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Linking rising pCO_2 and temperature to the larval development and physiology of the American lobster (*Homarus americanus*)

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Few studies have evaluated the joint effects of elevated temperature and pCO_2 on marine organisms. In this study we investigated the interactive effects of Intergovernmental Panel on Climate Change predicted temperature and pCO_2 for the end of the 21st century on key aspects of larval development of the American lobster, *Homarus americanus*, an otherwise well-studied, iconic, and commercially prominent species in the northeastern United States and Atlantic Canada. Our experiments showed that larvae (stages I–III) and postlarvae (stage IV) reared in the high temperature treatments (19 °C) experienced significantly lower survival, developed twice as fast, and had significantly higher oxygen consumption rates, than those in ambient treatments (16 °C). Larvae from the ambient temperature/high pCO_2 (750 ppm) treatment had significantly longer carapace lengths, greater dry masses in stages I–III and higher C: N ratios in stage IV than larvae from all other treatments. Stage IVs raised in the high pCO_2 treatment at 19 °C had significantly higher feeding rates and swimming speeds than stage IVs from the other three treatments. Together these results suggest that projected end-century warming will have greater adverse effects than increased pCO_2 on larval survival, and changing pCO_2 may have a complex effect on larval metabolism and behaviour. Understanding how the most vulnerable life stages of the lobster life cycle respond to climate change is essential in connecting the northward geographic shifts projected by habitat quality models, and the underlying physiological and genetic mechanisms that drive their ecology.

Keywords: climate change, crustacean larvae, *Homarus americanus*, lobster, ocean acidification.

Introduction

The input of anthropogenic carbon into the atmosphere has caused the ocean to warm and acidify, but the joint effects of these changes remain unclear for the majority of marine species (Caldeira and Wickett, 2003, Browman, 2016). Changes in pH and temperature are not uniform across the marine environment. Higher latitudes are experiencing both sharper decreases in pH and more rapid increases in temperature than lower latitudes (Fabry *et al.*, 2009). The Northwest Atlantic and the Gulf of Maine in particular is warming rapidly with its warmest year on record occurring in 2012 (Mills *et al.*, 2013). A relatively small number of ocean acidification (OA) studies have been conducted on species in the Northwest Atlantic and even fewer have included relevant co-stressors such as increasing temperature.

Although temperature is a well-studied determinant of growth, development and survival in crustaceans (Ross *et al.*, 1988; Anger, 2001; Weiss *et al.*, 2009; Swingle *et al.*, 2013), studies of responses to an elevated partial pressure of carbon dioxide (pCO_2) have only emerged in recent years. It has become clear that these responses are species and life-stage specific (reviewed by Whitley, 2011; Gledhill *et al.*, 2015). For example, Tanner crab (*Chionoecetes bairdi*) juveniles and Dungeness crab (*Cancer magister*) larvae experience decreased rates of survival in high pCO_2 environments, while other crustacean species show no change (Arnberg *et al.*, 2013; Byrne and Przeslawski, 2013; Long *et al.*, 2013; Schiffer *et al.*, 2013; Miller *et al.*, 2016).

This study focuses on the response of the larval American lobster (*Homarus americanus*) to elevated temperature and pCO_2. To date, only two studies have evaluated the impacts of increasing pCO_2 on the American lobster and only one on the larval stages (Ries *et al.*, 2009; Keppel *et al.*, 2012). The single larval investigation found that elevated pCO_2 led to smaller carapace lengths and slowed development in planktonic larvae (Keppel *et al.*, 2012). Recent studies of European lobster (*Homarus gammarus*) larvae have produced mixed results. In one study elevated pCO_2 resulted in carapace deformities (Agnalt *et al.*, 2013), whereas another reported no significant effect on carapace length or wet body mass (Small *et al.*, 2015). Only exposures to extremely high pCO_2 (9000 ppm) have produced adverse effects in benthic juvenile *H. gammarus* (Small *et al.*, 2016).

Although the effects of temperature on larval growth and development of the American lobster are well studied (MacKenzie, 1988; Fitzgibbon and Battaglene, 2012), no studies have examined the potential interactive effects of elevated pCO_2 and temperature on any life stage. This study was designed to fill a knowledge gap in our understanding of how predicted increases in pCO_2 and temperature will affect the survival, development and physiology of the larval stages of *H. americanus*. In addition to being culturally and economically important, the American lobster also has a rich history as a model organism in crustacean physiology and ecology (Herrick, 1911; Factor, 1995; Wahle *et al.*, 2013). This research legacy provides valuable background on basic biology, physiology and most recently genetic controls unavailable for most marine taxa, making it an ideal model organism to investigate impacts of various pCO_2 and warming scenarios on crustacean development and physiology.

The goal of this study is to examine the response of *H. americanus* larval stages to end-century pCO_2 and temperature. We raised newly hatched lobster larvae under conditions representing ambient and projected end-century temperatures and pCO_2s for the Gulf of Maine under a moderate CO_2 emissions scenario modeled by the Intergovernmental Panel on Climate Change (IPCC, 2013). We compare our results to the limited number of existing studies on the development of *H. americanus* and *H. gammarus* larvae and those of other crustaceans under elevated temperature and pCO_2. These measurements of larval and stage IV growth, development, survival, and oxygen consumption, as well as stage IV swimming speed and feeding rate provide valuable insight into the future of larval development under changing oceanic conditions.

Material and methods

Larval collection and hatching

Ten ovigerous females were collected from midcoast Maine by local lobstermen and the Maine Department of Marine Resources. Females were kept in the seawater lab at the University of Maine's Darling Marine Center, Walpole, Maine. The hatch tank (2 m diametre, 0.75 m deep) received a continuous supply of ambient coarsely filtered sea water and was fitted with 1 mm mesh screen to prevent larvae from being drawn into the overflow at a central standpipe. We housed four to five egg-bearing females, with claws banded, in this system before larvae were collected. We inspected the tank every 6 h until all females had hatched all eggs. Larvae were collected and transported the short distance to Bigelow Laboratory. To be clear with our terminology, larvae of *Homarus* progress through three instars, referred to as stages I–III, after which they metamorphose to the postlarval form, commonly referred to as stage IV (Herrick, 1895).

Ocean acidification facilities and carbonate chemistry analysis

Experiments were conducted at Bigelow Laboratory for Ocean Sciences, East Boothbay, Maine using 24 38 L conical tanks with a mesh-lined bottom. We stocked each tank with 250 larvae, an initial density of 6.6 larvae/l. Larvae hatched within the same 6-h time period, from at least three ovigerous females, were placed in the same experimental tank and acclimated to each treatment for a period of 6–8 h. Within each pCO_2/°C treatment, three tanks were assigned to assess survival and three to assess development time, carapace length, dry mass, carbon and nitrogen mass, oxygen consumption rates (OCRs), swimming speed, and feeding rate. The larvae in the tanks dedicated to survival measurements were not handled daily and were not used for any other experimental parameters. Larvae were fed daily with live *Artemia salina* nauplii to maintain a concentration of 5 *A. salina* nauplii/ml.

The control temperature of 16 °C reflects the average summer temperature in midcoast Maine, and 19 °C was selected to represent the increase predicted by a moderate IPCC warming scenario for the year 2100 (IPCC, 2013). All tanks were kept in a temperature controlled room at 16 °C while half of the tanks were heated to 19 °C with Hydor submersible glass aquarium heaters. Tanks were cleaned every 48 h and filled with 0.2 µm filtered seawater pre-equilibrated to each pCO_2 and temperature treatment. Tanks were bubbled with compressed air at pCO_2s of 380 and 750 ppm generated at Bigelow. Compressed air was stripped of CO_2 (>10 ppm) with a Puregas VCD CO_2 Adsorber. By using this method, the daily fluctuations in the input airstream did not impact set pCO_2 values in each tank. Gases were mixed using Aarlburg mass flow controllers with "blocks" for adjusting CO_2 (GFC no. 37; 0–10 ml min^{-1}) and air (GFC no. 17; range 0–5 l min^{-1}). The pCO_2 of each mixture were measured periodically using Licor model 820 CO_2 sensors with an accuracy of ±1 ppm prior to delivery to each individual tank. Temperature, pH and salinity were measured daily to ensure that the chemistry within the tank remained stable. Daily pH measurements were made with a pH electrode (Thermo Orion 3-star, temperature compensated system) calibrated with pH NIST buffer standards (Mettler-Toledo, Leicester, UK). Salinity was measured with an Oakton SALT handheld metre. To validate and add precision to daily pH measurements, pH was also measured twice a week using spectrophotometry (Hitachi U3010 spectrophotometer) and the pH sensitive indicator dye *m*-Cresol purple sodium salt (Sigma-Aldrich) following SOP (standard operating procedure 6b: Dickson *et al.*, 2007). Samples of seawater were collected with polypropylene syringes and 40 ml of water was filtered through 0.2 µm syringe filters. Samples were analysed immediately after collection. For each pH measurement, 10 ml of each sample was slowly pipetted into two quartz cuvettes with a 1 cm path length. The cuvettes were held at 16 or 19 °C in the temperature controlled chamber of the spectrophotometer. 20 mM *m*-Cresol purple (10 µl) was added to the sample cuvette, while the second cuvette served as a reference. Absorbance was measured at 578 nm (A1), and 434 nm (A2) and 730 nm (background). We used equations in SOP 6b (section 8.3) to correct A1/A2 for the addition of the *m*-Cresol purple dye. The final pH value (total scale, pH_T) was determined from Liu *et al.* (2011).

To measure total alkalinity (A_T), seawater samples (40 ml) were taken twice a week and fixed with saturated $HgCl_2$ to eliminate all biological activity (Riebesell *et al.*, 2010). Samples were kept in dark and cool conditions until the sample was titrated with 0.01 N HCl using a Metrohm 888 Titrando controlled by Tiamo software to perform automated Gran titrations. Certified reference material (Andrew Dickson—Scripps Institution of Oceanography, San Diego, USA) was used to calibrate A_T measurements in our system. Carbonate chemistry parameters (pCO$_2$,[HCO$_3^-$], [CO$_3^{2-}$], Ω_{Ar}, Ω_{Ca}) within the tanks were calculated using CO2SYS2.1 (Lewis *et al.*, 1998) with the standard set of carbonate system equations using constants from Mehrbach (1973) and refit by Dickson and Millero (1987), $KHSO_4$ from Dickson (1990) and $[B]_T$ from Uppstrom (1974) . Data input into CO2SYS2.1 included temperature, salinity, pH_T and A_T. All chemistry results for each pCO$_2$/°C treatment during the experiment are reported in Table 1.

Measurements of survival, development, carapace length, and mass

Survival assessments were conducted daily and the number of larvae in each tank was recorded when larvae reached stage IV. Each day a 1 l sub-sample was removed from each tank. The number of larvae alive was counted via visual inspection to minimize handling of larvae. Dead larvae were counted and removed. Any observations of cannibalism (partially eaten larvae) were also noted. The percent of larvae alive each day was calculated by dividing the estimated number of larvae alive in each tank by the number of larvae used to stock the tank.

Carapace length from 10 haphazardly selected larvae was measured daily from each experimental tank within a treatment. Every larva was examined under a dissecting microscope to assess developmental stage based on stage-specific features (Herrick, 1895). The first appearance of each developmental stage in each treatment was recorded. The mean development time for each treatment was calculated from the three replicate tanks. Each larva was photographed using an Olympus SZ61 dissecting scope fitted with a 2X magnifier and a Canon T3i digital camera. The photographs were analysed for carapace length using NIH-ImageJ (NIH, USA). Carapace length was measured as the distance from the back of the eye socket to the end of the carapace. Larvae were returned alive to the appropriate tank after measurement.

Every 3 days, starting with eggs on the day of hatching, a minimum of three larvae from each experimental replicate tank were removed to measure dry weight as well as carbon and nitrogen mass. Eggs and individual larvae were rinsed three times in deionized water and placed into pre-weighed tin boats. These boats were placed in a 40 °C oven for a minimum of 48 h before being weighed. The samples were analysed by Bigelow Laboratory Analytical Services and combusted with a Costech ECS (Elemental Combustion System) 4010. All masses were an order of magnitude above the instrumental threshold of sensitivity and corrected using blank samples containing only the deionized rinse water.

Measurement of oxygen consumption and feeding rate

OCRs were measured at each stage. Larvae of the same developmental stage were individually selected from experimental tanks and placed into a 50 ml container with water from the appropriate treatment. The container was sealed with a ground glass stopper with a small (400 μm) hole in the top to accommodate the microelectrode. The number of larvae in the container ranged from 15 for stages I and II, 10 for stage III and 1–2 for stage IV. Measurements were made at 16 °C or 19 °C (±0.01 °C) using a Forma Scientific CH/P water bath (model 2006). Oxygen concentration was measured over the course of an hour and never decreased more than 20% below saturation. Control OCR replicates containing only water from each treatment provided background OCRs that included microbial respiration. A control OCR replicate was run immediately after each experimental OCR replicate. A minimum of three experimental OCR replicates and three OCR controls were measured at each stage from each experimental tank within a treatment. OCRs were calculated from the slope of the decrease in oxygen concentration over time, after accounting for oxygen consumption in the control replicates. Mass-specific OCRs were calculated using the dry mass of the larvae used. Dissolved oxygen was measured with a Clark-type oxygen microelectrode (Unisense; Aarhus, Denmark). Electrodes were calibrated prior to all measurements with 0.2 μm filtered seawater bubbled for a minimum of 1 hour to set the probe to the 100% dissolved oxygen measurement. The anoxic measurement was set by placing seawater into a silicone tube that was immersed in a solution of 0.1 M sodium ascorbate and 0.1 M sodium hydroxide for 4 h or more. OCR trials were conducted for only one hour to maintain the chemistry of each pCO$_2$/°C treatment. This trial duration aligns with other studies that measured OCRs of planktonic crustaceans in response to acute toxicity exposures (Capuzzo, 1977; Fields *et al.*, 2015).

Feeding rate was measured in stage IVs from all treatments 48 h after molting. The length of the experiment was optimized to allow enough time for the stage IVs to ingest a measurable number of prey (>5%), but not drastically reduce the available food supply (no >30% of the initial concentration). A single stage IV was selected from each experimental replicate tank and placed into a 1 l polypropylene container with seawater pre-equilibrated to the appropriate treatment. Using a dissecting microscope, 100 live *A. salina* (48-h old) nauplii were counted and placed into the polypropylene container. Each container with the stage IV and *A. salina* was sealed and kept in a dark incubator during the 6 h feeding period. At the end of this period, we removed the stage IV from the container and counted the remaining *A. salina*. A total of 10 feeding measurements, using ten different stage IVs (3–4 individuals from each experimental replicate tank), were made for each treatment.

Table 1. Water chemistry results over the course of this experiment

Treatment combination	p CO$_2$ input (ppm)	Temperature (°C)	Salinity (ppt)	$pH_{(T)}$	A_T (μmolkg^{-1})	HCO_3^- (μmolkg^{-1})	CO_3^{2-} (μmolkg^{-1})	ΩCa	ΩAr	Calculated p CO$_2$ (ppm)
Ambient CO$_2$–16 °C	380	16.2 ± 0.2	31.5 ± 0.3	8.066 ± 0.01	2042 ± 28	1699 ± 31	137 ± 2.2	3.35± 0.1	2.14 ± 0.03	360 ± 17
High CO$_2$–16 °C	750	16.1 ± 0.2	31.5 ± 0.2	7.886 ± 0.05	2131 ± 29	1873 ± 33	103 ± 11	2.53 ± 0.3	1.62 ± 0.17	593 ± 74
Ambient CO$_2$–19 °C	380	19.1 ± 0.1	31.5 ± 0.1	8.091 ± 0.10	2090 ± 16	1673 ± 90	168 ± 34	4.12 ± 0.8	2.65 ± 0.34	347 ± 94
High CO$_2$–19 °C	750	19.2 ±0.1	31.5 ± 0.1	7.836 ± 0.08	2100 ± 18	1845 ± 47	102 ±17	2.61 ±0.5	1.67 ± 0.29	647 ± 50

Data are means with SD for all parameters throughout the experimental period.

Measurement of swimming speed

Swimming speed was analysed in stage IVs (all 48-h post-molt) from the two pCO_2 treatments only at 19 °C. Five stage IVs from each °C/pCO_2 treatment, at least one from each experimental replicate tank, were analysed for the measurement of swimming speed. Swimming speed measurements were also conducted with stage IVs at 16 °C, but there was an insufficient number of swimming events to analyse. Although over 5 h of video was recorded on stage IV individuals from the 16 °C treatments, only one swimming event was recorded. Video observations used to calculate stage IV swimming speed were made through a 2-l Plexiglas vessel. The filming was done using shadow videography with an overall magnification of 5–10×. The video cameras consisted of two perpendicularly mounted Point grey HD highspeed (60 Hz) cameras with 105-mm Nikon lenses. Through this configuration, the X Z coordinates and the Y Z views of the larvae were recorded. Filming simultaneously from two perpendicularly mounted cameras provided 3D coordinates of the stage IV within the field of view. The 2-l filming volume (10 × 10 × 20 cm: length × width × height) was illuminated using two 632-nm LED diodes each expanded to a 150 mm diameter beam using an Oriel 150mm lens (Newport, Stratford, CT, USA). Images were recorded on a single Dell computer to provide synchronized images from both views. Individual video frames (0.016 s) were analysed using NIH-ImageJ. The coordinates for each video frame was referenced by the starting location of the stage IV's rostrum. The base of the rostrum was denoted (X_o,Y_o,Z_o) and swimming distance (D) was measured from the starting point of the stage IV's rostrum to the final location of the rostrum (X_f, Y_f, Z_f). Distance was calculated through the following equation:

$$D = \sqrt{(X_f - X_0)^2 + (Y_f - Y_0)^2 + (Z_f - Z_0)^2}$$

Data analysis

We used two- and three-way analysis of variance (ANOVA) to investigate the joint effects of developmental stage, temperature and pCO_2 on average development time, carapace length, carbon and nitrogen mass, dry mass, OCRs, mass-specific OCRs at all four stages, and feeding rate in stage IVs. Separate experimental replicate tanks represented the initial unit of treatment replication. For each of these experimental measurements we found that the difference between replicate tanks within a treatment was not significant ($p > 0.05$). Therefore, the factor replicate tank was excluded from our analysis (Feder and Collins, 1982). Levene's mean test was used to test for equal variance, and a Shapiro-Wilk test was used to test for a normal distribution. The Holm-Sidak method was used to conduct *post-hoc* analyses. When a data set was non-normal, a log transformation was applied. To account for a skewed distribution, all development time, dry mass, survival and OCR data were log transformed prior to analysis. Carapace length data were transformed using a Box-Cox statistical transformation. Survival was analysed with a repeated measures ANOVA to reflect repeated observations. Swimming speeds were analysed with a one-way repeated measures ANOVA since measurements were made from a single larva over the course of an hour. All statistical analyses were performed in SigmaPlot 11.0 or 'R' statistical and programming environment (R Development Core Team, 2009).

Results

Survival

Larvae raised at 19 °C experienced significantly lower survival than those at 16 °C (ANOVA, $F = 4.86$, $p < 0.05$, Supplementary Table S1), but we observed no significant effect of elevated pCO_2 (ANOVA, $F_1 = 0.28$, $p > 0.05$, Figure 1, Supplementary Table S1). Half-way through the total time of the trial, larvae in the 19 °C treatments were in stage IV and only 2.6% (± 0.9 SD) of the initial number of larvae were alive whereas larvae in the 16 °C treatments were in stage II and an average of 19% (±2.8 SD) were still alive. A primary source of mortality was cannibalism. We observed, but did not directly quantify, large decreases in survival when the first larvae at stage III began to cannibalize earlier stages (day 7 at 19 °C, day 10 at 16 °C, Figure 1). The larvae from the high pCO_2 treatment at16 °C had the highest average survival to stage IV (1.6%), and the larvae from the high pCO_2 treatment at 19 °C had the lowest average survival to that stage (0.79%).

Development time

Development time from hatching to stage IV ranged from 10 to 32 d. The mean development time was calculated from each replicate tank and then by treatment. Larvae raised at 19 °C developed more than twice as fast as those at 16 °C (Figure 2). However, within these temperature treatments we observed no significant difference in development time between the two pCO_2 treatments (ANOVA, $F = 0.03$, $p > 0.05$, Supplementary Table S1). Larvae developed significantly faster at 19 °C regardless of the pCO_2 (ANOVA, $F = 221.25$, $p < 0.01$, Supplementary Table S1).

Carapace length, dry mass, and C: N

Stage I lengths ranged from 2.3 to 2.4 mm and stage IV carapace lengths ranged from 4.4 to 4.8 mm in all treatments (Figure 3). Stages I–III larvae from the high pCO_2 treatment raised at 16 °C had significantly longer carapace lengths (ANOVA, $F = 2.71$, $p < 0.05$, Supplementary Table S2). No other main effects or interactions were significant.

H. americanus larvae all exhibited exponential increases in dry mass and C:N ratio from stage I to IV (Figures 4 and 5).

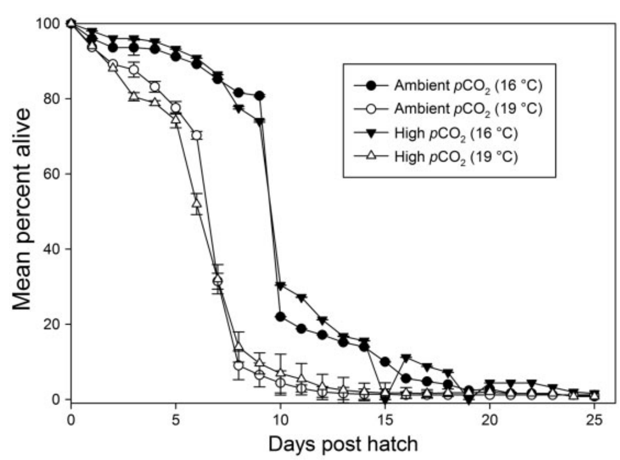

Figure 1. *H. americanus* larval survival (% alive) in each treatment each day after hatching (±SD).

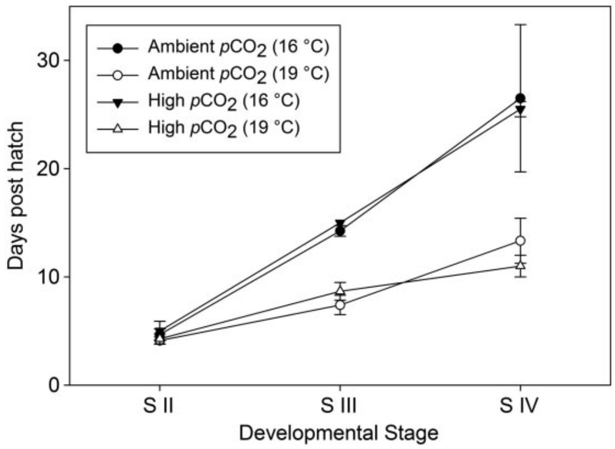

Figure 2. Day to first appearance of each *H. americanus* developmental stage (means ± SD). SII, stage II; SIII, stage III; SIV, stage IV.

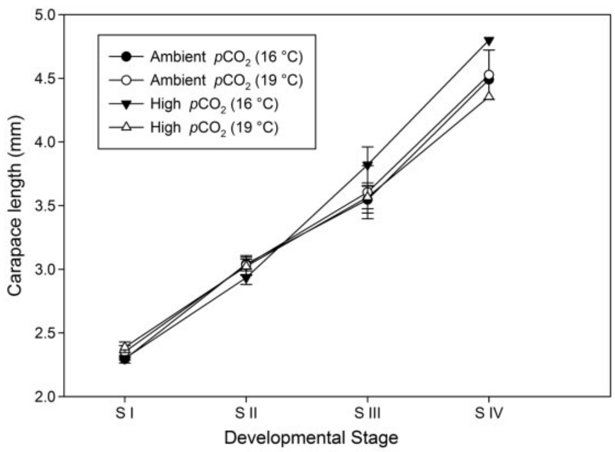

Figure 3. Effect of temperature and pCO_2 on larval *H. americanus* carapace length (mm, mean ± SD) at each developmental stage.

Measurements of these parameters could only be collected from stage IVs in the 16 °C treatments due to low survival in the 19 °C treatments. Larvae from the high pCO_2 treatment raised at 16 °C had significantly heavier stages I–III larvae compared with their counterparts in any other treatment (ANOVA, $F = 4.29$, $p = 0.05$, Supplementary Table S2). Our analysis of stages I–IV from the 16 °C treatments showed only the expected significant effect of stage on body mass (ANOVA, $F = 593.75$, $p < 0.01$, Supplementary Table S2), as well as a marginally significant positive effect of elevated pCO_2 (ANOVA, $F = 3.91$, $p = 0.06$, Supplementary Table S2).

Average C: N values were highest in eggs (5.35 ± 0.33 SD) and stage IVs from the high pCO_2 treatment raised at 16 °C (5.0 ± 0.04 SD). Larvae in stages I–III in all treatments showed a narrow range of C: N values (3.9–4.3) although C: N values were significantly higher in stage II and III larvae from the ambient pCO_2 treatments (ANOVA, $F = 5.90$, $p < 0.05$, Figure 5, Supplementary Table S2). At 16 °C elevated pCO_2 treatment had a positive effect on C: N of stage IVs (Mann-Whitney Ranks Sum, $T = 10$, $U = 0.0$, $p = 0.029$).

Oxygen consumption rates

Whole body OCRs increased from stage I to IV across all treatments presumably because of the increase in body mass (Figure 6a). We found a statistically significant positive temperature effect (ANOVA, $F = 12.97$, $p < 0.01$, Supplementary Table S3), but no significant pCO_2 effect (ANOVA, $F = 1.35$, $p > 0.05$, Supplementary Table S3). Stage III larvae at 19 °C had OCRs three times as high as larvae raised at 16 °C. Stage IV OCRs from the 19 °C treatments were about 25% higher than stage IV OCRs from the 16 °C treatments.

Mass-specific OCRs, however, remained relatively constant for larval stages I–III, but dropped dramatically by about 50% after the metamorphosis to stage IV (Figure 6b). We found no statistically significant effect of temperature or pCO_2 treatments at any stage, however ($p > 0.05$; Figure 6b, Supplementary Table S3).

Swimming speed and feeding rate

The swimming speed of stage IVs raised at 19 °C in the high pCO_2 treatment was about 40% higher than for those raised at ambient pCO_2 at the same temperature (ANOVA, $F = 24.48$, $p < 0.01$; Figure 7, Supplementary Table S4). We also found a significant positive effect of elevated pCO_2 on stage IV feeding rate at 19 °C but not at 16 °C (ANOVA, $F = 13.87$, $p < 0.01$; Figure 8, Supplementary Table S4). Stage IVs raised in the high pCO_2 treatment at 19 °C fed at rates some 40% higher than in the other three treatments.

Discussion

Our results infer that projected end-century warming may be more consequential than end-century pCO_2 for larval development in the American lobster. Relative to the effects of elevated pCO_2, increased temperature has a more marked impact on many aspects of *H. americanus* larval development. Our results indicate that the higher temperature that lobster larvae may experience in the year 2100 caused accelerated development and increased OCRs, but reduced survival between stages I and IV. Elevating pCO_2 from ambient to ~750 ppm had no direct impact on these critical aspects of larval survival. Nonetheless, we found a significant interactive effect of pCO_2 and temperature in the dry mass, carapace length, stage IV swimming speed and feeding rate. It is important to note that, as with most other studies, we report the results of acute, short-term experimental treatments that may not reflect the response of a species to environmental change over many generations. Still, these results offer unique insight into how the interactive effects of warming and rising pCO_2 may alter short-term larval development.

Survival

We observed no adverse effect of elevated pCO_2 on larval survival in either temperature treatment. Survival in many larval crustaceans has not consistently proven to be sensitive to elevated pCO_2 at ambient and experimental temperatures: European lobster, *H. gammarus* (Small *et al.*, 2015), Northern shrimp, *Pandalus borealis* (Bechmann *et al.*, 2011), spider crab, *Hyas araneus* (Schiffer *et al.*, 2013), and tanner crab, *C. bairdi* (Long *et al.*, 2016). Larval survival in our study was strongly temperature dependent. In contrast, a previous comparison of American lobster larval development in which larvae were reared individually reported no consistent relationship between temperature and survival over temperatures ranging from 9.8 to 22 °C (MacKenzie, 1988). Similarly, survival of

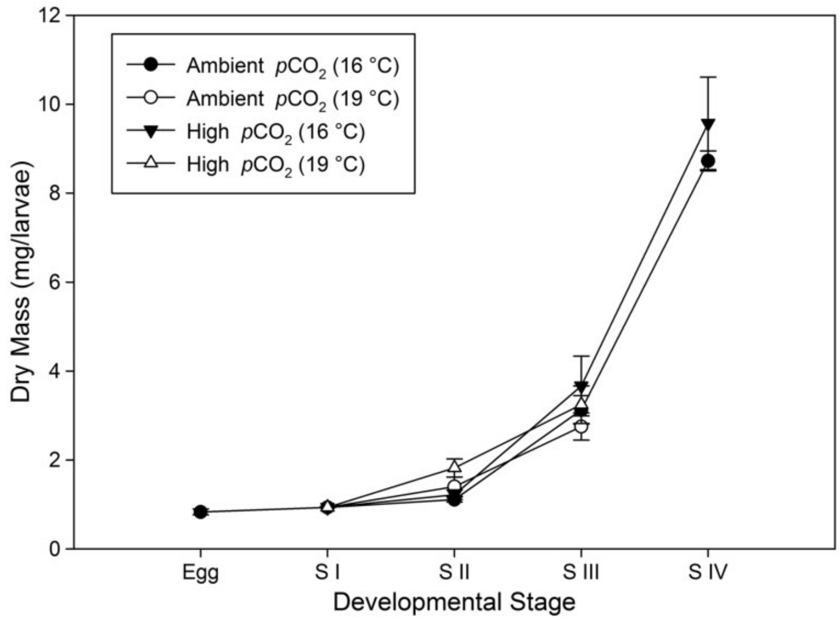

Figure 4. Dry mass of larval *H. americanus* (means ± SD) raised in the four treatments at each developmental stage.

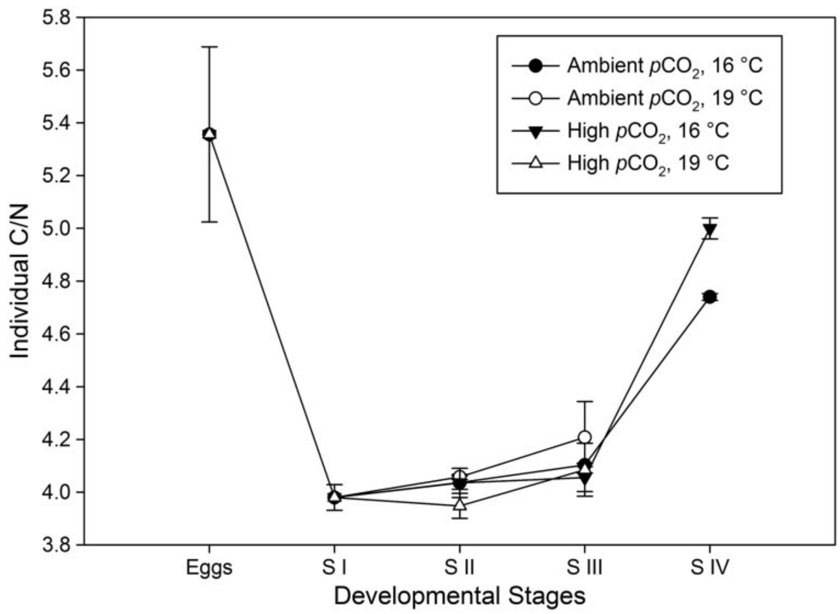

Figure 5. Carbon: nitrogen ratio of *H. americanus* (means ± SD) raised in the four treatments at each larval stage.

H. gammarus and velvet crab (*Necora puber*) larvae were unaffected by temperatures below 24 °C under saturated food conditions (Jackson *et al.*, 2014). The difference between those results and the results seen in this study may be related to the culturing method.

Our relatively low survival rates are comparable to previously published communal larval rearing experiments of *Homarus* larvae (Beal and Chapman, 2001; Small *et al.*, 2015). We attribute the low survival we observed from stage III onward to cannibalism. We observed that heightened larval mortality occurred when more advanced larval stages began to cannibalize earlier stage larvae. Cannibalism is likely an artifact of the high densities. Natural larval densities are generally several orders of magnitude lower in the wild, which would greatly diminish the impact of cannibalism in natural populations (Incze *et al.*, 2010). We therefore infer that the pCO_2 expected by the end of the century may not have as great an impact as end-century warming. We fully recognize that in nature larvae likely encounter widely varying temperatures due to small scale differences in seasonal warming and stratification and undertake movements to optimize their exposure to favourable temperatures (Boudreau *et al.*, 1992; Annis, 2005).

Developmental rates

The rate of larval development showed significant temperature effects but no direct or indirect effect of pCO_2. These results are

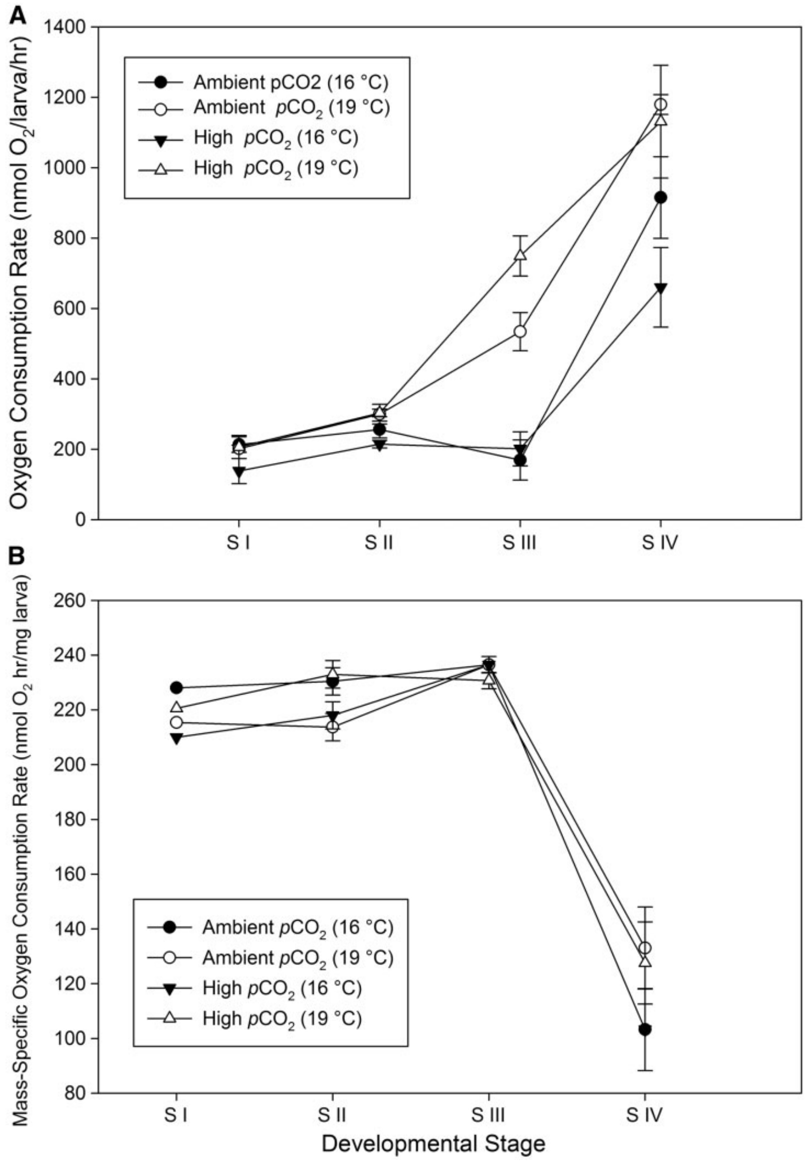

Figure 6. Whole body **(a)** and mass-specific **(b)** OCRs (means ± SD) of *H. americanus* larvae raised in each treatment at each developmental stage.

consistent with the previous findings of Arnold *et al.* (2009) and Small *et al.* (2015) who found no significant impact of pCO_2 on larval development time in *H. gammarus* larvae, but run contrary to those of Keppel *et al.* (2012) for *H. americanus*. The high temperatures (~20 °C) used by Keppel *et al.* (2012) may contribute to this disparity since temperatures of 20–21 °C are deleterious to American lobster larvae (MacKenzie 1988). Previous research has demonstrated that exposure to elevated pCO_2 can lower the thermal tolerance of the edible crab, *Cancer pagurus*, and the spider crab, *H. araneus* (Metzger *et al.*, 2007; Walther *et al.*, 2009; Whiteley, 2011). It may be that at high pCO_2, temperatures of 20-21 °C have a stronger adverse effect on *H. americanus* larval development.

OCR and behaviour

OCR is a key measure of metabolic rate. Larvae raised in the high temperature treatments had significantly higher OCRs than larvae at the low temperature. No difference was found in OCRs as a function of the pCO_2 treatment. These results are consistent with experiments performed with nauplii of the Arctic copepod, *Calanus glacialis* (Bailey *et al.*, 2016) and Northern shrimp larvae, *P. borealis* (Arnberg *et al.*, 2013). Although OCR increased as a function of developmental stage, when adjusted for dry mass, mass-specific OCRs declined as the animals grew larger. There were no significant differences between treatments or stages I–III. When the lobsters molted to stage IV, mass-specific OCRs decreased >50%, signaling greater metabolic efficiency.

The higher OCRs at warmer temperature are likely the result of increased metabolic costs associated with faster growth rates. At 16 °C, larvae increase their dry mass at a rate of 0.24 mg/d. At 19 °C, the growth rate increases by 54% to 0.37 mg/d. To fuel higher growth rates larvae must increase their feeding rates which in turn, require higher swimming speeds. The metabolic costs of increased activity are hard to measure directly. However, some

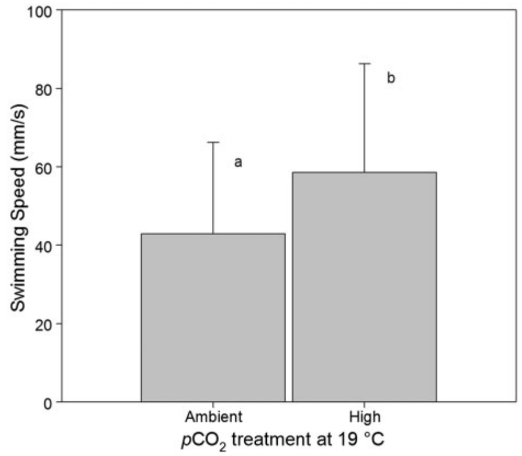

Figure 7. Swimming speed of *H. americanus* stage IVs raised under two pCO_2s at 19 °C (means ± SD). Significant differences are denoted by letters.

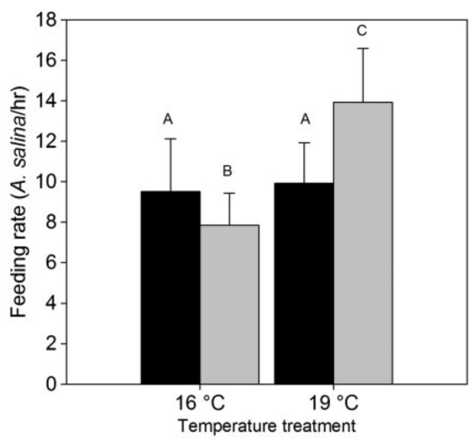

Figure 8. Feeding rate of *H. americanus* stage IVs raised in each of the four treatments (means ± SD). Significant differences are denoted by letters. Black bars represent ambient pCO_2 treatments (input of 380 ppm) and grey bars represent high pCO_2 treatments (input of 750 ppm).

model results suggest that the energetic cost of swimming in other planktonic crustaceans, such as copepods, is relatively expensive (Morris *et al.*, 1985). Furthermore, the act of feeding itself causes a rise in metabolic rate stemming from the activities involved in the ingestion, digestion, absorption, and assimilation of the prey. Thus the increases in metabolic costs associated with temperature are complex and typically nonlinear.

Direct and interactive effects

We saw significant interactive effects in our measurements of larval length, dry mass, stage IV C:N and stage IV feeding and swimming. Increased pCO_2, at ambient temperature, produced larger and heavier larvae while having no direct effect on the rates of development or OCR. This increase in length and mass may be attributed to elevated rates of calcification, a result seen in juvenile American lobsters under high pCO_2 (Ries *et al.*, 2009; Ries, 2011). Interestingly, stage IVs at 19 °C had swimming speeds and feeding rates that were nearly 50% higher in the high pCO_2 treatment, despite showing no concomitant increase in OCR. This may be partially explained by the smaller swimming volume used in the OCR measurements.

A meta-analysis of climate change studies showed that environmental stressors can have significant interactive effects that often go undetected in single-stressor experiments (Wernberg *et al.*, 2012). Our suite of results showed that larvae from the high pCO_2 treatment at 16 °C were longer, heavier, developed slower, consumed less oxygen and ate less than larvae raised in the high pCO_2 and 19 °C treatment. Some results differ from previous studies on European lobster larvae and juveniles, highlighting the importance of multi-stressor studies and understanding stage-specific, species-specific responses to warming and OA (Arnold *et al.*, 2009; Whiteley, 2011; Agnalt *et al.*, 2013; Small *et al.*, 2016). Energy and mineral budgets were not monitored in this study, so how rising pCO_2 and warming may impact the overall composition, distribution and longevity of *H. americanus* specifically is still not known. Notwithstanding the possibility of long term, multigenerational adaptation, the effects of increasing pCO_2 and temperature could have wide-ranging ecological implications including changes in local fitness, shifting biogeographical ranges and community composition (Somero, 2002; Pörtner and Farrell, 2008; Pörtner and Peck, 2010).

Future study and implications

There is growing evidence that ocean warming and increased pCO_2 could have complex effects on the development and survival of *Homarus* larvae and juveniles (this study, Arnold *et al.*, 2009; Keppel *et al.*, 2012; Agnalt *et al.*, 2013; Small *et al.*, 2015, 2016). Increased research on the interactive effects of climate change on physiology, exoskeleton minerology and gene expression are still needed. Analysis of gene expression in particular could reveal homeostatic mechanisms that make it possible for larvae to combat the adverse effects of increased pCO_2. In addition, most experiments investigating effects of environmental stressors on growth and development of larval stages are conducted at non-limiting food concentrations. More natural food concentrations may prevent animals from obtaining sufficient energy to cover the added energetic costs of overcoming physiological stress (Whiteley, 2011). As oceans around the globe continue to warm and acidify, it is crucial to understand how these factors will impact the development and survival of marine organisms.

Supplementary data

Supplementary material is available at the *ICESJMS* online version of the manuscript.

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