreases in growth and shell size of softshell clam (*Mya arenaria*) (Green et al., 2009, 2013; Clements and Hunt, 2014), northern quahog (Talmage and Gobler, 2009; Gobler and Talmage, 2014), eastern oyster (Talmage and Gobler, 2009), and Pacific oyster (*C. gigas*) (Waldbusser and Salisbury, 2014; Waldbusser et al., 2015). However, no effects at ρCO_2 concentrations of the RCP 8.5 scenario have been reported for the Olympia oyster (*Ostrea lurida*) (Waldbusser et al., 2016), Antarctic bivalve (*Laternula elliptica*) (Bylenga et al., 2015), or blue mussel (Gazeau et al., 2013). A hormetic response to OA exposure in bivalves, although not often reported, was recently reported for juvenile Atlantic surfclam (Pousse et al. 2020) and has been noted in other marine species, including copepod (Li and Gao, 2012; Thor and Oliva, 2015) and finfish (Miller et al., 2013, 2016) species.

Other bivalve species may experience a hormetic response, but it may be masked by the ρCO_2 levels chosen for other experiments, choices that can result in only a decrease being observed. For example, in a repeat study conducted in 2014 with eastern oyster by using a finer exposure resolution than that in our study, a maximal hormetic response at a ρCO_2 of 380 µatm was found for growth and percentage of larvae that completed metamorphosis (Gobler and Talmage, 2014). Unlike larvae of estuarine bivalve species, the larval Atlantic surfclam in our experiment were able to tolerate moderate OA levels. Future research should include a finer exposure resolution to determine the width of hormetic response in larvae of Atlantic surfclam.

In previous studies conducted with estuarine bivalves, larvae of most species were more sensitive to increased ρCO_2 than juveniles or adults. For example, for eastern oyster exposed to conditions of the RCP 6.0 scenario, larvae had reduced rates of growth rates and survival and a lower percentage of larvae completed metamorphosis (Talmage and Gobler, 2009), and for juveniles no significant difference was observed in growth at the ρCO_2 level of the RCP 6.0 scenario (Dickinson et al., 2012) or levels greater than that of the RCP 8.5 scenario (1700 µatm; Young and Gobler, 2018). For adult eastern oyster in other studies, no effects on growth or gaping were observed at ρCO_2 levels as high as 8000 µatm (Clements et al., 2017, 2018). A similar trend has been observed for blue mussel with larvae being more sensitive than adults (Thomsen et al., 2017).

Even though the results of this study were surprising, with increased growth at ρCO_2 levels predicted for the RCP 6.0 scenario, growth decreased at levels predicted for the RCP 8.5 scenario. Results of an experiment with juveniles of this subspecies indicate a similar hormetic response to that of larvae in our study (Pousse et al., 2020). Pousse et al. (2020) found metabolic depression in juvenile Atlantic surfclam exposed to ρCO_2 levels of 1350 µatm, making them more susceptible than Pacific oyster (Lannig et al., 2010) and blue mussel (Thomsen and Melzner, 2010). The larval Atlantic surfclam in our study were not exposed to the higher levels of ρCO_2 used on juveniles in the Pousse et al. (2020) study, but it would be interesting to determine when metabolic depression occurred in larvae considering the similar trends observed between these 2 studies. We cannot explain why larval and juvenile Atlantic surfclam may behave similarly or why larval Atlantic surfclam appear to tolerate higher levels of ρCO_2 than larvae of some other estuarine species like the eastern oyster; however, a possible reason may relate to how Atlantic surfclam allocate energy for growth and development. Bioenergetic studies, including those that include dynamic energy budget modeling, would provide insight into whether the small changes in growth observed in our study and in the study of Pousse et al. (2020) would have a long-term effect over the time period required for Atlantic surfclam to reach harvest size and would help to clarify variation in responses between bivalve species.

Parsons (2001) suggests that a hormetic response may reflect evolutionary adaptation of metabolic systems to environmental variables and may link to Darwinian fitness; however, there is little quantitative evidence currently available to confirm this hypothesis. Recent research on the response of bivalve larvae to OA has focused on population-level responses and the potential for evolutionary adaptation. Increased growth of larval Atlantic surfclam at elevated ρCO_2 concentrations during our study may reflect adaptation of adults in the source population. The wild adults used in this study were exposed to conditions during the summer in LIS, where in situ sediment levels of ρCO_2 can periodically range from 689 to 1828 µatm (Perry et al., 2015; Meseck et al., 2018; Snyder et al., 2019). Researchers have found that survival of larvae varied significantly between populations of the Sydney rock oyster (*Saccostrea glomerata*) (Parker et al., 2011) and Chilean mussel (*M. chilensis*), on the basis of the history of adult exposure to OA (Duarte et al., 2015). These findings indicate potential physiological and metabolic adaptations of shellfish populations to OA conditions.

It was beyond the scope of this study to determine the ability of Atlantic surfclam to adapt and evolve to shifting environmental conditions; however, future evolutionary adaptation studies should include both subspecies of Atlantic surfclam. There is an evolutionary divergence of 13.9% between the northern and southern subspecies, indicating long-term reproductive isolation of these subspecies (Hare and Weinberg, 2005; Hare et al., 2010) that may have allowed each to develop differential tolerances to OA conditions. A comparison between specimens of the 2 subspecies from the same location, exposed to increasing ρCO_2, might resolve whether there is an evolutionary divergence in OA tolerance between the subspecies, and the results of such an investigation could support more refined management of populations of Atlantic surfclam in New England.

Slower growth rates and smaller shell height in larvae from the high- and low-ρCO_2 treatments indicate that suboptimal conditions for larval Atlantic surfclam may stimulate a shift in allocation of energetic resources

away from allometric growth toward maintenance of metabolic or physiological homeostasis. Ocean acidification in marine environments can cause invertebrates to slow metabolism and may result in reduced growth and smaller body size (Pörtner et al., 2005; Gobler and Talmage, 2014). The hormetic response we observed in growth and time to metamorphosis of Atlantic surfclam may result from energetic changes reflected in lipid metabolism. Although there was no significant difference between the lipid levels in specimens among treatments, there was a consistent trend in the concentrations of phospholipids, sterols, and triacylglycerols and in the ratio of sterols to phospholipids, with the same hormetic response as that observed for growth. The trend we observed in these lipid levels among treatments may indicate an adaptive response of membranes to different environmental conditions, including temperatures and pressures (Crockett, 1998; Pernet et al., 2006, 2007), a process known as homeoviscous adaptation (HVA).

Homeoviscous adaptation in other marine organisms has been reported previously as an adaptive response to OA (Turk et al., 2007; Bennett et al., 2018). Slight decreases in phospholipid, sterol, and triacylglycerol levels and in the sterol-to-phospholipid ratio at low and high ρCO_2 levels, like those observed in our study, may provide a metabolically less expensive and energy-conserving mechanism to reduce expenditure of adenosine triphosphate (Crockett, 1998) that facilitates use of different ion exchange pathways (Kusumi et al., 1986). Pousse et al. (2020) found an HVA response in respiration rates and food selection efficiency of juvenile Atlantic surfclam. Further research on how HVA pathways are used in larvae under OA conditions should be pursued with the additional measurements of respiration rate, feeding rate, and scope for growth to help determine if OA can facilitate different metabolic pathways.

Movement of populations of Atlantic surfclam northward and into deeper water can be attributed to warming temperatures (Weinberg, 2005; Munroe et al., 2016). Limited ρCO_2 data, combined with modeling results, indicate that decreases in pH and $\Omega_{aragonite}$ occur in the areas to which Atlantic surfclam are moving (Wang, 2016; Saba et al., 2019; Friedland et al., 2020; Siedlecki et al., 2021). This study focused on only the ρCO_2 levels predicted for the RCP 6.0 and RCP 8.5 scenarios; we did not look at the role of increasing temperature. Under the RCP 6.0 and RCP 8.5 scenarios, increases in temperature are expected to occur concurrently with increased ρCO_2 levels. Future research should include examination of the response of Atlantic surfclam to OA under different temperatures to determine if the combined effects of temperature and OA changes their tolerance to ρCO_2 concentrations.

Atlantic surfclam can live up to 35 years and become harvestable within 5–7 years, and this study focused on a short portion of their lifespan. More information about the ρCO_2 levels experienced by larval, juvenile, and adult Atlantic surfclam during their lifetime is needed to better define the range of ρCO_2 concentrations on which future studies should focus. Further research should emphasize the bioenergetic pathways governing larval response to OA and address adaption and evolution by including genetic analysis. Finally, incorporating increased ρCO_2 levels into future research would provide information beneficial to fisheries management efforts.

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