The University of Maine
DigitalCommons@UMaine

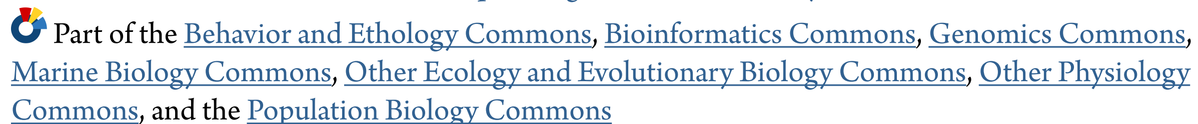
Electronic Theses and Dissertations | Fogler Library

Summer 8-2019

Response of Early Life Stage Homarus americanus to Ocean Warming and Acidification: an Interpopulation Comparison

Maura K. Niemisto
University of Maine, maura.niemisto@maine.edu

Follow this and additional works at: https://digitalcommons.library.umaine.edu/etd


Part of the Behavior and Ethology Commons, Bioinformatics Commons, Genomics Commons, Marine Biology Commons, Other Ecology and Evolutionary Biology Commons, Other Physiology Commons, and the Population Biology Commons

Recommended Citation
Niemisto, Maura K., "Response of Early Life Stage Homarus americanus to Ocean Warming and Acidification: an Interpopulation Comparison" (2019). *Electronic Theses and Dissertations*. 3076.
https://digitalcommons.library.umaine.edu/etd/3076

This Open-Access Thesis is brought to you for free and open access by DigitalCommons@UMaine. It has been accepted for inclusion in Electronic Theses and Dissertations by an authorized administrator of DigitalCommons@UMaine. For more information, please contact um.library.technical.services@maine.edu.

RESPONSE OF EARLY LIFE STAGE *HOMARUS AMERICANUS* TO OCEAN WARMING AND ACIDIFICATION: AN INTERPOPULATION COMPARISON

By:

Maura Kathleen Niemisto

B.S. University of Michigan, 2013

A THESIS

Submitted in Partial Fulfillment of the

Requirements of Degree of

Master of Science

(in Marine Biology)

The Graduate School

University of Maine

May 2019

Advisory Committee:

Richard Wahle, Research Professor, University of Maine, Co-advisor

David Fields, Senior Research Scientist, Bigelow Laboratory for Ocean Sciences, Co-advisor

Spencer Greenwood, Professor in Biomedical Science, University of Prince Edward Island

Paul Rawson, Associate Professor of Marine Biology, University of Maine

RESPONSE OF EARLY LIFE STAGE *HOMARUS AMERICANUS* TO OCEAN WARMING AND ACIDIFICATION: AN INTERPOPULATION COMPARISON

By: Maura Kathleen Niemisto

Thesis Co-Advisors: Dr. Richard Wahle & Dr. David Fields

An Abstract of the Thesis Presented
in Partial Fulfillment of the Requirements for the
Degree of Master of Science
(in Marine Biology)
May 2019

Anthropogenic carbon released into the atmosphere is driving rapid, concurrent increases in temperature and acidity across the world's oceans, most prominently in northern latitudes. The geographic range of the iconic American lobster (*Homarus americanus*) spans a steep thermal gradient and one of the most rapidly warming oceanic environments. Understanding the interactive effects of ocean warming and acidification on this species' most vulnerable early life stages is important to predict its response to climate change on a stage-specific and population level. This study compares the responses of lobster larvae from two sub-populations spanning New England's north-south temperature gradient (southern New England and eastern Gulf of Maine) to the joint effects of elevated pCO_2 and temperature. Using a full factorial experimental design, we subjected planktonic larval and post-larval stages to different combinations of ambient, end-century projected, and extreme pCO_2 concentrations (400 ppm, 750 ppm, 1200 ppm), and ambient and projected end-century temperatures (16°C and 19°C). We measured larval growth (both linear and dry weight), oxygen consumption, and elemental composition. In addition, with postlarvae, we quantified swimming speed and feeding

behavior. Our experiments suggested that elevated temperature has a larger influence over measured parameters than elevated pCO_2 alone, resulting in faster development times of all larvae. However, responses in other measured parameters were location and stage-specific, suggesting that larvae from contrasting thermal regimes respond differently to elevated pCO_2 and temperature. Larvae from southern New England were more responsive to temperature than pCO_2 stress, resulting in a longer carapace and greater dry, carbon and nitrogen weights in stage II when reared at elevated temperature. Larvae from eastern Gulf of Maine, however, more often showed no treatment effects, grew to longer carapace lengths, had greater elemental and dry weights and exhibited higher rates of oxygen consumption than southern New England larvae, despite starting at the same elemental weight and carapace length at stage I.

In addition, this study also investigated the interactive effects of ocean warming and acidification on the gene expression response of the planktonic postlarval lobster from southern New England. Using a full factorial experimental design, lobsters were raised in ambient and elevated pCO_2 concentrations (400ppm, 1200 ppm) and temperatures (16°C and 19°C). When temperature alone was elevated (19°C), larvae downregulated genes related to cuticle development; when pCO_2 alone was elevated (1200 ppm), larvae upregulated chitinase as well as genes related to stress response and immune function. The joint effects of end-century stressors (19°C, 1200ppm) resulted in the upregulation of those same genes, as well as cellulase, and the downregulation of calcified cuticle proteins, and a greater upregulation in genes tied to immune response and functioning. These first results of the impact of varying conditions on larval lobster gene expression suggest the existence of compensatory mechanisms in response to stressors resulting from a rapidly changing environment.

ACKNOWLEDGEMENTS

Thank you to all the staff and interns at the Darling Marine Center and Bigelow Laboratory of Ocean Science for all of the support and guidance throughout this project. Thank you so much to Charlie Hudson for helping with the hatchery system, as well as Katherine Thompson, Kathleen Reardon, and Scott Olszewski for supplying us with our eggers. This could not have been possible without the help of many interns at both laboratories to keep this project afloat: Darcia Gonzalez, Devin Domeyer, Sarah Caron, Yoojin Cha, Adrian Contreras, Sarah Risley, Abby Shaughnessy, David Gauld, Alex Ascher. I would like to especially thank Jesica Waller, who paved the way for the project and offered advice and commiseration. Thanks to Fraser Clark for his patience and help with the transcriptomic and bioinformatic process. Thank you to the members of the Wahle lab and my patient and supportive committee members Spencer Greenwood and Paul Rawson. Finally, a special thank you to my co-advisors Rick & David, who trusted in my ability to tackle the extremely steep learning curve that is this project. This research was supported by NOAA's Ocean Acidification Program and Northeast Regional SeaGrant Program (NOAA grant #NSCNA140AR4170072). Because this project was aided by so many hands and was very much a team effort, I intentionally use the first person plural pronoun in the narrative. Thank you so much to everyone who helped with this project.

.

TABLE OF CONTENTS

ACKNOWLEDGEMENTS ..... **III**

LIST OF TABLES ..... **VI**

LIST OF FIGURES ..... **IX**

LIST OF EQUATIONS ..... **XII**

CHAPTER

1. INTRODUCTION **1**

2. COMPARISON OF PHYSIOLOGICAL RESPONSES BETWEEN LARVAE FROM TWO THERMALLY DISTINCT LOCATIONS **6**

2.1 INTRODUCTION 6

2.2 METHODS 8

2.2.1 Description of Study Species *8*

2.2.2 Hatching & Rearing *10*

2.2.3 Treatments & Water Quality *11*

2.2.4 Measurements on all stages *13*

2.2.5 Statistical Analysis *16*

2.3 RESULTS 16

2.3.1 Development Time *16*

2.3.2 Carapace Length *18*

2.3.3 Dry Weight *21*

2.3.4 Carbon Weight *25*

2.3.5 Carbon content as a proportion of dry weight *29*

2.3.6 Nitrogen Weight *33*

2.3.7 Carbon to Nitrogen Ratio *37*

2.3.8 Oxygen Consumption Rate (OCR) *41*

2.3.9 Mass-specific OCR *45*

2.3.10 Swimming Speed *49*

2.3.11 Feeding Rate *50*

2.4 DISCUSSION 51

2.4.1 Growth and Development *51*

2.4.2 Oxygen Consumption *54*

2.4.3 Behavior *55*

2.4.4 Conclusion *55*

3. GENE REGULATORY RESPONSE IN POSTLARVAE 57

3.1 INTRODUCTION 57

3.2 METHODS 59

3.3 RESULTS 61

3.4 DISCUSSION 71

BIOGRAPHY OF AUTHOR 86

LIST OF TABLES

Table 2.1. Water chemistry parameters during the course of experiment for southern New England, and eastern Gulf of Maine trials. 13

Table 2.2. Summary of three-way ANOVA used to evaluate the effect of temperature, $p\mathrm{CO_2}$ and subpopulation location of *H. americanus* on the first day of SIV appearance...........17

Table 2.3. Summary of statistics comparing means of carapace lengths between two populations (a) and summary of two-way ANOVA used to evaluate the effect of temperature and $p\mathrm{CO_2}$ of *H. americanus* on carapace length at each stage within each population (b)..20

Table 2.4. Summary of statistics comparing means of dry weights between two populations (a) and Summary of two-way ANOVA used to evaluate the effect of temperature and $p\mathrm{CO_2}$ of *H. americanus* on dry weight at each stage within each population (b)..24

Table 2.5. Summary of statistics comparing means of carbon weight between two populations (a) and Summary of two-way ANOVA used to evaluate the effect of temperature and $p\mathrm{CO_2}$ of *H. americanus* on dry weight at each stage within each population (b)..28

Table 2.6. Summary of statistics comparing means of carbon weight: dry weight between two populations (a) and Summary of two-way ANOVA used to evaluate the effect of temperature and $p\mathrm{CO_2}$ of *H. americanus* on carbon: dry weight at each stage within each population (b)..32

Table 2.7. Summary of statistics comparing means of nitrogen weight (mg) between two populations (a) and Summary of two-way ANOVA used to evaluate the effect of temperature and pCO_2 of *H. americanus* on nitrogen weight at each stage within each population (b)....36

Table 2.8. Summary of statistics comparing means of carbon: nitrogen ratio between two populations (a) and Summary of two-way ANOVA used to evaluate the effect of temperature and pCO_2 of *H. americanus* on nitrogen weight at each stage within each population (b)....40

Table 2.9. Summary of statistics comparing means of whole body OCR ratio between two populations (a) and Summary of two-way ANOVA used to evaluate the effect of temperature and pCO_2 of *H. americanus* on OCR t at each stage within each population (b)....44

Table 2.10. Summary of statistics comparing means of mass normalized OCR ratio between two populations (a) and Summary of three way ANOVA used to evaluate the effect of temperature and pCO_2 of *H. americanus* on OCR t at each stage within each population (b)....48

Table 2.11. Summary of statistics comparing means of swimming speeds between two populations (a) and Summary of two-way ANOVA used to evaluate the effect of temperature and pCO_2 of *H. americanus* on swimming speed of Stage IV postlarvae within each population (b)....49

Table 2.12. Summary of two-way ANOVA used to evaluate the effect of temperature and pCO_2 of *H. americanus* on swimming speed of Stage IV postlarvae from EGoM. 50

Table 3.1. Water Chemistry parameters during course of experiment. 60

LIST OF FIGURES

Figure 2.1. Distinguishing features of *Homarus americanus* larval and postlarval stages........ 9

Figure 2.2. Number of days post-hatching (DPH) to first appearance of stage IV (means ± SE) from two populations of *H. americanus* under different pCO_2 and temperature conditions 17

Figure 2.3. Effects of pCO_2 and temperature on carapace length (mm, mean ± SE) at across stages by location (a), and within stages.. 18

Figure 2.4. Effects of pCO_2 and temperature on dry weight of *H. americanus* (mg, mean ± SE) across stages by location (a), and within stages. 22

Figure 2.5. Effects of pCO_2 and temperature on carbon weight of *H. americanus* (mg, mean ± SE) across stages by location (a), and within stages. 26

Figure 2.6. Effects of pCO_2 and temperature on proportion of carbon to dry weight of *H. americanus* (mean ± SE) across stages by location (a), and within stages. 30

Figure 2.7. Effects of pCO_2 and temperature on nitrogen weight of *H. americanus* (mg, mean ± SE) across stages by location (a), and within stages 34

Figure 2.8. Effects of pCO_2 and temperature on carbon to nitrogen weight ratio of *H. americanus* (mean ± SE) across stages by location (a), and within stages. 38

Figure 2.9. Effects of pCO_2 and temperature on whole body oxygen consumption rate of *H. americanus* (µmol/L/hr, mean ± SE) across stages by location (a),
and within stages 42

Figure 2.10. Effects of *p*CO_2 and temperature on mass-specific oxygen consumption rate of *H. americanus* (μmol/mg/L/hr, mean ± SE) across stages by location (a), and within stages 46

Figure 2.11. Effects of *p*CO_2 and temperature on swimming speed of stage IV postlarval *H. americanus* (mm/s, mean ± SE) by location. 49

Figure 2.12. Effects of *p*CO_2 and temperature on feeding rate of stage IV postlarval *H. americanus* (mm/s, mean ± SE) from EGoM. 50

Figure 3.1. Heat map depicting expression of 1,108 differentially expressed genes relative to ambient treatment of 400ppm, 16°C. 62

Figure 3.2. Percent of total (a) and annotated (b) differentially expressed genes as quantified by DESeq2 analysis within elevated temperature treatment (400 ppm 19°C), elevated *p*CO_2 treatment (1200ppm 16°C), and joint temperature and *p*CO_2 treatment (1200ppm 19°C) 63

Figure 3.3. MA plots exhibiting the strength of significance (-Log10(p-values)) of particular genes against the Log2 fold change of each gene across samples of treatments 65

Figure 3.4. Comparison of number of annotated, differentially expressed genes identified by DESeq2 and edgeR analysis 66

Figure 3.5. Response of Genes of Interest of carapace formation to elevated temperature and pCO_2. Log2 Fold change of 28 GOI's within postlarvae 68

Figure 3.6. Response of Genes of Interest of immune response and function to elevated temperature and pCO_2 70

LIST OF EQUATIONS

Equation 2.1. Postlarval Swimming Speed..15

CHAPTER 1

INTRODUCTION

The release of anthropogenic carbon emissions into the Earth's atmosphere has resulted in large scale changes in oceanic temperature and pH that have serious implications for marine ecosystems. Atmospheric concentrations of $p\mathrm{CO_2}$-atm have increased by 40% since the start of the industrial revolution and the rate of increase has been fastest (2.0 +/- 0.1 ppm/yr) in recent years (IPCC, 2013). IPCC scenarios project $p\mathrm{CO_2}$-atm will continue to climb and by the end of the century result in an average sea surface temperature increase of 0.6°-2.0°C and a pH drop of 0.06-0.32 units, depending on emissions (IPCC, 2013; Blunden & Arndt, 2014). Under no IPCC scenario does the ocean temperature or acidity decrease or remain constant.

However, these rapid changes will not occur uniformly across marine environments. Climate models predict that higher latitudes will warm faster and experience quicker declines in pH than lower latitudes, putting marine ecosystems and fisheries in these regions at greater risk (Fabry *et al*., 2009; Gledhill *et al.*, 2015). The Northwest Atlantic and Gulf of Maine have warmed 0.026°C per year since 1980, a rate faster than that of the majority of the world's oceans (Balch et al., 2012; Pershing *et al.*, 2015). Furthermore, coastal areas within this region are susceptible to higher rates of acidification, due to the ocean's buffering capacity being depressed by freshwater inputs of rivers and incoming currents (Salisbury *et al*., 2008; Fabry *et al*., 2009; Gledhill *et al*., 2015). In light of these rapid, concurrent changes, concern is growing with regard to the implications for marine organisms and fisheries in the region.

Since many physiological processes are closely tied to temperature in marine ectotherms, oceanic warming has the potential to stress marine ecosystems by way of shifts in species ranges that alter community structure and food webs (Mills, *et al.*, 2013; Somero *et al.*,

2017). The physiological responses of marine organisms to change in temperature have been a long-standing topic of study (Shatzlein and Costlow, 1978; MacKenzie, 1988; Ross *et al.*, 1998; Anger, 2001; Helmuth, 2009; Weiss *et al.*, 2009; Somero, 2010; Swingle *et al.*, 2013; Tepolt & Somero, 2014), but interactive effects with other stressors are often overlooked. Recent concern over the direct effects of elevated CO_2 in seawater and the concomitant decrease in pH has motivated an accelerated pace of ocean acidification study over the past decade (Browman, 2016). The absorption of CO_2 into the ocean results in an increase of free hydrogen ions, thereby lowering the pH. The net result is a decrease in biologically-available carbonate. This affects the energetics of calcifying organisms that rely on carbonate in order to build hard body structures (Kroeker *et al.,* 2013; Gledhill *et al.*, 2015). The rapid rate of change in carbonate chemistry is a pressing concern in the Northwest Atlantic and Gulf of Maine, which relies disproportionately on marine calcifiers as targets of valuable fisheries (Gledhill *et al*., 2015).

However, recent research suggests that crustaceans, as a group, are relatively resistant to acidification though the response varies by species (Whiteley, 2011; Wernburg *et al.*, 2012; Gledhill *et al*, 2015). For example, although larval Dungeness crab (*Cancer magister*) and juvenile tanner crab (*Chionoecetes bairdi*) show decreased survival in high pCO_2 environments, many other crustacean species are unaffected by projected end-century pH (Arnberg *et al*., 2013; Byrne *et al.,* 2013; Long *et al*., 2013; Shiffer *et al*., 2013; Miller *et al*., 2016). On the other hand, increased CO_2 can have sublethal effects on a variety of species that are manifested by changes in cellular respiration, enzyme regulation, and biomineralization (Anger, 1987; Portner *et al*, 2010; Kunkel *et al.,* 2012).

Experiments evaluating the impact of multiple environmental stressors can reveal interactive effects that would be undetectable in single-stressor studies (Wernberg *et al*., 2012). For example, in some invertebrates, such as coralline algae (*Lithophyllum cabiochae,* Martin & Gattuso, 2009), spider crabs (*Hyas araneus,* Walther *et al.,* 2009), and larval lobsters (*Homarus* americanus, Waller *et al.* 2016), the effects of elevated pCO_2 have been shown to intensify with elevated temperatures.

The American lobster (*Homarus americanus)* comprises the most valuable single-species fishery in North America, and thus is one of particular socio-economic importance (NOAA 2019, DFO 2019). The species ranges geographically from Labrador to the US mid-Atlantic, but since the 1960s, its center of distribution has shifted northward in response to warmer temperatures (Pinsky et al. 2013), a process that is projected to continue (LeBris *et al*., 2018). Warming temperatures have also been implicated in the downward shift in the size of maturity of this species (Le Bris *et al*., 2016; Haar *et al*., 2017). Surprisingly, little else is known about how this species will react to projected end-century pCO_2 levels and temperatures, but it is suggested that the rapidly molting, pelagic larval and postlarval stages might be particularly vulnerable to these oceanic changes (Kurihara *et al*., 2007; Pörtner & Farrell, 2008; Gledhill *et al*., 2015).

Homarus americanus begins life as one of many eggs in a clutch anchored to the abdomen of a benthic, adult female. After hatching, larvae transition through three instars (Stage I, Stage II, Stage III), that are fully pelagic, followed by a metamorphosis to the postlarval stage (Stage IV) which settles to the seabed (Factor, 1995). Upon settlement, postlarvae seek out suitable substrate to transition to the first benthic instar, which represents the recruitment stage of the fishery (Factor, 1995; Incze *et al*, 1997). Transitions between these

stages are associated with high mortality and thus represent important biological bottlenecks within this species' life cycle (MacKenzie, 1988; Factor, 1995). In addition, larval and postlarval stages of this species are more likely to experience stressful conditions because they occupy the thermally-variable upper water column (MacKenzie, 1988; Byrne, 2011; Gledhill *et al.,* 2015). At this time, we do not know how projected oceanic changes will affect crucial transitions for this species and therefore recruitment into the fishery (Walther *et al*., 2010).

Previous research on larval stages of the congeners *H. americanus* and *H. gammarus,* indicates that the effects of climate change on this early life stages are complex. Metabolic demand differs between life stages, indicating stage-specific vulnerability to increases in temperature and decreases in pH (Hines *et al*., 2013; Small *et al.* 2015; Waller *et al.*, 2017). Studies that have examined the effects of OA have observed varying degrees of impact on carapace formation and development (Ries *et al.*, 2009; Keppel *et al*., 2012; Agnalt *et al*., 2013).

By the end of the 21st century, seawater temperatures in the Gulf of Maine are predicted to be 3°C warmer and atmospheric pCO_2 levels are projected to nearly double. But to date, only three studies have examined the joint effects of elevated pCO_2 and temperature on the earliest life stages of lobster (Agnalt *et al*., 2013; Small *et al.,* 2015; Waller *et al.,* 2017). These studies suggest that many physiological parameters of larval lobster are more sensitive to the end-century increase in temperature than they are to the corresponding increase in pCO_2. In addition, elevated temperature and pCO_2 can interact to cause further changes in behavior, carapace length, carbon content, and development time than either stressor alone, although reports are conflicting in some cases (Small *et al.* 2015; Waller *et al.*, 2017). The inconsistency between studies makes the task of projecting future impacts of the changing climate on lobster even

more challenging. Investigations of the impact of changing temperature and pCO_2 on gene expression are in their infancy. Early studies on the American lobster suggest the response to elevated pCO_2 may be greater than the morphological or behavioral performance metrics suggest (Waller, 2016).

The primary goal of this study is to examine aspects of the physiological and gene expression response of larval stages of *Homarus americanus* when reared in end century projected temperature and pCO_2. However, it is important to highlight that the geographic range of *H. americanus* occupies one of the steepest gradients of sea surface temperatures in the world, ranging from a summertime average of 12°C in the Bay of Fundy to 20°C in southern New England (Longhurst, 1998). Therefore, our secondary goal is to determine if larvae of subpopulations from different thermal regimes respond differently to these predicted changes.

Chapter two reports the results of a full-factorial experiment that compares morphological, physiological and behavioral response of the earliest life stages of *H. americanus* to predicted end-century temperature and pCO_2. This study is the first to compare physiological, developmental, behavioral and gene regulatory response of lobster larval stages from two sub-populations occupying oceanographically contrasting thermal regimes along the New England coast.

Chapter three describes the differential expression of physiologically relevant genes in postlarvae reared from hatch to evaluate the single and joint effects of elevated pCO_2 and temperature on gene expression. This study adds new information to our understanding of the effects of multiple climate stressors on *H. americanus* and provides new insight on phenotypic response within the context of molecular response and compensation.

CHAPTER 2

COMPARISON OF PHYSIOLOGICAL RESPONSES BETWEEN LARVAE FROM TWO THERMALLY DISTINCT LOCATIONS

2.1 Introduction

The projected rate of ocean warming (OW) and acidification (OA) in the Northwest Atlantic and Gulf of Maine has important implications for the production and distribution of marine organisms, particularly marine calcifiers (IPCC, 2013; Gledhill *et al*., 2015; Le Bris *et al*., 2018). The metabolic budget of ectotherms is closely tied with temperature. Reports are accumulating that OW and OA have varying individual and combined effects on marine calcifiers such as corals, bryozoa, molluscs and crustaceans (Reynaud *et al*., 2003; Anthony *et al*., 2008; Rodolfo-Metalpa *et al,* 2010, 2011; Whiteley, 2011). However, predicting effects of concurrent OA and OW is important in order to project how individual species will fare, because the two can work either antagonistically, resulting in a mitigating effect, or synergistically though an accumulation of stress effects (Anthony *et al*., 2008; McCulloch *et al*., 2012).

In clawed lobsters, as in most crustaceans, the interactive effects of temperature and pCO_2 are species and life-stage specific (Whiteley, 2011; Wernburg *et al.*, 2012; Gledhill *et al*, 2015). The pelagic larval and postlarval stages of *Homarus* are suspected to be especially vulnerable to the effects of OW and OA, since they undergo rapid development with physiologically demanding ecdyses and metamorphoses, within the variable upper water column (Kurihara *et al*., 2007; Pörtner & Farrell, 2008; Gledhill *et al*., 2015). Understanding the energetic and metabolic demands of increased temperature and decreased pH on early developmental stages could help predict where climate change induced bottlenecks might occur in the life cycle.

Recent studies have provided data on the physiological and behavioral responses of larval *Homarus* congeners to OW and OA, (Small *et al.*, 2015; Waller *et al*, 2016). This present study will investigate hypothesized differences in the response of *H. americanus* larvae from populations occupying thermally contrasting segments of the species' geographic range. The range of *H. americanus* spans one of the ocean's steepest latitudinal thermal gradients (Longhurst, 1998). Geographic differences in allele frequencies of thermally sensitive genes suggest that genetic differentiation has occurred among *H. americanus* sub-populations along this range (Ketchington *et al.*, 2009; Benestan *et al.,* 2015; Benestan *et al.,* 2016) and that subpopulations along this thermal gradient are subject to selection for thermally adaptive traits. Selective pressures due to environmental gradients can manifest as micro-evolutionary adaptive shifts in life history, behavior, and physiological traits (Conover,1995), and have been exhibited in differential tolerance to heat stress across latitudinal clines in several crustacean species (Sanford *et al*., 2006; Kelly *et al*., 2012; Tepolt and Somero, 2014). The question of thermal adaptation across subpopulations has remained largely unexplored in *Homarus americanus.*

While multi-generational studies can provide much insight into the micro-evolutionary response to environmental change (Gibbin *et al*., 2016), such studies are not practical with long-lived species like *Homarus,* which has a generation time on the order of 5-8 years. In lieu of long-term, multigenerational studies, there is opportunity to compare individuals sourced from different thermal regimes. New England's steep thermal gradient offers a living laboratory to look for evidence of thermal adaptation on a local scale.

This study investigates stage-specific responses to end-century-predicted temperature and pCO_2, and compares those responses between larvae from two thermally distinct regimes along the lobster's geographic range: southern New England (SNE) and eastern Gulf of Maine

(EGoM). Measuring a suite of biological end-points, we sought to identify possible trade-offs, energetic compensation and to compare acclimation capacity between larvae from two populations. Differences in biological endpoints between treatments and locations would suggest larvae are differentially stressed based on region of origin or environmental condition.

2.2 Methods

2.2.1 Description of Study Species

The American lobster has three planktonic larval stages (I-III) terminated by metamorphosis to a still-planktonic postlarval stage (IV). Larvae are similar in morphology: a large carapace, segmented abdomen, and pronounced rostrum. Size and specific morphological features aid in identifying each stage (Fig. 1). The time of development between stages varies based on temperature, food availability, and other abiotic factors, and each stage duration is successively longer than the last (MacKenzie, 1988). Abiotic factors can have a large effect on larval duration, creating a range of 11 to 25 days to pass through the first three stages to molt into postlarvae. Planktonic duration can have a significant effect on survival in the wild, where larvae are exposed to predation and highly variable water conditions (Hudon & Fradette, 1988).

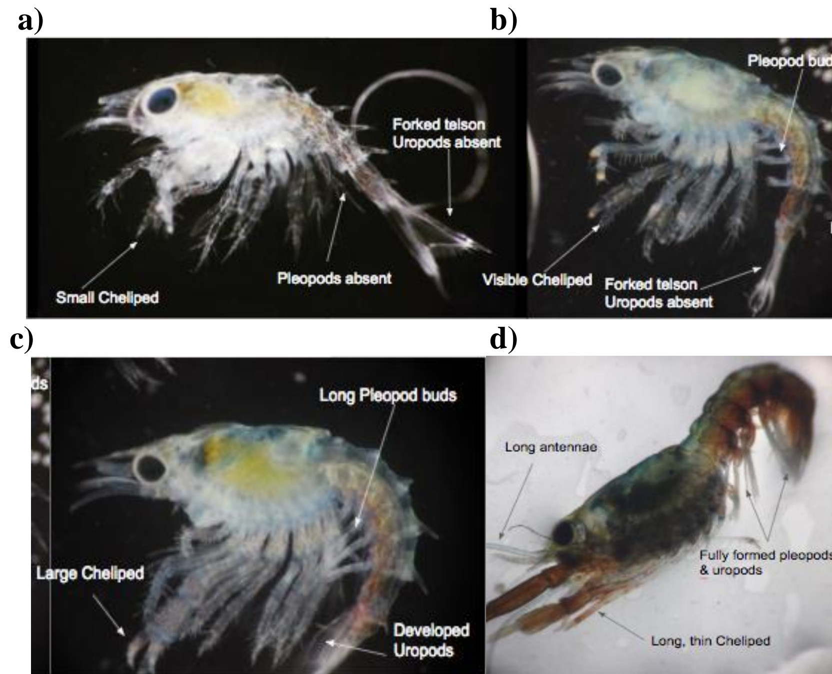


Figure 2.1. Distinguishing features of *Homarus americanus* larval and postlarval stages. a) Stage I larvae, b) Stage II larvae, c) Stage III larvae, and d) Stage IV postlarval

Stage I-III larvae undergo three successive molts to reach the postlarval stage. A metamorphosis occurs with the transition into the postlarval instar that resembles the adult (Fig.1). About half-way through the postlarval stage they also begin a behavioral transition whereby the seek bottom substrate to settle and molt to a benthic juvenile.

Relative to larval stages, postlarvae exert more control in their vertical placement in the water column, preferring to occupying waters above 12°C (Annis, 2005). Larvae can delay timing of settlement and subsequent development to juvenile stages if a suitable substrate and temperature are not found (Lawton & Lavalli, 1995)

Larval growth is achieved by ecdysis. Many abiotic factors play critical roles in development and survival of larvae through successive molts, most notably temperature. For example, larvae will only advance to juvenile stages at temperatures exceeding 10°C, with stage duration decreasing as temperature increases to approximately 22°C. Above this temperature,

survival rates decrease rapidly (MacKenzie, 1988). Survivorship in the wild has been shown to favor lobsters that hatch earlier in the season, taking advantage of rapidly warming surface waters, and reducing the exposure to predators in the planktonic phase (Hudon & Fradette, 1988).

2.2.2 Hatching *& Rearing*

Data for this project were collected over three summers across two populations located in oceanographically contrasting ends of the U.S. range of *Homarus americanus.* Measurements of larval stages I-III were taken from summer of 2017. Stage IV postlarval behavioral measurements were taken from larvae raised in identical conditions in the summer of 2016 (southern New England) and the summer of 2018 (eastern Gulf of Maine). In mid-May, seven ovigerous females, provided by Rhode Island's Department of Environmental Management's Fisheries Section, were collected off of the coast of Rhode Island. Likewise, in mid-July, seven ovigerous females were collected from the eastern Gulf of Maine , provided by Maine Department of Marine Resources. Egg bearing females were held in 300L hatching tanks filled with coarsely filtered seawater at the Darling Marine Center for a maximum of three weeks. Water temperature averaged ~15°C. Tanks were monitored every six hours until at least three females had hatched out eggs.

Over the course of 2-3 days, newly hatched larvae were transported to Bigelow Laboratory of Ocean Science and distributed randomly in 18 20-L buckets pre-equilibrated to the experimental treatments. Progeny from 4-6 females were used to stock our tanks. Larvae hatched within the same 6-h time frame were grouped within buckets as a cohort, to ensure that they would be on the same developmental trajectory. Each bucket was stocked with 250 larvae,

resulting in an average starting density of 12.5 larvae/L. Larvae were allowed 24 hours to acclimate before any measurements were taken.

2.2.3 Treatments & Water Quality

Tanks were maintained in a full factorial three-way ANOVA design. Temperatures represented average present-day sea surface temperature and a 3°C increase for the end of the century projected under IPCC Model RCP8.5, a moderate carbon emissions scenario (16°C & 19°C, respectively; IPCC, 2013). All tanks were held in a temperature controlled room at 16°C. We used Hydor submersible aquarium heaters to create the warmer treatments of 19°C.

Three pCO_2 treatments (400, 750 and 1200 ppm) were achieved by bubbling the tanks with air mixed with specific concentrations of CO_2 using Aarlburg mass flow controllers (Waller *et al*., 2017). The three pCO_2 concentrations represented current atmospheric concentration (400 ppm), moderate emission scenario end-century predicted average oceanic concentration (750 ppm), and an extreme pCO_2 concentration to generate pH values representative of estuarine and coastal regions (1200ppm) (IPCC, 2013; Gledhill *et al.*, 2015). Each of the six treatment combinations were maintained in triplicate (18 tanks in total).

We monitored water quality daily. Salinity was measured using an Oakton SALT meter, and pH and temperature were measured using a Thermo Orion 3-star benchtop pH probe, calibrated using NIST buffers. Water within the tank was replaced every 3 d with fresh, pre-equilibrated water.

In addition to daily monitoring, spectrophotometric pH and alkalinity was measured weekly to validate daily readings. The pH(tot) was measured spectrophotometrically (Hitachi U-310 dual-beam, Hitachi, Japan) using the pH sensitive indicator dye m-cresol purple (Sigma-Aldrich) following SOP (standard operating procedure (SOP) 6b: Dickson, 2007).

We drew 40mL of seawater from each treatment and filtered it through a 0.2µm syringe filter. We dyed 10mL of each sample with *m*-Cresol purple sodium salt as a pH indicator dye. Dyed and undyed reference samples were pipetted into a 1 cm path quartz cuvette and placed in a temperature controlled chamber of the spectrophotometer. We measured absorbance at wavelengths of 578 nm, 434 nm and 739 nm. Addition of *m*-Cresol purple dye was corrected using SOP 6b (Dickson, 2007). Final pH was determined from (Liu *et al.,* 2011).

Total alkalinity (A_T) was measured using a Metrohm 888 Titrando by Tiamo software (Metrohm, USA). We extracted 40 mL per week from each treatment position and fixed with saturated $HgCl_2$ to eliminate biological activity and hold A_T constant. The system was calibrated using certified reference material from the Dickson laboratory at Scripps Institute of Oceanography. Using temperature, salinity, pH and A_T inputs, we calculated carbonate chemistry parameters (pCO_2, [HCO_3^-], [CO_3^{2-}], Ω_{Ar}, Ω_{Ca}) using the CO2SYS2.1 system (Lewis & Wallace, 1998; Table 2.1).

Table 2.1. Water chemistry parameters during the course of experiment for (a) southern New England, and (b) eastern Gulf of Maine trials. All parameters list mean ± SD through the experimental period.

a.

Treatment	Temperature (°C)	Salinity (ppt)	pH	ΩCa	ΩAr
400ppm 16C	16.6 ± 0.5	30.3 ± 0.8	7.94 ± 0.06	2.34 ± 0.10	1.45 ± 0.06
750ppm 16C	17.1 ± 0.4	29.8 ± 0.4	7.75 ± 0.03	1.68 ± 0.12	1.05 ± 0.07
1200ppm 16C	17.0 ± 0.4	30.0 ± 0.8	7.56 ± 0.01	1.16 ± 0.025	0.72 ± 0.02
400ppm 19C	18.7 ± 0.4	30.2 ± 0.9	7.89 ± 0.03	2.44 ± 0.25	1.52 ± 0.16
750ppm 19C	19.3 ± 1.0	30.2 ± 1.0	7.81 ± 0.04	2.23 ± 0.23	1.38 ± 0.14
1200ppm 19C	19.5 ± 1.0	29.9 ± 0.7	7.63 ± 0.01	1.42 ± 0.004	0.88 ± 0.002

b.

Treatment	Temperature (°C)	Salinity (ppt)	pH	ΩCa	ΩAr
400ppm 16C	16.4 ± 0.3	30.8 ± 0.6	7.98 ± 0.01	2.92 ± 0.11	1.81 ± 0.07
750ppm 16C	17.0 ± 0.5	30.6 ± 0.5	7.81 ± 0.04	2.00 ± 0.18	1.245 ± 0.11
1200ppm 16C	16.6 ± 0.4	29.8 ± 0.9	7.60 ± 0.03	1.24 ± 0.13	0.77 ± 0.08
400ppm 19C	19.1 ± 1.0	30.7 ± 0.7	8.00 ± 0.05	3.28 ± 0.30	2.04 ± 0.19
750ppm 19C	19.9 ± 0.8	30.5 ± 0.5	7.82 ± 0.06	2.13 ± 0.22	1.32 ± 0.14
1200ppm 19C	19.2 ± 0.9	30.1 ± 0.9	7.68 ± 0.02	1.57 ± 0.05	0.97 ± 0.03

2.2.4 Measurements on all stages

We collected 30 eggs from each subpopulation to measure dry weight, as well as carbon and nitrogen content. For the first three pelagic larval stages I-III, we quantified developmental rate, dry weight and carapace length as parameters of growth and life history. For these stages we also measured carbon and nitrogen content and respiration rates as indicators of metabolic rate of individuals. We also compared swimming speed and feeding rate for postlarvae (SIV) between the two locations. This stage represents a pivotal physiological and morphological transition, to a life stage that actively seeks benthic habitat to transition to a juvenile. Within this life stage we examined feeding rate and swimming speeds under treatment conditions.

In each treatment replicate, the day and time of first appearance of each larval stage was recorded. Each day, five randomly selected individuals from each treatment replicate were examined and photographed (Canon EOS Rebel T3i, Japan) under a dissecting microscope

(Olympus SX 61). Carapace length was measured in profile from the back of the eye to the furthest edge of the carapace using NIH-ImageJ software (NIH, USA). Individuals were returned to tanks after being photographed.

Samples for dry weights and elemental (CHN) composition were collected for each stage from each replicate tank 48 h after the first individual of that stage was recorded. Individuals were rinsed three times in deionized water to remove salt, and then placed into pre-weighed tin boats. The number of individuals evaluated per tin boat depended on the stage (eggs: n=10, SI: n=5, SII: n=3, SIII: n=2, SIV: n=1). Samples were desiccated at 40 °C for 48+ hours. Samples were weighed for a final dry weight and then analyzed by Bigelow Analytical Services using a Costech Elemental Combustion System 4010 to determine carbon and nitrogen elemental weights (Costech, USA).

Oxygen consumption rates (OCR) were monitored at each stage from each experimental replicate. Individuals of the same stage were placed into 50 mL jars filled with pre-equilibrated water of the appropriate treatment, and sealed with a ground glass stopper with a 400 μm hole at the top (SI: n=10, SII: n=5, SIII: n=2-3, SIV: n=1). All measurements were taken in a water bath (Thermo-Fisher Scientific, USA) at the treatment temperature and pCO_2. Oxygen concentration was measured using a clark-type oxygen microelectrode (Unisense, Denmark) over a 1-2 h period. OCR measurements were taken from each treatment condition at every stage. OCRs were calculated from the slope of decrease in oxygen concentration over time. Mass-specific OCRs were calculated using the measured carbon weight of individuals.

Feeding rate was measured for all surviving postlarvae 48 h after molt for larvae from EGoM only. Larvae were placed in sealed, 15L lidded dark buckets filled with pre-equilibrated seawater set to their treatment conditions along with 375 live *Artemia salina* (25 prey/L). Tanks

were kept at temperature in the dark for 6 h, after which point the water was passed through a 20 µm mesh and the remaining *A. salina* were counted. Feeding rate was calculated as the number of *Artemia* consumed per hour.

Swimming speed of postlarvae from both populations was measured using silhouette video imaging. The optical setup provides fine-scale behavioral observations with an image quality that is unaffected by ambient light levels (described in Fields *et al.,* 2012). The system consists of two orthogonally-oriented Point grey HD highspeed (60Hz) video cameras with 105-mm Nikon lenses. Silhouette illumination was provided by a far red light emitting diode (LED) placed at the focal point of a biconvex collimating lens. The output beam (15 cm diameter) was passed through a 4L plexiglass aquarium placed at the intersection of the two beams. Surrounding the filming chamber was a 40L temperatures bath that was maintained at treatment temperature. Larval position, speed and distance traveled was measured using the NIH Image J software package. The base of the tail provided the reference point by measuring the Distance (D) from its initial location (X_0, Y_0, Z_0) at the start of a swimming event, to an end point (X_f, Y_f, Z_f). Total distance traveled between sequential video frames was calculated through the following equation:

Equation 2.1. Postlarval Swimming Speed

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

2.2.5 Statistical Analysis

Each of the three replicate tanks within each treatment was used as a unit of replication, and no difference was found between replicate tanks for any trial ($p>0.05$). Data were analyzed using Sigmaplot (11.0). A 3-way ANOVA was used to evaluate the effects of location, temperature and pCO_2 on development time to the postlarval stage. The remaining statistical analyses were done in a two-step process for each larval stage, whereby location effects were evaluated first with a standard t-test. For stages that failed normality, a Whitney rank sum test was used. We then conducted a two-way ANOVA to assess temperature and pCO_2 effects on carapace length, dry weight, carbon and nitrogen elemental weights, OCRs, mass-specific OCRs, carbon to nitrogen ratio, and carbon to dry weight ratio for stages I-III, and swimming speed for postlarvae within each population. We used Shapiro-Wilkes test and Levene's mean test to assess the assumption of normality and homogeneity of variance, respectively. Any parameter that failed either assumption was log-transformed to meet assumptions. Post-hoc analysis for differences between treatments and stages was conducted using Tukey's test. Postlarval feeding rate was only measured for southern New England, using a 2-way ANOVA.

2.3 Results

2.3.1 Development Time

The development time of larvae from hatch to postlarvae ranged from 12 to 36 days. Larvae raised at 19°C experienced a shorter development time to reach the postlarval stage than at 16°C, and there was no significant direct or interactive effect of pCO_2 on development time (Table 2.2). Development time also differed significantly between populations (Figure 2.2.). Larvae from SNE developed 25% (4.5 days) faster than larvae from EGoM, (Table 2.2).

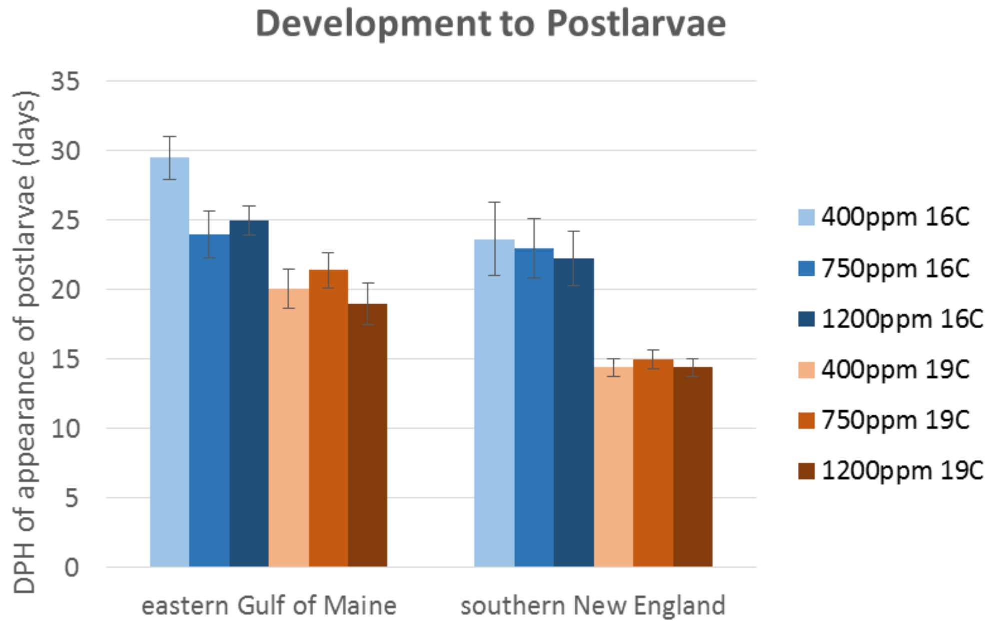


Figure 2.2. Number of days post-hatching (DPH) to first appearance of stage IV (means ± SE) from two populations of *H. americanus* under different pCO_2 and temperature conditions.

Table 2.2. Summary of three-way ANOVA used to evaluate the effect of temperature, pCO_2 and population location of *H. americanus* on first day of SIV appearance. Significant values ($p<0.05$) are bolded.

Day to first appearance of Stage IV					
Source of Variation	DF	SS	MS	F	P
Population	1	280.901	280.901	21.68	**<0.001**
pCO2	2	30.314	15.157	1.17	0.318
Temperature	1	756.775	756.775	58.407	**<0.001**
Population x pCO2	2	14.259	7.13	0.55	0.58
Population x temp	1	20.556	20.556	1.586	0.213
pCO2 x temp	2	41.514	20.757	1.602	0.211
Population x pCO2 x temp	2	19.769	9.885	0.763	0.471
Residual	55	712.626	12.957		
Total	66	1936.269	29.337		

2.3.2 Carapace Length

The overall average carapace length for stage I larvae of both populations did not differ between populations (Table 2.3a; Figure 2.3a, 23b). Larvae from EGoM exhibited significantly longer carapace lengths than SNE (Table 2.3a; Figure 2.3a). There was an interactive effect between temperature and pCO_2 in EGoM larvae, resulting in significantly longer larvae from the most extreme treatment (Table 2.3b; Figure 2.3c). From SNE, larvae grew longer carapaces within the warm temperature treatment (Table 2.3b; Figure 2.3d). In Stage III larvae, no treatment effects were present for either location (Table 2.3b; Figure 2.3e). Larvae from EGoM maintained longer carapace lengths within this stage (Table 2.3a; Figure 2.3a).

a

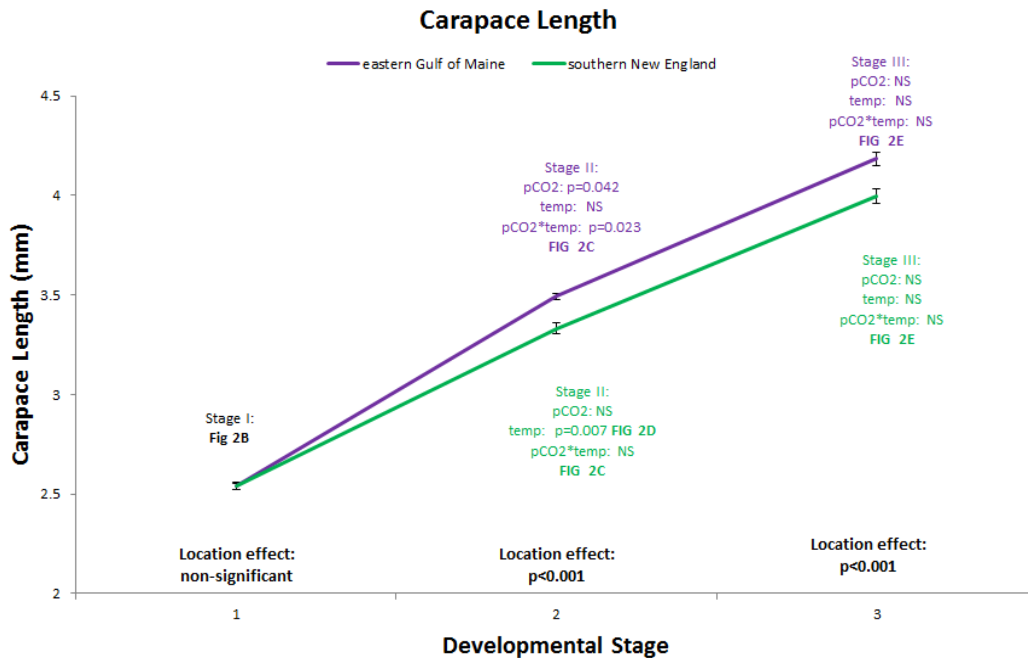


Figure 2.3. Effects of pCO_2 and temperature on carapace length (mm, mean ± SE) at across stages by location (a), and within stages. Overall treatment effect graphs are shown for each population at each stage (mm, mean ± SE) , and also unambiguous main effects: Stage I by location (b); stage II from EGoM and SNE by temperature and pCO_2 treatment (c); stage II from SNE by temperature main effect (d); stage III from EGoM and SNE by temperature and pCO_2 treatment effect (e)

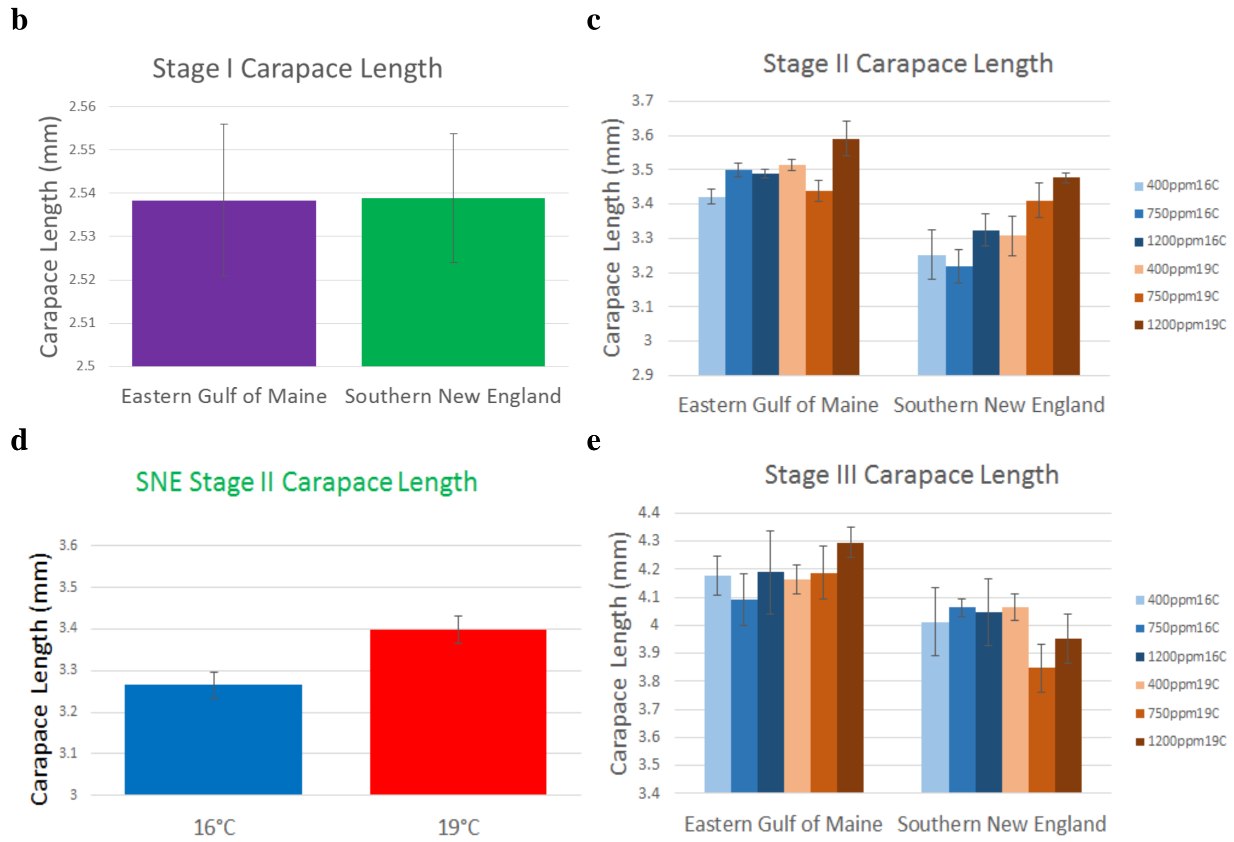


Figure 2.3. continued

Table 2.3. Summary of statistics comparing means of carapace lengths between two populations (a) and Summary of two-way ANOVA used to evaluate the effect of temperature and pCO_2 of *H. americanus* on carapace length at each stage within each population (b). Significant values ($p<0.05$) are bolded.

a

Location Comparison for Carapace Length							
Stage	Test	Source of Variation	t statistic	DF	U Statistic	T	p-value
I	*Mann-Whitney*	*Location*			*124.00*	*371.00*	*0.235*
II	*Mann-Whitney*	*Location*			*34.00*	*205.00*	***<0.001***
III	*Independent T-test*	*Location*	*-3.757*	*34*			***<0.001***

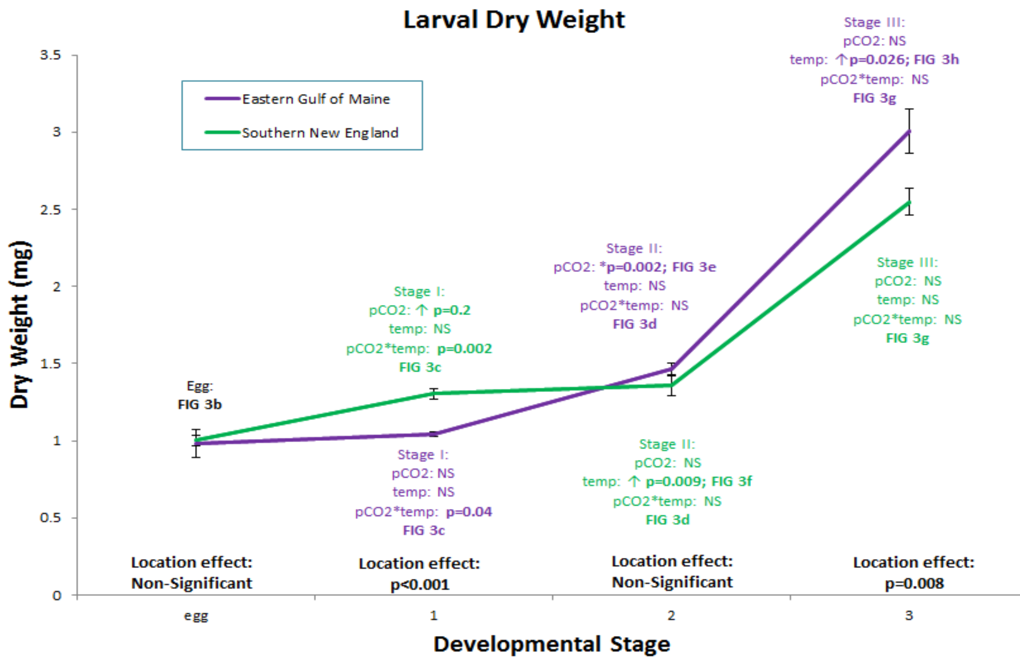
b

ANOVAs for Carapace Length						
Stage	Population	Source of Variation	DF	Mean Square	F	P
II	*Northern Maine*	*pCO2*	*2*	*0.010*	*4.172*	***0.042***
		temp	*1*	*0.009*	*3.752*	*0.077*
		pCO2 x temp	*2*	*0.013*	*5.275*	***0.023***
		Residual	*12*	*0.002*		
		Total	*17*	*0.005*		
	Rhode Island	*pCO2*	*2*	*0.023*	*3.010*	*0.087*
		temp	*1*	*0.081*	*10.480*	***0.007***
		pCO2 x temp	*2*	*0.008*	*0.992*	*0.399*
		Residual	*12*	*0.008*		
		Total	*17*	*0.014*		
III	*Northern Maine*	*pCO2*	*2*	*0.017*	*0.680*	*0.525*
		temp	*1*	*0.018*	*0.726*	*0.411*
		pCO2 x temp	*2*	*0.007*	*0.270*	*0.768*
		Residual	*12*	*0.025*		
		Total	*17*	*0.021*		
	Rhode Island	*pCO2*	*2*	*0.010*	*0.435*	*0.657*
		temp	*1*	*0.033*	*1.378*	*0.263*
		pCO2 x temp	*2*	*0.027*	*1.147*	*0.350*
		Residual	*12*	*0.024*		
		Total	*17*	*0.023*		

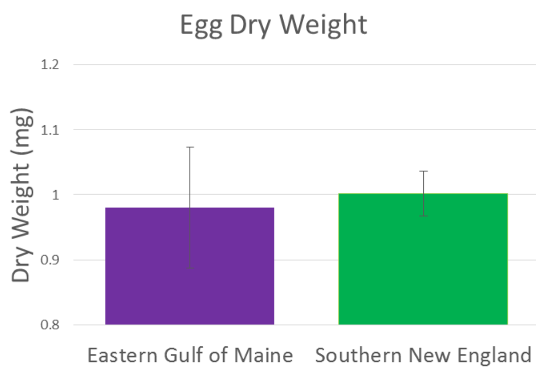
2.3.3 Dry Weight

There was no statistical difference in egg dry weight between populations (Figure 2.4a; Table 2.4a).Stage I larvae from EGoM had a lower dry weight than those from SNE (Table 2.4a; Figure 2.4a), although there was an interaction between pCO_2 and temperature leading to an ambiguous main effect for both locations (Table 2.4b; Figure 2.4c). This interactive effect occurred specifically at the 19°C treatment, where 1200 ppm resulted in heavier weight in SNE larvae (Table 2.4b; Figure 2.4c), and 750 ppm resulted in heavier EGoM larvae (Table 2.4b; Figure 2.4b). Average dry weight for stage II larvae was 1.41mg +/- 0.04mg with no statistical difference between locations (Table 2.4a; Figure 2.4a). EGoM larvae were affected by pCO_2, where 750 ppm resulted in the highest weight, but 1200 ppm resulted in the lowest (Table 2.4b; Figure 2.4e). Higher temperature resulted in heavier larvae in SNE individuals (Table 2.4b; Figure 2.4d, 4f). By Stage III the mass of larvae from EGoM larvae surpassed those from SNE (Table 2.4a; Figure 2.4a), and EGoM stage III larvae raised at warmer temperatures were heavier (Table 2.4b; Figure 2.4h). SNE larvae weighed less and had no treatment effects evident (Table 2.4b; Figure 2.4g).

a



b



c

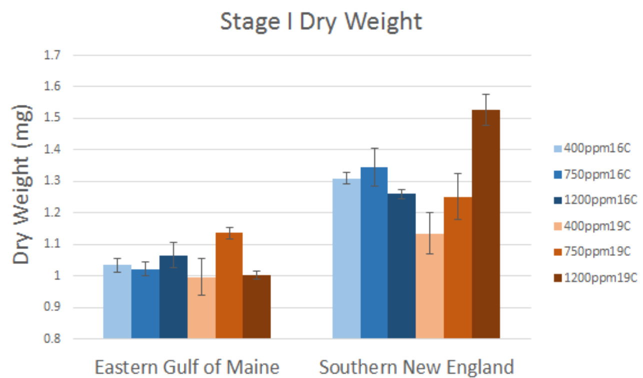


Figure 2.4. Effects of pCO_2 and temperature on dry weight of *H. americanus* (mg, mean ± SE) across stages by location (a), and within stages. Overall treatment effect graphs are shown for each population at each stage (mg, mean ± SE) , and also unambiguous main effects: egg weight by location (b); stage I from EGoM and SNE by temperature and pCO_2 treatment (c); stage II from EGoM and SNE by temperature and pCO_2 treatment (d) EGoM by pCO_2 main effect (e); stage II from SNE by temperature main effect (f); stage III from EGoM and SNE by temperature and pCO_2 treatment effect (g) and stage III from EGoM by temperature effect (h)

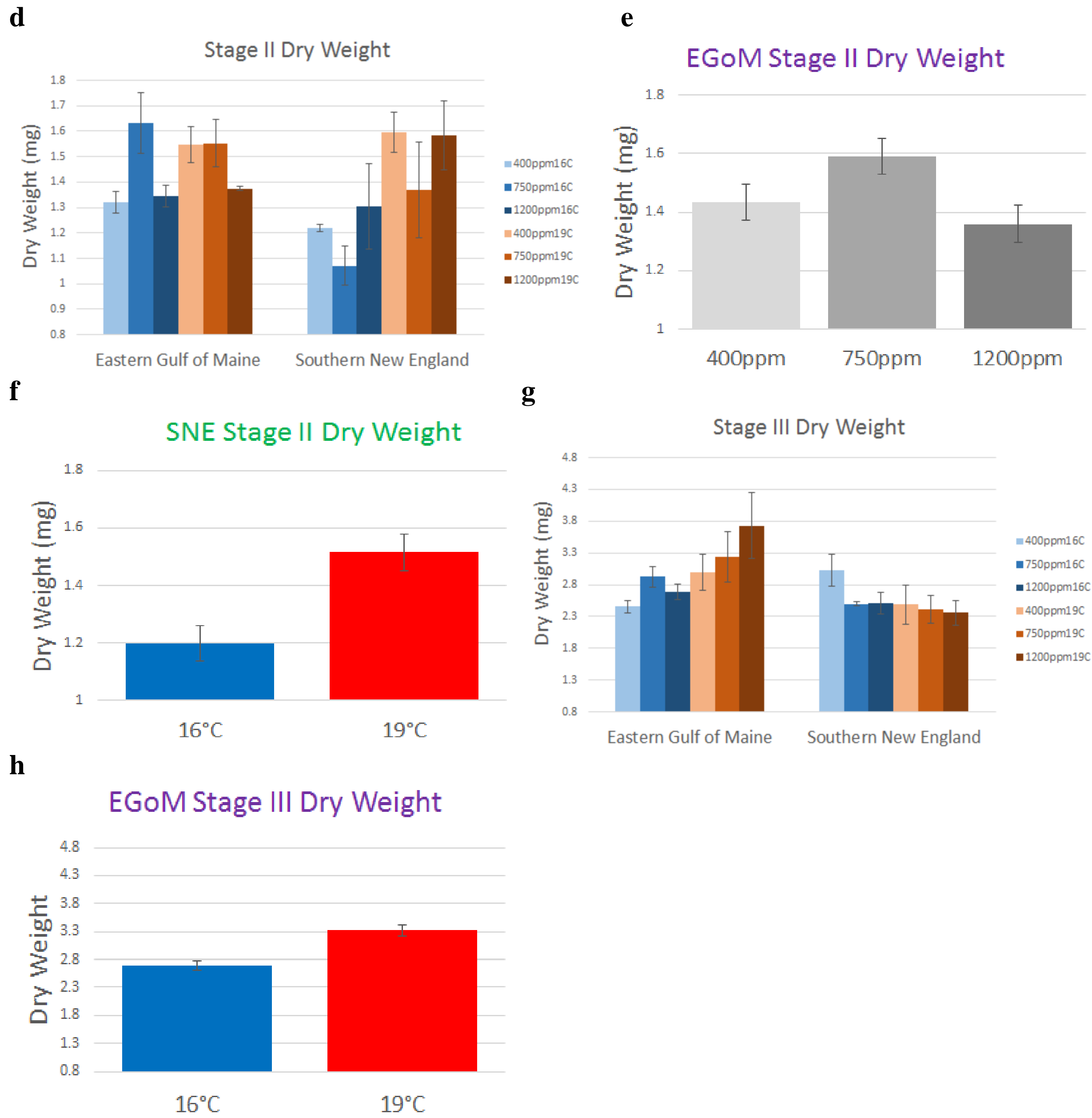


Figure 2.4. continued

Table 2.4. Summary of statistics comparing means of dry weights between two populations (a) and Summary of two-way ANOVA used to evaluate the effect of temperature and pCO_2 of *H. americanus* on dry weight at each stage within each population (b). Significant values (p<0.05) are bolded.

a

Location Comparison for Dry Weight							
Stage	Test	Source of Variation	t statistic	DF	U Statistic	T	p-value
Eggs	*Independent T-test*	*Location*	*0.215*	*4*			*0.84*
I	*Independent T-test*	*Location*	*6.988*	*34*			***<0.001***
II	*Mann-Whitney*	*Location*			*109.00*	*280.00*	*0.097*
III	*Mann-Whitney*	*Location*			*77.00*	*248.00*	***0.008***

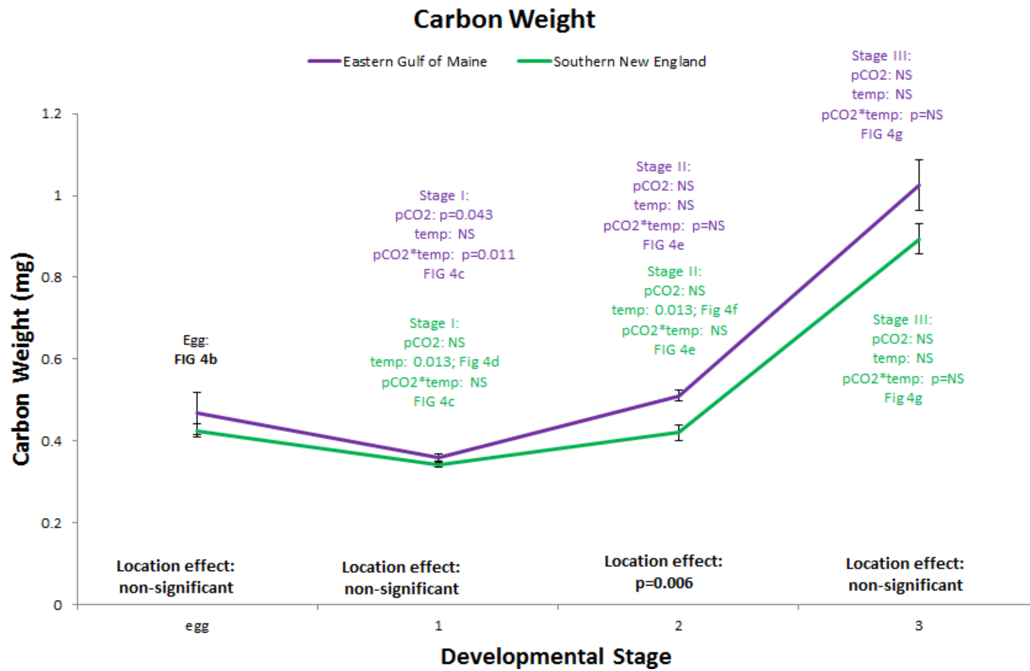
b

ANOVAs for Dry Weight						
Stage	Population	Source of Variation	DF	Mean Square	F	P
I	*Northern Maine*	*pCO2*	*2*	*0.007*	*2.025*	*0.175*
		temp	*1*	*0.000*	*0.020*	*0.890*
		pCO2 x temp	*2*	*0.014*	*4.281*	***0.040***
		Residual	*12*	*0.003*		
		Total	*17*	*0.005*		
	Rhode Island	*pCO2*	*2*	*0.044*	*5.494*	***0.020***
		temp	*1*	*0.000*	*0.000*	*0.985*
		pCO2 x temp	*2*	*0.083*	*10.311*	***0.002***
		Residual	*12*	*0.008*		
		Total	*17*	*0.021*		
II	*Northern Maine*	*pCO2*	*2*	*0.085*	*5.337*	***0.022***
		temp	*1*	*0.015*	*0.948*	*0.349*
		pCO2 x temp	*2*	*0.036*	*2.253*	*0.148*
		Residual	*12*	*0.016*		
		Total	*17*	*0.026*		
	Rhode Island	*pCO2*	*2*	*0.087*	*1.862*	*0.198*
		temp	*1*	*0.451*	*9.674*	***0.009***
		pCO2 x temp	*2*	*0.004*	*0.087*	*0.918*
		Residual	*12*	*0.047*		
		Total	*17*	*0.070*		
III	*Northern Maine*	*pCO2*	*2*	*0.374*	*1.346*	*0.297*
		temp	*1*	*1.780*	*6.403*	***0.026***
		pCO2 x temp	*2*	*0.206*	*0.740*	*0.498*
		Residual	*12*	*0.278*		
		Total	*17*	*0.369*		
	Rhode Island	*pCO2*	*2*	*0.197*	*1.482*	*0.266*
		temp	*1*	*0.305*	*2.289*	*0.156*
		pCO2 x temp	*2*	*0.091*	*0.680*	*0.525*
		Residual	*12*	*0.133*		
		Total	*17*	*0.146*		

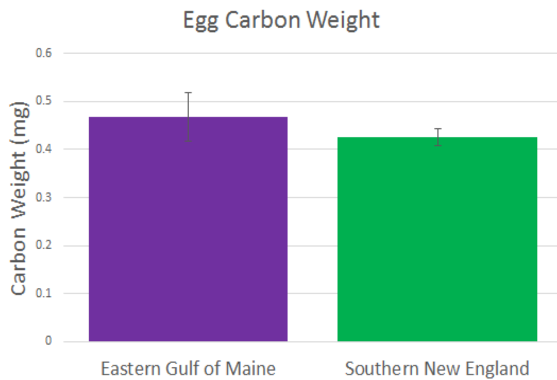
2.3.4 Carbon Weight

Carbon weight within eggs was not statistically different between the two populations (Figure 2.5b; Table 2.5a). Stage I larval carbon weight also did not differ between locations (Figure 2.5a; Table 2.5a), however, there was an interactive effect of temperature and pCO_2 for this stage in the EGoM population; the 750 ppm, 19°C treatment had more carbon weight than any other treatment (Table 2.5b; Figure 2.5c). The SNE population showed a temperature treatment effect in this stage with larvae raised at warmer temperatures having more carbon (Table 2.5b; Figure 2.5d). Within stage II EGoM larvae had more carbon content than SNE (Table 2.5a; Figure 2.5a), however there were no temperature or pCO_2 effects detected in this location (Table 2.5b; Figure 2.5e). Stage II larvae from SNE continued to display a temperature effect; larvae raised at 19°C had more carbon than larvae raised at 16°C (Table 2.5b; Figure 2.5f). Stage III larvae exhibited no treatment nor location effects (Table 2.5a, 5b; Figure 2.5a, 5g).

a



b



c

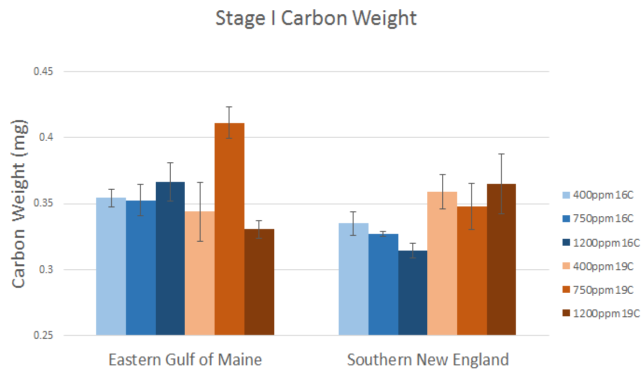
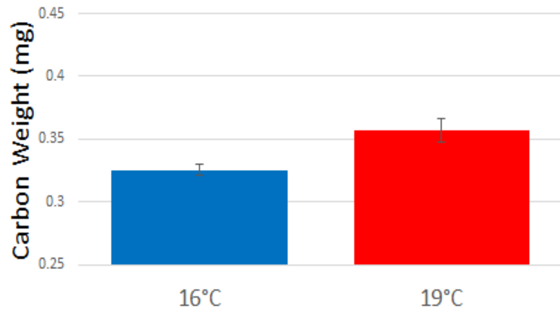
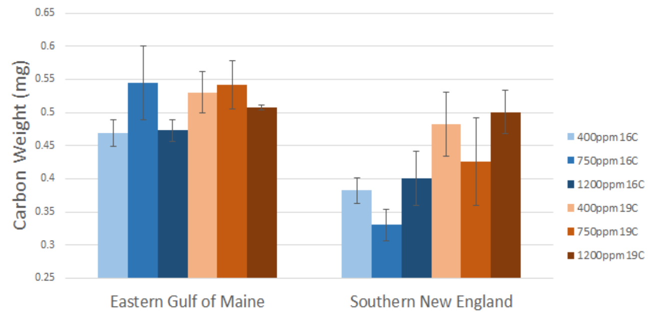


Figure 2.5. Effects of pCO_2 and temperature on carbon weight of *H. americanus* (mg, mean ± SE) across stages by location (a), and within stages. Overall treatment effect graphs are shown for each population at each stage (mg, mean ± SE) , and unambiguous main effects: egg weight by location (b); stage I from EGoM and SNE by temperature and pCO_2 treatment (c); stage I from SNE by temperature main effect (d);stage II from EGoM and SNE by temperature and pCO_2 treatment (e); stage II from SNE by temperature main effect (f); stage III from EGoM and SNE by temperature and pCO_2 treatment effect (g).

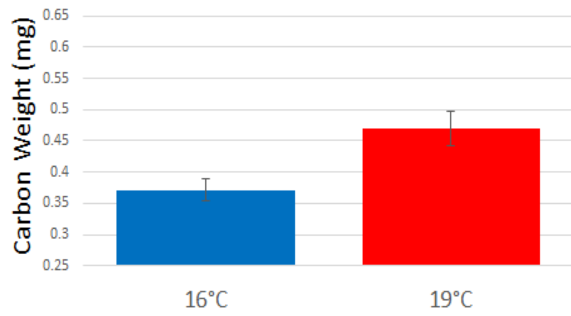
d



e



f



g

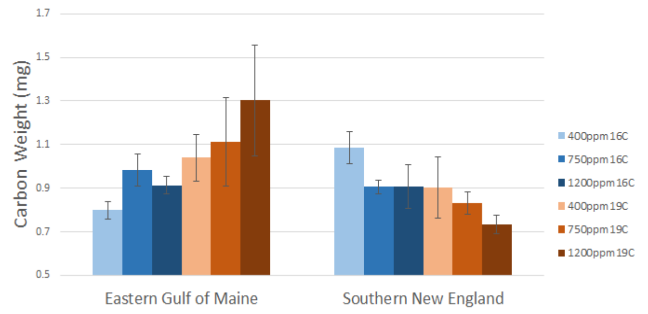


Figure 2.5. continued

Table 2.5. Summary of statistics comparing means of carbon weight between two populations (a) and Summary of two-way ANOVA used to evaluate the effect of temperature and pCO$_2$ of *H. americanus* on dry weight at each stage within each population (b). Significant values ($p<0.05$) are bolded.

a

Location Comparison for Carbon Weight							
Stage	Test	Source of Variation	t statistic	DF	U Statistic	T	p-value
Eggs	*Independent T-test*	*Location*	*-0.092*	*4*			*0.473*
I	*Independent T-test*	*Location*	*-1.85*	*34*			*0.073*
II	*Mann-Whitney*	*Location*			*75.00*	*246.00*	***0.006***
III	*Mann-Whitney*	*Location*			*110.00*	*281.00*	*0.103*

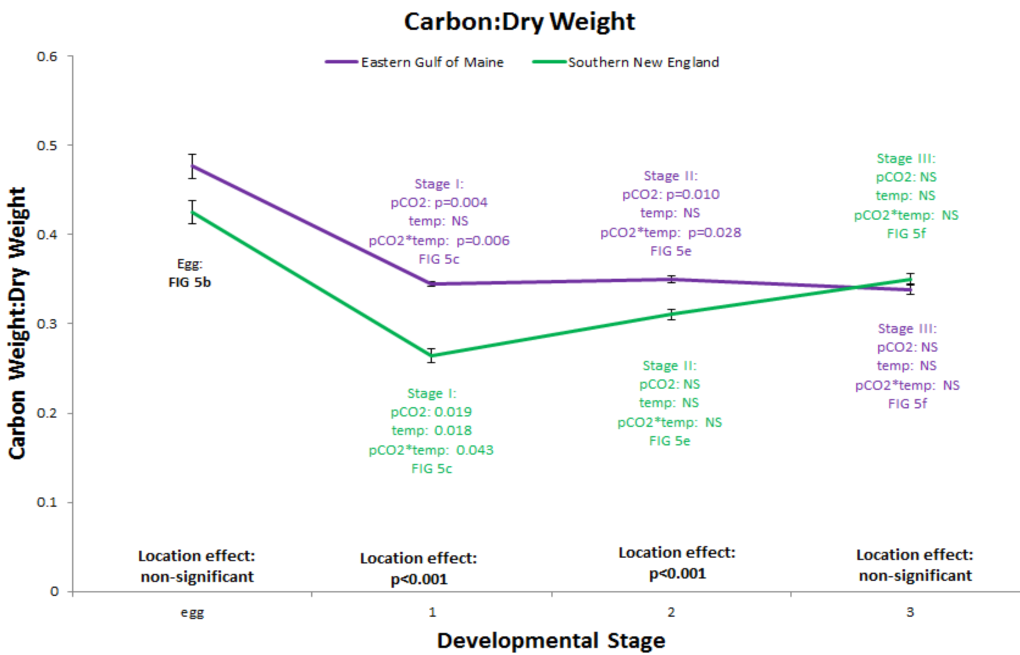
b

ANOVAs for Carbon Weight						
Stage	Population	Source of Variation	DF	Mean Square	F	P
I	*Northern Maine*	*pCO2*	*2*	*0.002*	*4.141*	***0.043***
		temp	*1*	*0.000*	*0.142*	*0.712*
		pCO2 x temp	*2*	*0.004*	*6.713*	***0.011***
		Residual	*12*	*0.001*		
		Total	*17*	*0.001*		
	Rhode Island	*pCO2*	*2*	*0.000*	*0.282*	*0.759*
		temp	*1*	*0.005*	*8.427*	***0.013***
		pCO2 x temp	*2*	*0.001*	*0.726*	*0.504*
		Residual	*12*	*0.001*		
		Total	*17*	*0.001*		
II	*Northern Maine*	*pCO2*	*2*	*0.005*	*1.549*	*0.252*
		temp	*1*	*0.004*	*1.428*	*0.255*
		pCO2 x temp	*2*	*0.002*	*0.521*	*0.607*
		Residual	*12*	*0.003*		
		Total	*17*	*0.003*		
	Rhode Island	*pCO2*	*2*	*0.009*	*1.645*	*0.234*
		temp	*1*	*0.044*	*8.383*	***0.013***
		pCO2 x temp	*2*	*0.000*	*0.001*	*0.999*
		Residual	*12*	*0.005*		
		Total	*17*	*0.007*		
III	*Northern Maine*	*pCO2*	*2*	*0.055*	*0.875*	*0.442*
		temp	*1*	*0.288*	*4.579*	*0.054*
		pCO2 x temp	*2*	*0.026*	*0.409*	*0.673*
		Residual	*12*	*0.063*		
		Total	*17*	*0.071*		
	Rhode Island	*pCO2*	*2*	*0.048*	*2.394*	*0.133*
		temp	*1*	*0.092*	*4.569*	*0.054*
		pCO2 x temp	*2*	*0.005*	*0.253*	*0.781*
		Residual	*12*	*0.020*		
		Total	*17*	*0.026*		

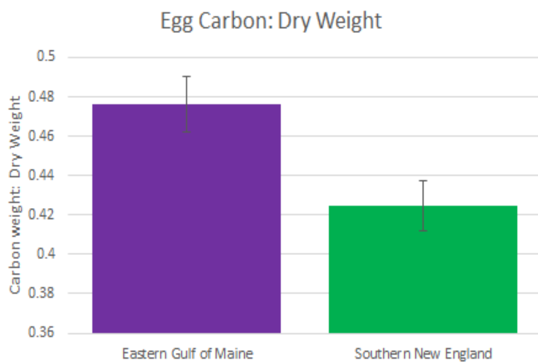
2.3.5 Carbon content as a proportion of dry weight

Eggs from the EGoM had a larger proportion of carbon in their eggs than those from SNE by a marginal level of significance (Table 2.6a; Figure 2.6a, 2.6b) Within Stage I, EGoM had a higher proportion of carbon than SNE larvae (Table 2.6a; Figure 2.6a). Stage I EGoM larvae raised at 750 ppm had a larger proportion carbon within this location (36% +/- 0.6%), but larvae raised at 1200 ppm, 19°C had the lowest (33% +/-0.3%), resulting in a significant interactive effect (Table 2.6b; Figure 2.6c). SNE also had an interactive effect of temperature and pCO_2 (Table 2.6b; Figure 2.6c); larvae raised at 400 ppm 19°C had a higher proportion of carbon to their body weight than other treatments (32% +/- 2.6%). Among Stage II, EGoM larvae maintained a higher proportion of carbon in Stage II (Table 2.6a; Figure 2.6a). Though there were no treatment effects present in SNE larvae (Table 2.6b; Figure 2.6e), EGoM larvae raised at 1200ppm, 19°C overtook other treatments and resulted in the highest proportion carbon within this stage (Table 2.6b; Figure 2.6e). In stage III, all treatment effects and location effects disappeared, resulting in the same proportion carbon for all larvae: 34% +/- 0.39% (Table 2.6a, 6b; Figure 2.6a, 2.6f).

a



b



c

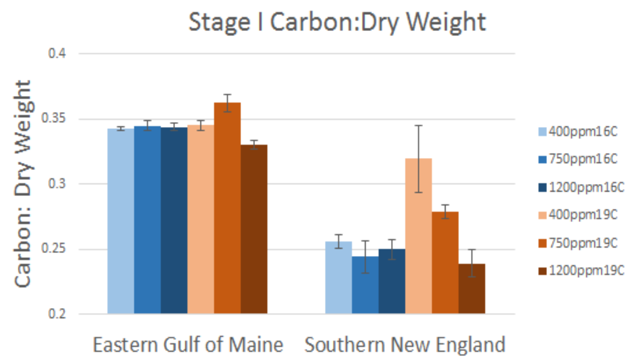
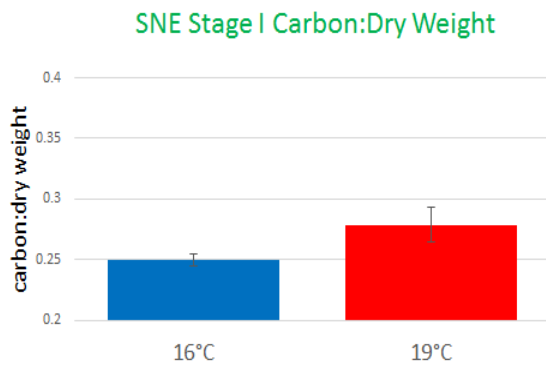
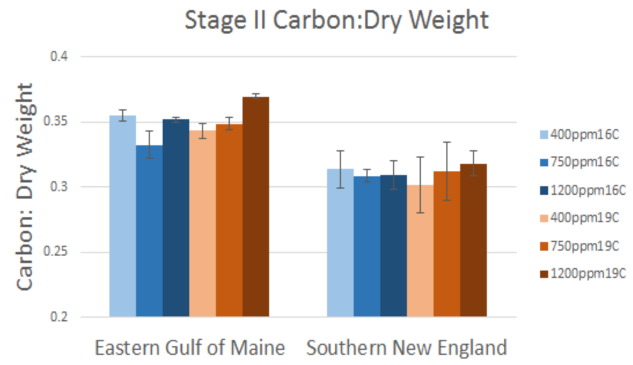


Figure 2.6. Effects of pCO_2 and temperature on proportion of carbon to dry weight of *H. americanus* (mean ± SE) across stages by location (a), and within stages. Overall treatment effect graphs are shown for each population at each stage (mean ± SE) , and unambiguous main effects: egg weight by location (b); stage I from EGoM and SNE by temperature and pCO_2 treatment (c); stage I from SNE by temperature main effect (d);stage II from EGoM and SNE by temperature and pCO_2 treatment (e); stage III from EGoM and SNE by temperature and pCO_2 treatment effect (f).

d



e



f

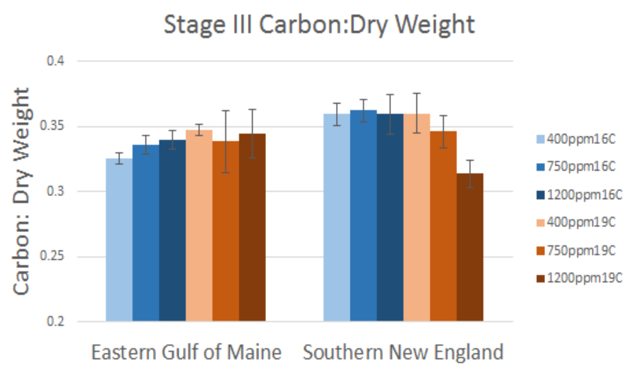


Figure 2.6. continued

Table 2.6. Summary of statistics comparing means of carbon weight: dry weight between two populations (a) and Summary of two-way ANOVA used to evaluate the effect of temperature and pCO_2 of *H. americanus* on carbon: dry weight at each stage within each population (b). Significant values ($p<0.05$) are bolded.

a

Location Comparison for Proportion Carbon							
Stage	Test	Source of Variation	t statistic	DF	U Statistic	T	p-value
Eggs	*Independent T-test*	*Location*	*-2.711*	*4*			*0.054*
I	*Mann-Whitney*	*Location*			*19.00*	*190.00*	***<0.001***
II	*Independent T-test*	*Location*	*-6.238*	*34*			***<0.001***
III	*Independent T-test*	*Location*	*1.536*	*34*			*0.134*

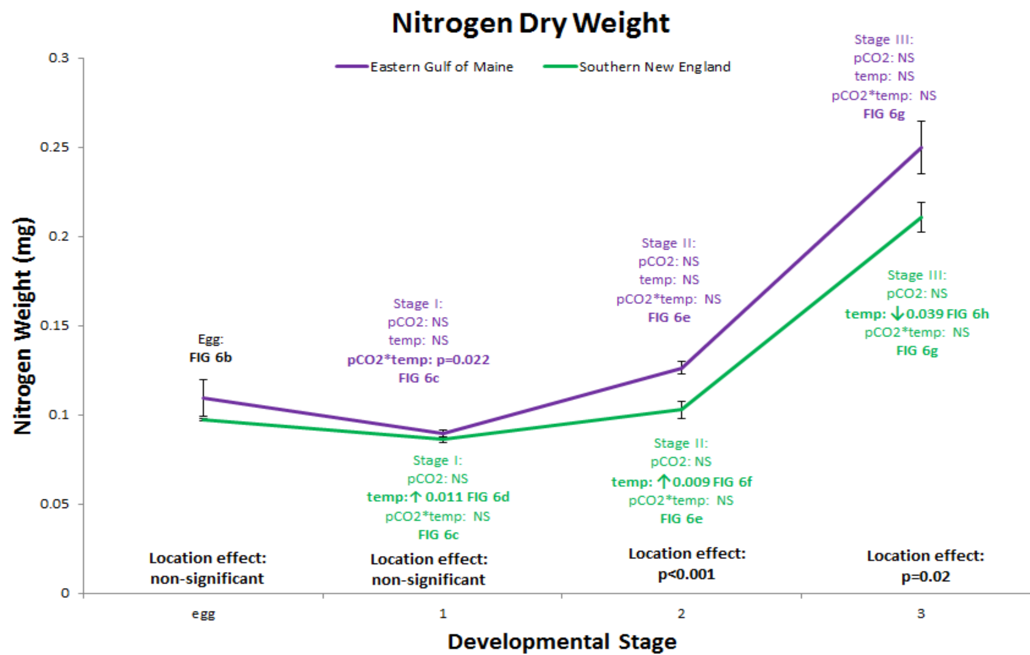
b

ANOVAs for Proportion Carbon						
Stage	Population	Source of Variation	DF	Mean Square	F	P
I	*Northern Maine*	*pCO2*	*2*	*0.000*	*9.299*	***0.004***
		temp	*1*	*0.000*	*0.365*	*0.557*
		pCO2 x temp	*2*	*0.000*	*8.093*	***0.006***
		Residual	*12*	*0.000*		
		Total	*17*	*0.000*		
	Rhode Island	*pCO2*	*2*	*0.003*	*5.571*	***0.019***
		temp	*1*	*0.004*	*7.423*	***0.018***
		pCO2 x temp	*2*	*0.002*	*4.120*	***0.043***
		Residual	*12*	*0.001*		
		Total	*17*	*0.001*		
II	*Northern Maine*	*pCO2*	*2*	*0.001*	*7.027*	***0.010***
		temp	*1*	*0.000*	*2.820*	*0.119*
		pCO2 x temp	*2*	*0.000*	*4.891*	***0.028***
		Residual	*12*	*0.000*		
		Total	*17*	*0.000*		
	Rhode Island	*pCO2*	*2*	*0.000*	*0.082*	*0.922*
		temp	*1*	*0.000*	*0.000*	*0.993*
		pCO2 x temp	*2*	*0.000*	*0.249*	*0.783*
		Residual	*12*	*0.001*		
		Total	*17*	*0.001*		
III	*Northern Maine*	*pCO2*	*2*	*0.000*	*0.118*	*0.890*
		temp	*1*	*0.000*	*0.811*	*0.386*
		pCO2 x temp	*2*	*0.000*	*0.320*	*0.732*
		Residual	*12*	*0.001*		
		Total	*17*	*0.000*		
	Rhode Island	*pCO2*	*2*	*0.048*	*2.399*	*0.133*
		temp	*1*	*0.092*	*3.569*	*0.054*
		pCO2 x temp	*2*	*0.005*	*0.253*	*0.781*
		Residual	*12*	*0.020*		
		Total	*17*	*0.026*		

2.3.6 Nitrogen Weight

As with carbon and dry weight, nitrogen weight also did not differ between the eggs by location of larvae (Table 2.7a; Figure 2.7a, 2.7b). Stage I larvae did not differ in nitrogen weight between locations (Table 2.7a; Figure 2.7a). However, within Stage I, EGoM larvae maintained the higher overall nitrogen weight for larvae raised at 750ppm 19°C (Table 2.7b; Figure 2.7c), and SNE larvae s raised at 19°C had more nitrogen than those reared at 16°C (Table 2.7b; Figure 2.7c, 2.7d). Larvae from EGoM had a higher nitrogen weight at stage II than SNE larvae (Table 2.7a; Figure 2.7a). SNE larvae within this stage maintained a higher nitrogen content when raised at 19°C (Table 2.7b; Figure 2.7e, 2.7f). EGoM larvae saw no treatment effect in this stage (Table 2.7b; Figure 2.7e).In stage III, larvae from EGoM maintained a higher nitrogen weight than SNE larvae (Table 2.7a; Figure 2.7a). Although SNE Stage III larvae continued to exhibit a temperature effect, larvae raised at 16°C contained a higher nitrogen weight than those raised at 19°C (Table 2.7b; Figure 2.7g, 2.7h). EGoM larvae saw no treatment effect in this stage (Table 2.7b; Figure 2.7g).

a



b

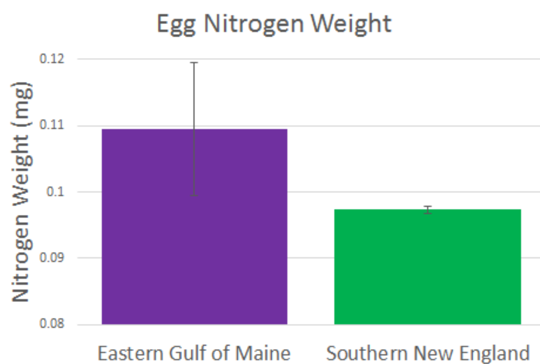
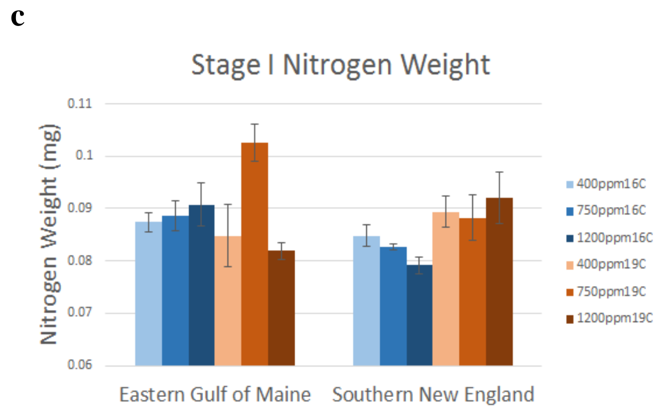
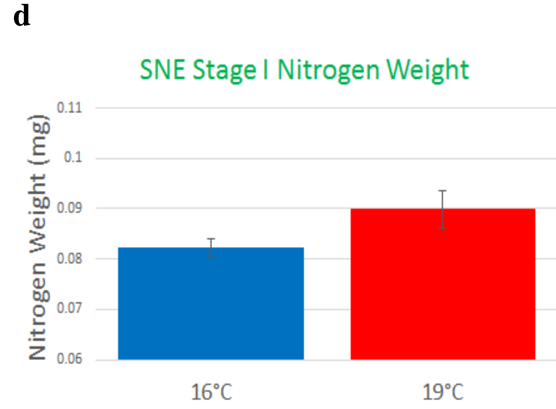


Figure 2.7. Effects of pCO_2 and temperature on nitrogen weight of *H. americanus* (mg, mean ± SE) across stages by location (a), and within stages. Overall treatment effect graphs are shown for each population at each stage (mg, mean ± SE) , and unambiguous main effects: egg nitrogen weight by location (b); stage I from EGoM and SNE by temperature and pCO_2 treatment (c); stage I from SNE by temperature main effect (d);stage II from EGoM and SNE by temperature and pCO_2 treatment (e); stage II from SNE by temperature main effect (f); stage III from EGoM and SNE by temperature and pCO_2 treatment effect (g); stage III from SNE by temperature main effect (h).

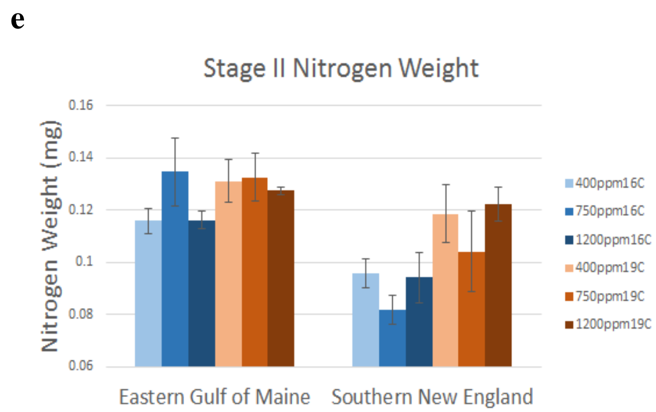
c



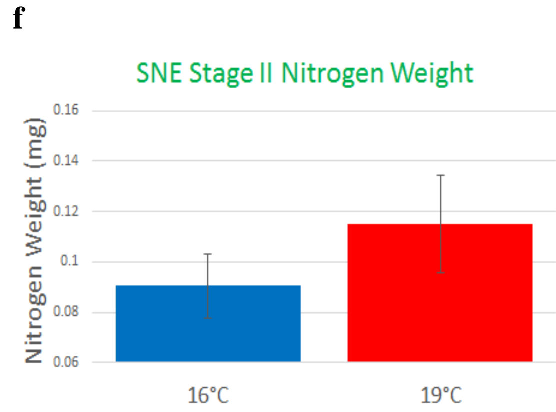
d



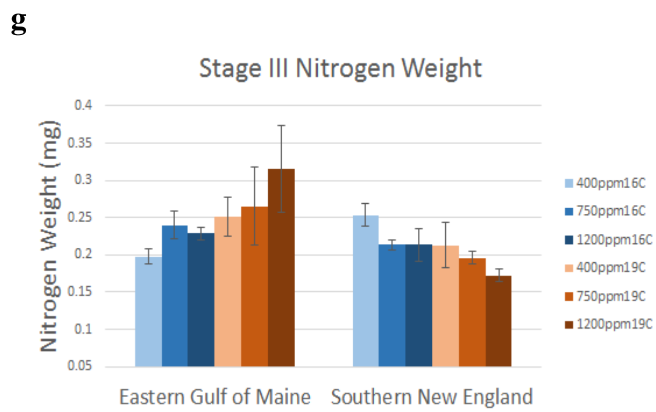
e



f



g



h

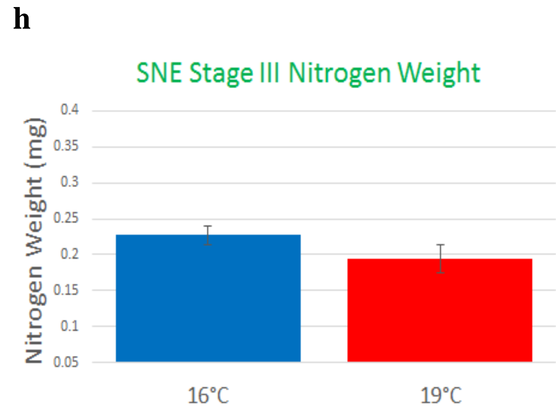


Figure 2.7. continued

Table 2.7. Summary of statistics comparing means of nitrogen weight (mg) between two populations (a) and Summary of two-way ANOVA used to evaluate the effect of temperature and pCO_2 of *H. americanus* on nitrogen weight at each stage within each population (b). Significant values ($p<0.05$) are bolded.

a

Location Comparison for Nitrogen Weight							
Stage	Test	Source of Variation	t statistic	DF	U Statistic	T	p-value
Eggs	*Independent T-test*	*Location*	*-1.204*	*4*			*0.295*
I	*Independent T-test*	*Location*	*-1.308*	*34*			*0.2*
II	*Independent T-test*	*Location*	*-4.089*	*34*			***<0.001***
III	*Mann-Whitney*	*Location*			*88.00*	*259.00*	***0.02***

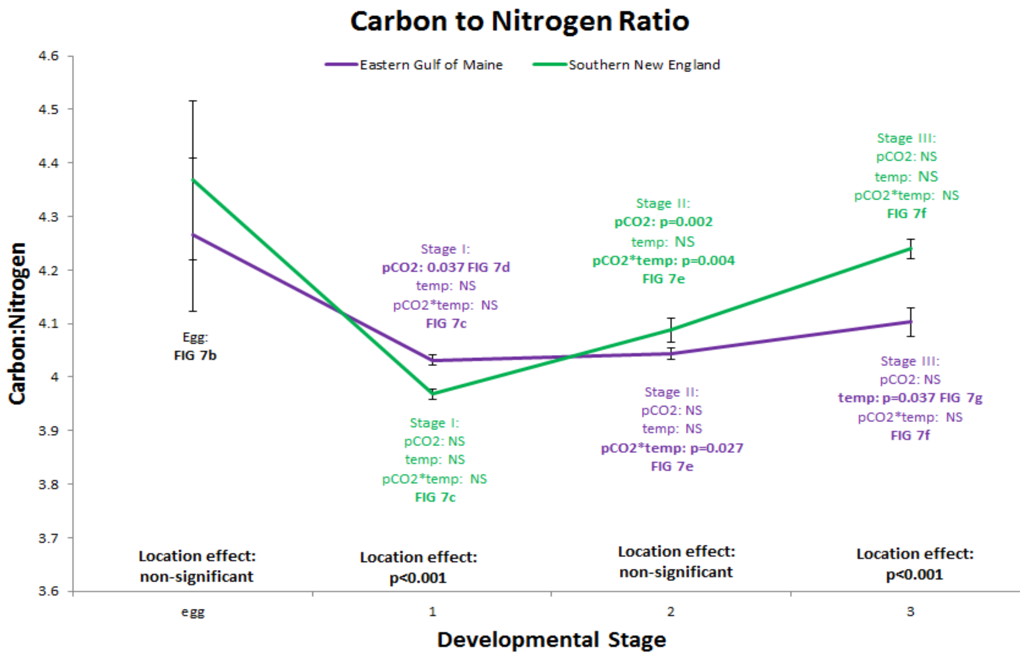
b

ANOVAs for Nitrogen						
Stage	Population	Source of Variation	DF	Mean Square	F	P
I	*Northern Maine*	*pCO2*	*2*	*0.000*	*4.465*	***0.036***
		temp	*1*	*0.000*	*0.087*	*0.773*
		pCO2 x temp	*2*	*0.000*	*5.339*	***0.022***
		Residual	*12*	*0.000*		
		Total	*17*	*0.000*		
	Rhode Island	*pCO2*	*2*	*0.000*	*0.165*	*0.850*
		temp	*1*	*0.000*	*8.946*	***0.011***
		pCO2 x temp	*2*	*0.000*	*1.046*	*0.381*
		Residual	*12*	*0.000*		
		Total	*17*	*0.000*		
II	*Northern Maine*	*pCO2*	*2*	*0.000*	*1.376*	*0.290*
		temp	*1*	*0.000*	*1.649*	*0.223*
		pCO2 x temp	*2*	*0.000*	*0.699*	*0.516*
		Residual	*12*	*0.000*		
		Total	*17*	*0.000*		
	Rhode Island	*pCO2*	*2*	*0.000*	*1.613*	*0.240*
		temp	*1*	*0.003*	*9.675*	***0.009***
		pCO2 x temp	*2*	*0.000*	*0.058*	*0.944*
		Residual	*12*	*0.000*		
		Total	*17*	*0.000*		
III	*Northern Maine*	*pCO2*	*2*	*0.003*	*0.938*	*0.418*
		temp	*1*	*0.014*	*3.703*	*0.078*
		pCO2 x temp	*2*	*0.001*	*0.388*	*0.687*
		Residual	*12*	*0.004*		
		Total	*17*	*0.004*		
	Rhode Island	*pCO2*	*2*	*0.003*	*2.760*	*0.103*
		temp	*1*	*0.005*	*5.350*	***0.039***
		pCO2 x temp	*2*	*0.000*	*0.284*	*0.757*
		Residual	*12*	*0.001*		
		Total	*17*	*0.001*		

2.3.7 Carbon to Nitrogen Ratio

Stage I carbon to nitrogen ratio (C:N) was higher in EGoM larvae (Table 2.8a; Figure 2.8a). Though there was no treatment effects in stage I larvae SNE larvae (Table 2.8b; Figure 2.8c), EGoM larvae saw a lower C:N at the 750 ppm treatment (Table 2.8b; Figure 2.8c, 2.8d). Stage II larvae were not statistically different between locations (Table 2.8a; Figure 2.8a), but both had interactive pCO_2*temp effects; SNE had a high C:N at the 1200ppm 16°C treatment (Table 2.8b; Figure 2.8e), whereas EGoM had a low C:N at the most extreme treatment, 1200ppm 19C (Table 2.8b; Figure 2.8e). SNE Stage III larvae had a statistically higher C:N Ratio than EGoM (Table 2.8a; Figure 2.8a), although no treatment effects for that location were observed (Table 2.8b; Figure 2.8f). EGoM did show a higher C:N ratio in warmer than cooler temperatures (Table 2.8b; Figure 2.8f, 2.8g).

a



b

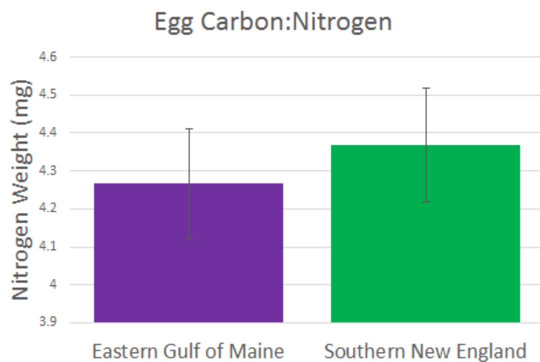
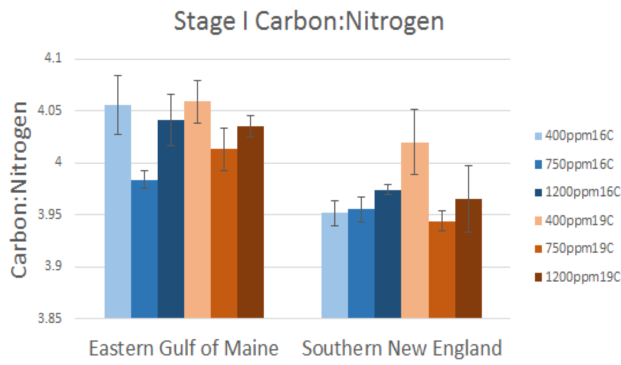
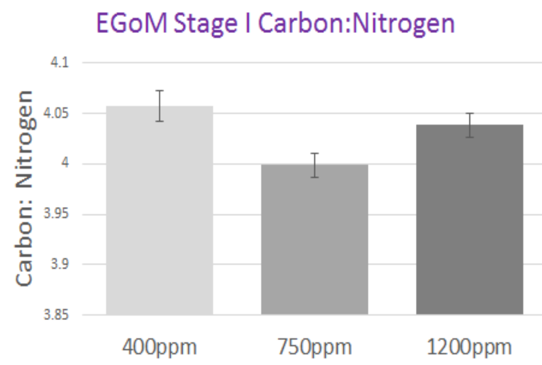


Figure 2.8. Effects of pCO_2 and temperature on carbon to nitrogen weight ratio of *H. americanus* (mean ± SE) across stages by location (a), and within stages. Overall treatment effect graphs are shown for each population at each stage (mean ± SE) , and unambiguous main effects: egg carbon: nitrogen by location (b); stage I from EGoM and SNE by temperature and pCO_2 treatment (c) and STAGE I from EGoM by pCO_2 main effect (d); stage II from EGoM and SNE by temperature and pCO_2 treatment (e); stage III from EGoM and SNE by temperature and pCO_2 treatment effect (f) and EGoM by temperature main effect (g).

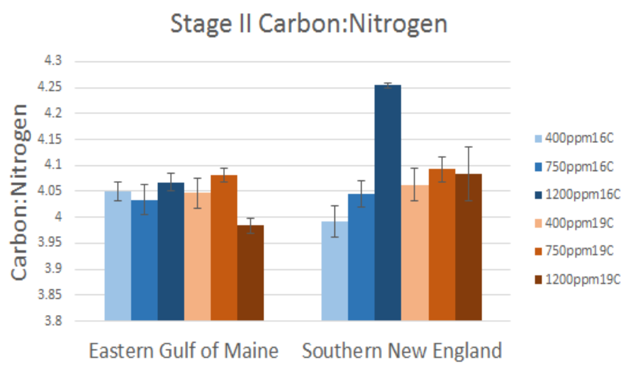
c



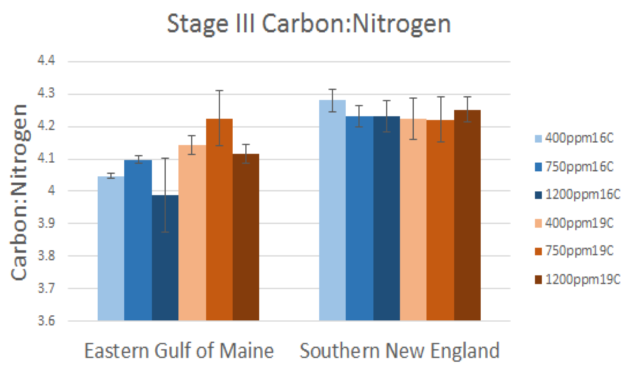
d



e



f



g

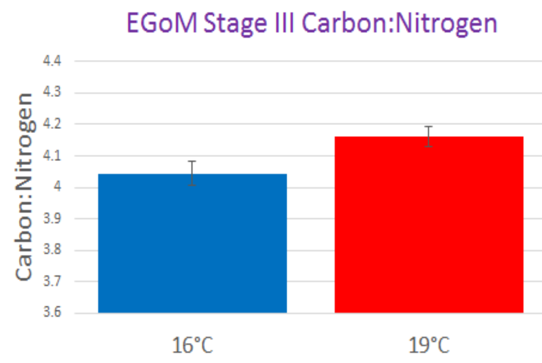


Figure 2.8. continued.

Table 2.8. Summary of statistics comparing means of carbon: nitrogen ratio between two populations (a) and Summary of two-way ANOVA used to evaluate the effect of temperature and $p\mathrm{CO_2}$ of *H. americanus* on nitrogen weight at each stage within each population (b). Significant values (p<0.05) are bolded.

a

Location Comparison for Carbon:Nitrogen							
Stage	Test	Source of Variation	t statistic	DF	U Statistic	T	p-value
Eggs	*Mann-Whitney*	*Location*			*2.00*	*13.00*	*0.4*
I	*Independent T-test*	*Location*	*-4.795*	*34*			***<0.001***
II	*Mann-Whitney*	*Location*			*118.00*	*377.00*	*0.169*
III	*Independent T-test*	*Location*	*4.183*	*34*			***<0.001***

b

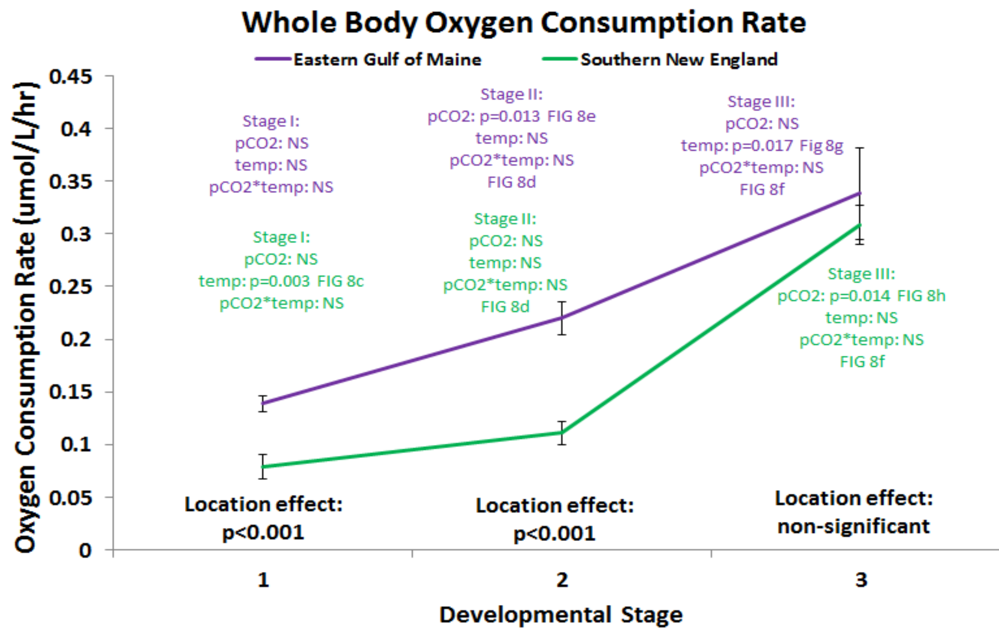
ANOVA's for Carbon:Nitrogen Ratio						
Stage	Population	Source of Variation	DF	Mean Square	F	P
I	*Northern Maine*	*pCO2*	*2*	*0.005*	*4.410*	***0.037***
		temp	*1*	*0.000*	*0.286*	*0.603*
		pCO2 x temp	*2*	*0.001*	*0.408*	*0.674*
		Residual	*12*	*0.001*		
		Total	*17*	*0.002*		
	Rhode Island	*pCO2*	*2*	*0.002*	*1.630*	*0.236*
		temp	*1*	*0.001*	*0.991*	*0.339*
		pCO2 x temp	*2*	*0.003*	*2.552*	*0.119*
		Residual	*12*	*0.001*		
		Total	*17*	*0.002*		
II	*Northern Maine*	*pCO2*	*2*	*0.002*	*1.213*	*0.331*
		temp	*1*	*0.001*	*0.576*	*0.462*
		pCO2 x temp	*2*	*0.007*	*4.974*	***0.027***
		Residual	*12*	*0.001*		
		Total	*17*	*0.002*		
	Rhode Island	*pCO2*	*2*	*0.032*	*10.934*	***0.002***
		temp	*1*	*0.001*	*0.487*	*0.499*
		pCO2 x temp	*2*	*0.027*	*9.076*	***0.004***
		Residual	*12*	*0.003*		
		Total	*17*	*0.009*		
III	*Northern Maine*	*pCO2*	*2*	*0.018*	*1.607*	*0.241*
		temp	*1*	*0.061*	*5.471*	***0.037***
		pCO2 x temp	*2*	*0.000*	*0.044*	*0.957*
		Residual	*12*	*0.011*		
		Total	*17*	*0.014*		
	Rhode Island	*pCO2*	*2*	*0.001*	*0.125*	*0.884*
		temp	*1*	*0.001*	*0.137*	*0.718*
		pCO2 x temp	*2*	*0.002*	*0.309*	*0.740*
		Residual	*12*	*0.008*		
		Total	*17*	*0.006*		

2.3.8 Oxygen Consumption Rate (OCR)

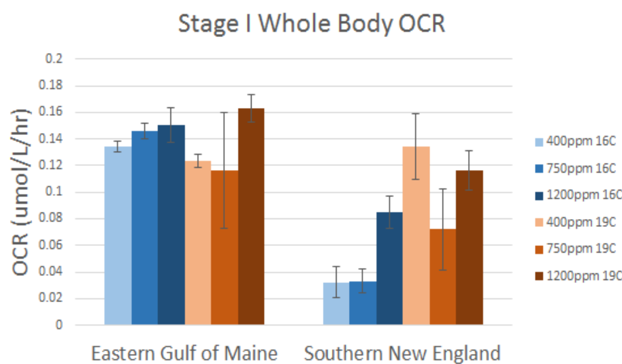
The oxygen consumption rate increased with stage. Stage I larvae from EGoM exhibited a higher OCR than SNE larvae (Table 2.9a; Figure 2.9a). However, EGoM didn't exhibit any treatment effects in Stage I (Table 2.9b; Figure 2.9b), whereas SNE larvae consumed more oxygen at higher temperatures (Table 2.9b; Figure 2.9b, 2.9c). In Stage II, EGoM larvae maintained higher OCRs than SNE (Table 2.9a; Figure 2.9a). In EGoM, OCR was affected by pCO_2; larvae at 750 ppm consumed more oxygen than the other pCO_2 treatments (Table 2.9b; Figure 2.9d, 2.9e). There were no treatment effects for SNE in this stage (Table 2.9b; Figure 2.9d).

In Stage III larvae and there was no statistically significant difference between locations (Table 2.9a; Figure 2.9a). Within Stage III, SNE larvae exhibited a pCO_2 effect, where larvae at 400ppm showed a higher OCR than in the EGoM population (Table 2.9b; Figure 2.9f, 2.9h). Likewise, EGoM larvae showed a temperature effect; larvae showed a greater OCR at 19°C compared to the 16°C treatment (Table 2.9b; Figure 2.9f, 2.9g).

a



b



c

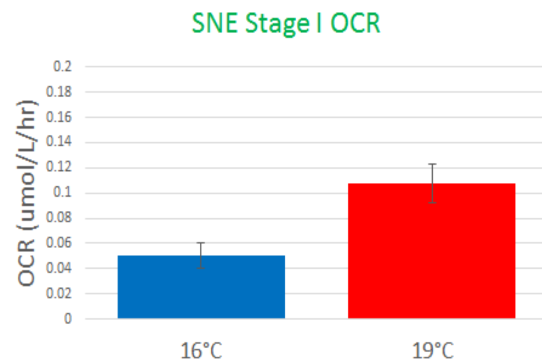
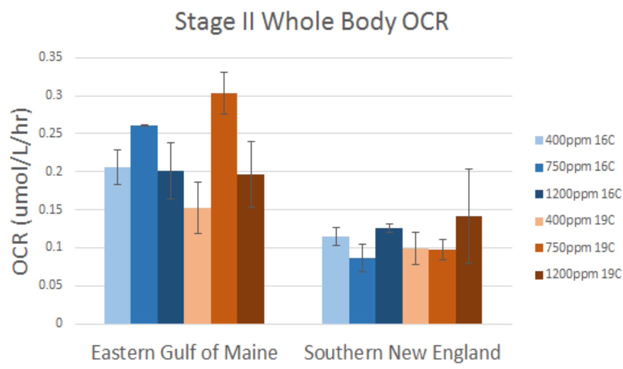
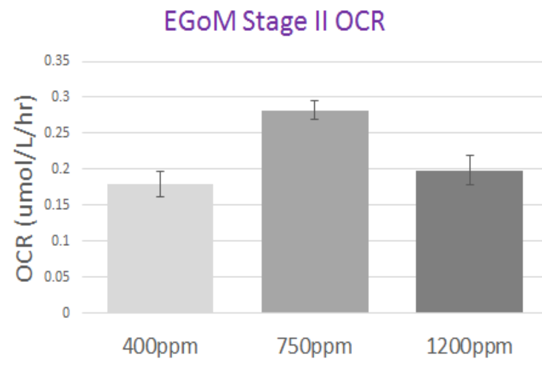


Figure 2.9. Effects of pCO_2 and temperature on whole body oxygen consumption rate of *H. americanus* (µmol/L/hr, mean ± SE) across stages by location (a), and within stages and locations (b-h). Overall treatment effect graphs are shown for each population at each stage (µmol/L/hr, mean ± SE) , and unambiguous main effects: stage I from EGoM and SNE by temperature and pCO_2 treatment (b); stage I from SNE by main temperature effect (c); stage II from EGoM and SNE by temperature and pCO_2 treatment (d) and stage II EGoM by main pCO_2 effect (e); stage III from EGoM and SNE by temperature and pCO_2 treatment effect (f); stage III from EGoM by temperature main effect (g); stage III from SNE by main pCO_2 effect (h).

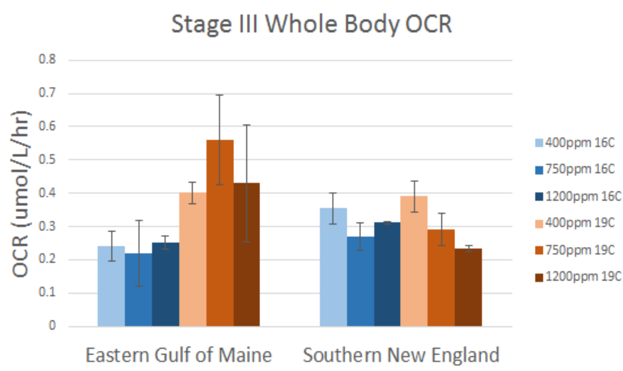
d



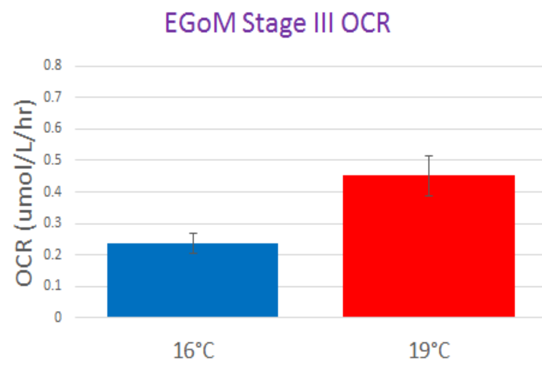
e



f



g



h

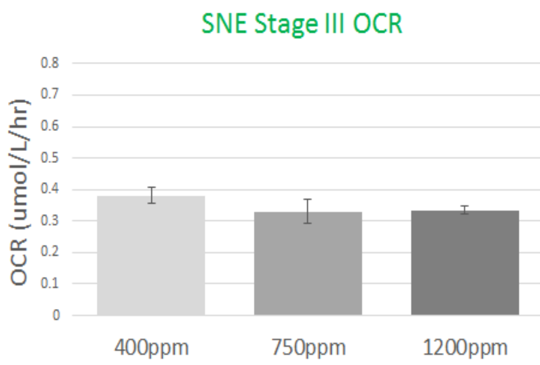


Figure 2.9. continued.

Table 2.9. Summary of statistics comparing means of whole body OCR ratio between two populations (a) and Summary of two-way ANOVA used to evaluate the effect of temperature and pCO_2 of *H. americanus* on OCR t at each stage within each population (b). Significant values ($p<0.05$) are bolded.

a

Location Comparison for whole body Oxygen Consumption Rate							
Stage	Test	Source of Variation	t statistic	DF	U Statistic	T	p-value
I	*Mann-Whitney*	*Location*			*53.50*	*224.50*	***<0.001***
II	*Independent t-test*	*Location*	*-5.684*	*34*			***<0.001***
III	*Mann-Whitney*	*Location*			*151.50*	*307.50*	*0.974*

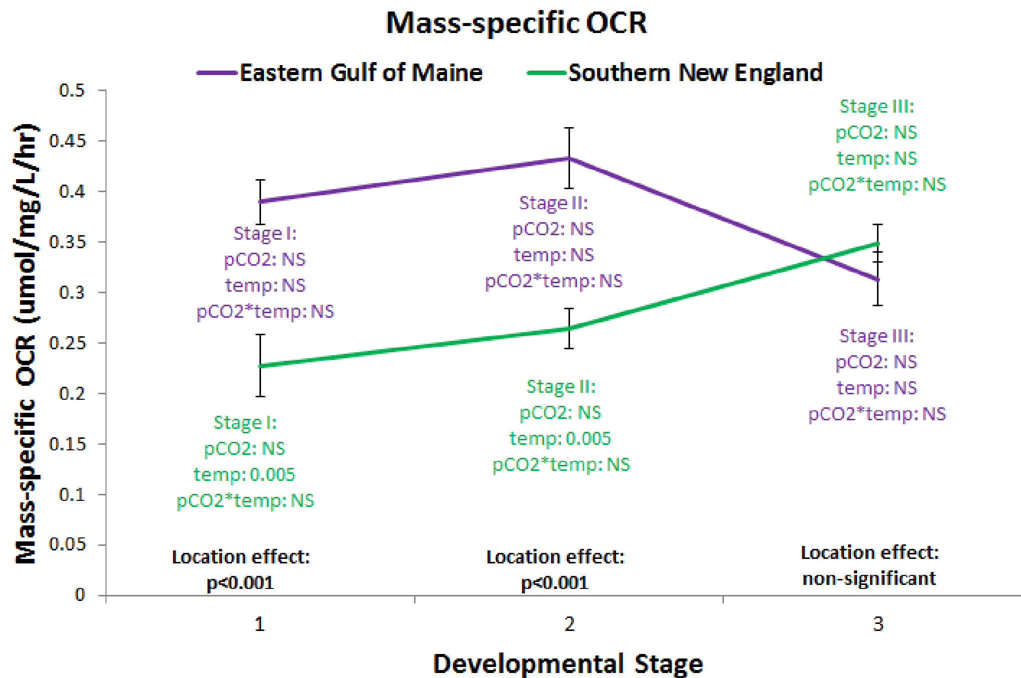
b

ANOVAs for whole body Oxygen Consumption Rate						
Stage	Population	Source of Variation	DF	Mean Square	F	P
I	*Northern Maine-failed normality & homogeneity*	*pCO2*	*2*	*0.001*	*1.295*	*0.309*
		temp	*1*	*0.000*	*0.338*	*0.572*
		pCO2 x temp	*2*	*0.001*	*0.596*	*0.566*
		Residual	*12*	*0.001*		
		Total	*17*			
	Rhode Island	*pCO2*	*2*	*0.004*	*3.301*	*0.072*
		temp	*1*	*0.015*	*13.951*	***0.003***
		pCO2 x temp	*2*	*0.002*	*2.111*	*0.164*
		Residual	*12*	*0.001*		
		Total	*17*			
II	*Northern Maine*	*pCO2*	*2*	*0.018*	*6.309*	***0.013***
		temp	*1*	*0.000*	*0.047*	*0.832*
		pCO2 x temp	*2*	*0.003*	*1.218*	*0.330*
		Residual	*12*	*0.003*		
		Total	*17*			
	Rhode Island-failed normality	*pCO2*	*2*	*0.025*	*0.684*	*0.523*
		temp	*1*	*0.001*	*0.041*	*0.843*
		pCO2 x temp	*2*	*0.009*	*0.246*	*0.786*
		Residual	*12*	*0.036*		
		Total	*17*			
III	*Northern Maine*	*pCO2*	*2*	*0.006*	*0.239*	*0.792*
		temp	*1*	*0.213*	*7.839*	***0.017***
		pCO2 x temp	*2*	*0.013*	*0.468*	*0.638*
		Residual	*12*	*0.027*		
		Total	*17*			
	Rhode Island	*pCO2*	*2*	*0.018*	*4.272*	***0.040***
		temp	*1*	*0.000*	*0.055*	*0.819*
		pCO2 x temp	*2*	*0.006*	*1.329*	*0.301*
		Residual	*12*	*0.004*		
		Total	*17*			

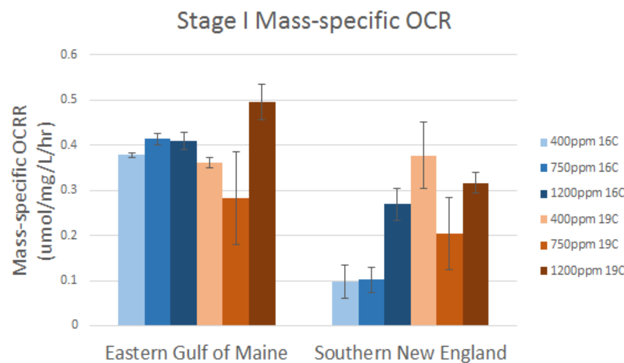
2.3.9 Mass-specific OCR

When corrected for body size, most treatment effects become non-significant for all EGoM larvae (Table 2.10a; Figure 2.10a). EGoM larvae have higher mass-corrected OCR at stage I than SNE larvae (Table 2.10a; Figure 2.10a). Although there are no treatment effects for EGoM larvae, SNE larvae, exhibit a temperature effect for stage I, where larvae reared at 19°C exhibit a higher mass-corrected OCR than those raised at ambient conditions (Table 2.10b; Figure 2.10b, 2.10c). EGoM larvae showed a higher mass specific OCR than SNE through stage II (Table 2.10a; Figure 2.10b). There were no treatment effects for either location (Table 2.10b; Figure 2.10d).By stage III, there was no statistically significant difference between locations (Table 2.10a; Figure 2.10a). There were no treatment effects for either location for stage III (Table 2.10b; Figure 2.10e).

a



b



c

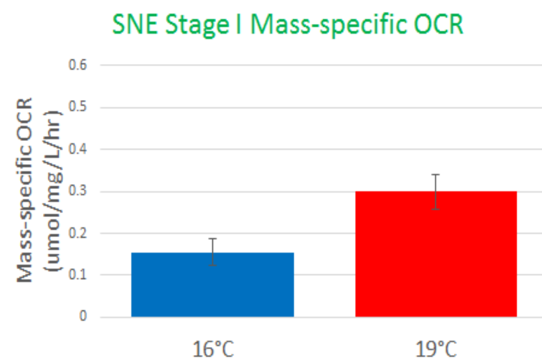
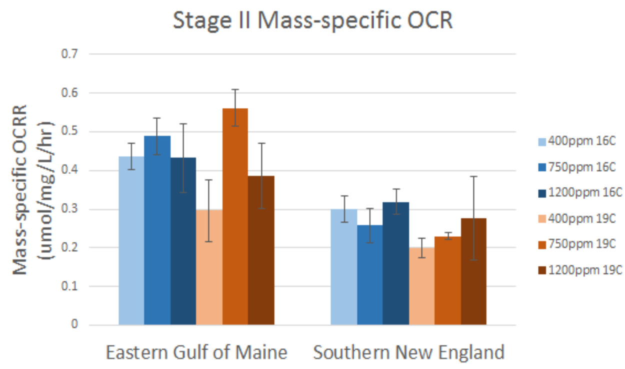


Figure 2.10. Effects of $p\mathrm{CO_2}$ and temperature on mass-specific oxygen consumption rate of *H. americanus* (µmol/mg/L/hr, mean ± SE) across stages by location (a), and within stages and locations (b-e). Overall treatment effect graphs are shown for each population at each stage (µmol/mg/L/hr, mean ± SE), and unambiguous main effects: stage I from EGoM and SNE by temperature and $p\mathrm{CO_2}$ treatment (b); stage I from SNE by main temperature effect (c); stage II from EGoM and SNE by temperature and $p\mathrm{CO_2}$ treatment (d); stage III from EGoM and SNE by temperature and $p\mathrm{CO_2}$ treatment effect (e).

d



e

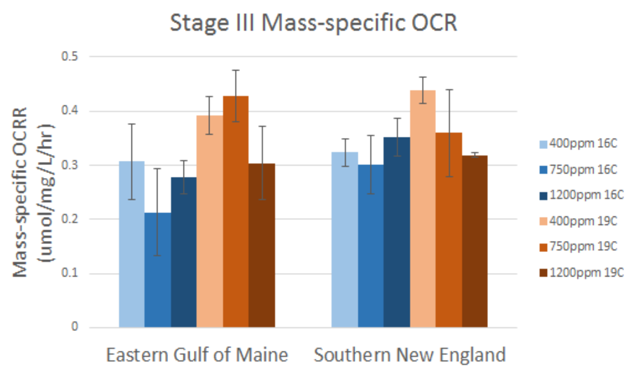


Figure 2.10. continued.

Table 2.10. Summary of statistics comparing means of mass normalized OCR ratio between two populations (a) and Summary of three way ANOVA used to evaluate the effect of temperature and pCO_2 of *H. americanus* on OCR t at each stage within each population (b). Significant values ($p<0.05$) are bolded.

a

Location Comparison for mass specific Oxygen Consumption Rate							
Stage	Test	Source of Variation	t statistic	DF	U Statistic	T	p-value
I	*Mann-Whitney*	*Location*			*46.00*	*217.00*	***<0.001***
II	*Independent T-test*	*Location*	*-4.596*	*34*			***<0.001***
III	*Independent T-test*	*Location*	*1.09*	*33*			*0.284*

b

ANOVAs for mass specific Oxygen Consumption Rate						
Stage	Population	Source of Variation	DF	Mean Square	F	P
I	*Northern Maine-failed normality & homogeneity*	*pCO2*	*2*	*0.018*	*2.839*	*0.098*
		temp	*1*	*0.002*	*0.311*	*0.587*
		pCO2 x temp	*2*	*0.018*	*2.761*	*0.103*
		Residual	*12*	*0.006*		
		Total	*17*	*0.009*		
	Rhode Island	*pCO2*	*2*	*0.030*	*3.739*	*0.055*
		temp	*1*	*0.093*	*11.686*	***0.005***
		pCO2 x temp	*2*	*0.022*	*2.806*	*0.100*
		Residual	*12*	*0.008*		
		Total	*17*	*0.017*		
II	*Northern Maine*	*pCO2*	*2*	*0.041*	*2.998*	*0.088*
		temp	*1*	*0.007*	*0.485*	*0.500*
		pCO2 x temp	*2*	*0.017*	*1.279*	*0.314*
		Residual	*12*	*0.014*		
		Total	*17*	*0.017*		
	Rhode Island	*pCO2*	*2*	*0.005*	*1.737*	*0.212*
		temp	*1*	*0.015*	*0.615*	*0.557*
		pCO2 x temp	*2*	*0.002*	*0.265*	*0.772*
		Residual	*12*	*0.008*		
		Total	*17*	*0.008*		
III	*Northern Maine*	*pCO2*	*2*	*0.005*	*0.486*	*0.628*
		temp	*1*	*0.049*	*4.735*	*0.052*
		pCO2 x temp	*2*	*0.012*	*1.179*	*0.344*
		Residual	*11*	*0.010*		
		Total	*16*	*0.012*		
	Rhode Island	*pCO2*	*2*	*0.005*	*1.664*	*0.221*
		temp	*1*	*0.010*	*0.799*	*0.472*
		pCO2 x temp	*2*	*0.008*	*1.435*	*0.276*
		Residual	*12*	*0.006*		
		Total	*17*	*0.006*		

2.3.10 Swimming Speed

There was no significance of location in swimming speed among Stage IV's (Table 2.11a; Figure 2.11), nor treatment effect for either location (Table 2.11b).

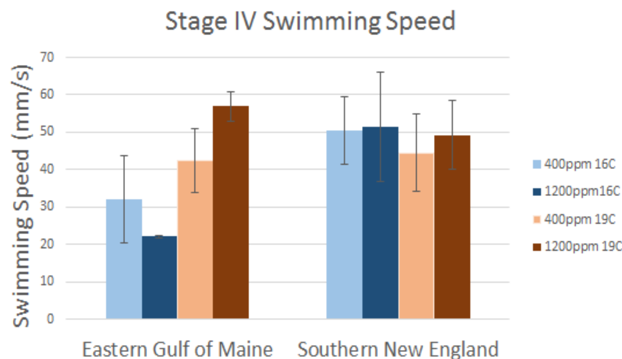


Figure 2.11. Effects of pCO_2 and temperature on swimming speed of stage IV postlarval *H. americanus* (mm/s, mean ± SE) by location.

Table 2.11. Summary of statistics comparing means of swimming speeds between two populations (a) and Summary of two-way ANOVA used to evaluate the effect of temperature and pCO_2 of *H. americanus* on swimming speed of Stage IV postlarvae within each population (b). Significant values (p<0.05) are bolded.

a

Location Comparison for Swimming Speed					
Stage	Test	Source of Variation	U Statistic	T	p-value
IV	Mann-Whitney	Location	53.00	131.00	0.285

b

ANOVAs for Swimming Speed						
Stage	Population	Source of Variation	DF	Mean Square	F	P
IV	Northern Maine	pCO2	1	10.440	0.036	0.854
		temp	1	1101.911	3.788	0.087
		pCO2 x temp	1	328.110	1.128	0.319
		Residual	8	290.899		
		Total	11	336.145		
	Rhode Island	pCO2	1	22.134	0.061	0.811
		temp	1	50.824	0.140	0.718
		pCO2 x temp	1	11.699	0.032	0.862
		Residual	8	363.703		
		Total	11	272.207		

2.3.11 Feeding Rate

There was no significant difference between temperature or pCO_2 treatments in feeding rates among Stage IV's from EGoM (Table 2.12, Figure 2.12).

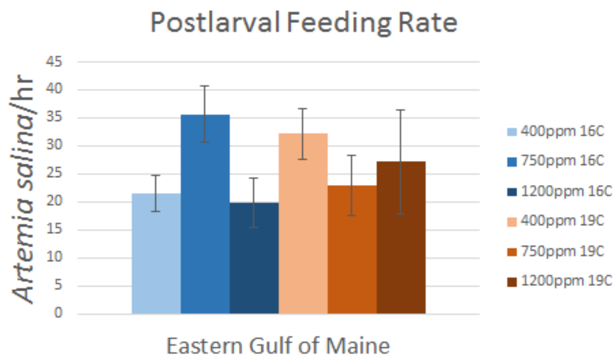


Figure 2.12. Effects of pCO_2 and temperature on feeding rate of stage IV postlarval *H. americanus* (mm/s, mean ± SE) from EGoM.

Table 2.12. Summary of two-way ANOVA used to evaluate the effect of temperature and pCO_2 of *H. americanus* on swimming speed of Stage IV postlarvae from EGoM. Significant values ($p<0.05$) are bolded.

ANOVA for Feeding Rate						
Stage	**Population**	**Source of Variation**	**DF**	**Mean Square**	**F**	**P**
IV	*Northern Maine*	*pCO2*	*2*	*59.058*	*0.486*	*0.622*
		temp	*1*	*17.864*	*0.147*	*0.705*
		pCO2 x temp	*2*	*370.174*	*3.048*	*0.069*
		Residual	*21*	*121.454*		
		Total	*26*	*133.135*		

2.4 Discussion

The response of *H. americanus* larvae to end-century-projected ocean warming and acidification is complex and varies by location of origin and larval stage. Our results suggest that larvae from contrasting thermal regimes respond to joint pCO_2 and temperature stressors differently, although we did not find that response to be consistent across larval stages.

Geographic gradients in temperature can lead to adaptive divergence in physiological and morphological traits at the population level (Sanford & Kelly, 2011). Here we report a strong effect of location of origin for most parameters at most stages, which is consistent with the hypothesis that the steep thermal gradient along the species' range is as a selective force on *H. americanus* larval physiology. Larvae from EGoM tended to be larger in carapace length, dry weight and elemental composition and consumed more oxygen than those from SNE, despite the similarity in initial eggs size.

While we mixed the progeny of several females from each region, we caution that our inferences about location effects are based on a relatively small sample of larvae from each population. We therefore cannot rule out the possibility that factors unrelated to population-level differences, such as maternal effects, may have contributed to variability in the measured parameters. Therefore, additional study is required to confirm population-level differences in the response to elevated temperature and pCO_2.

2.4.1 Growth and Development

As has been reported in previous studies of *Homarus*, the magnitude of the larval response to projected end-century increase in temperature was considerably greater than the response to the end-century levels of pCO_2 (Beal and Chapman, 2001; Arnold *et al*., 2009; Small

et al., 2015; Waller *et al.,* 2017). Larvae reared in the warmer treatment (19°C) reached the postlarval stage nearly seven days earlier than those held at the cooler temperature (16°C), regardless of their origin, whereas larvae reared at 750 ppm and 1200 ppm developed at approximately the same rate as those at 400 ppm. Differences in larval duration can have important implications for larval survival as it can determine their vulnerability to predation or advection (Ennis, 1995; Incze and Naime, 2000; Robert *et al*. 2007; Quinn, 2014). Within the experimental range of temperatures the shorter development time achieved in a warmer environment would likely be beneficial to larvae, allowing them to avoid the high predation rates in the upper water column (Hudon & Fradette, 1988; James-Pirri *et al.,* 1998).

In addition, we observed a strong location effect on the developmental rates of *H. americanus*. Larvae originating from the cooler regime of EGoM took 4.5 d longer to reach postlarval stage those from the warmer regime in SNE, regardless of rearing temperature. This result is consistent with that noted by Quinn *et al*. (2013, 2015) who observed a longer development time of cold-origin than warm-origin animals within the range of temperatures 15 - 21°C. However, outside this range, they observed that cold-origin larvae in colder temperatures developed considerably faster, demonstrating evidence of cold adaptation. Similarly, larvae of the fiddler crab, *Uca pugnax*, originating from cooler thermal regimes develop faster at any given temperature than those from warmer regimes (Sanford, 2006). These differences suggest a potentially adaptive capacity of larvae originating from cooler regimes to compensate for colder temperatures. Evidence of adaptation to warm regimes is less clear for the American lobster.

Growth in body size over time is an important performance metric as it relates to survival (Lawton and Lavalli, 1995; Waddy *et al.*, 1995). Molt increment is controlled by temperature and growth aspects preceding the molt, including cell division and nutritional content (Hartnoll,

1982). While growth studies with the American lobster have demonstrated maximum growth rates at 18°C, higher temperatures have been shown to result in smaller molt increments and higher mortality (Botsford *et al.,* 1974; Aiken and Waddy, 1976; Wahle *et al.,* 2013). The declines in growth rate above 18°C may therefore be indicative of the onset of physiological stress (MacKenzie, 1988; Quinn, 2017). Recent work by Menu-Courey *et al*. (2019) has indicated that carapace size may be affected by pCO_2 in stage V juveniles, but these conclusions are derived through linear regression analysis including acute pCO_2 levels up to 3000µatm. In accordance to our observations, these data do not indicate an effect at end-century levels.

Although we found no difference between the two populations with respect to egg weight or Stage I larval carapace length, we did find that body mass and elemental mass (C:N) of Stage II and III larvae from EGoM were significantly greater than those from SNE, regardless of temperature or pCO_2 treatment. The mechanism behind this faster growth is not well understood, but it may reflect a greater capacity of larvae originating from cooler regimes to achieve greater growth efficiencies within the experimental range of temperatures (Hartnoll, 1982). In addition, larvae from EGoM (cold-origin) and SNE (warm-origin) were similar in original elemental and dry weights of eggs, but diverged during the three larval stages, with EGoM maintaining greater weight in all parameters.

Overall, an increasing C:N ratio indicates higher muscle turnover and higher metabolic activity, presumably in the context of environmental stress (Walther *et al.*, 2010; Small *et al.* 2015). However, there was no consistent pattern to a treatment or origin effect. Because we measured overall carbon weight, this parameter could have been complicated by differing carbon content in tissue versus exoskeleton.

Within Stage I, EGoM showed a significant interactive effect between pCO_2 and temperature for every parameter of elemental and overall dry weight. The 19°C, 750 ppm treatment produced larger larvae that were higher in carbon and nitrogen content than any other treatment. This result drove a consistent interactive effect within this stage, and is difficult to explain, since it is a midpoint treatment. Also, larvae in this treatment were not consistently heavier in later developmental stages and therefore it is difficult to interpret this ambiguous result.

2.4.2 Oxygen Consumption

Oxygen consumption rate (OCR) is a conventional proxy for metabolic rate. We examined whole organism OCR and mass-specific (per unit carbon weight) OCR. Whole-body OCR increased exponentially with body size from SI to SIII as would be expected. Typically, ectotherms exhibit higher respiration rates in warmer water (Somero *et al*., 2017) but this was only apparent in stage I larvae from SNE and stage III larvae from EGoM. It is possible that individual variability overpowered this treatment effect, a documented challenge to larval work (Somero *et al*., 2017).

We observed elevated oxygen consumption rates at high pCO_2 in Stage II larvae from EGoM with a higher OCR in our intermediate pCO_2 treatment (750 ppm), as well as in our SNE low pCO_2 treatment larvae (400ppm), but this phenomenon was neither consistent across stages or subpopulations. These results are similar to the OCRs of *H. americanus* from the midcoast region of Maine (Waller, *et al*., 2016) and other crustaceans such as the copepod *Calanus glacialis* and shrimp larvae *P. borealis*, which have shown little to no effect of pCO_2 on respiration rates (Arnberg *et al*., 2013; Bailey *et al*., 2016).

2.4.3 Behavior

This study measured two metrics of behavior: swimming speed and feeding behavior. Increased swimming speed can be metabolically costly to planktonic crustaceans (Morris *et al*. 1985), but may increase the encounter rate with potential prey. However we saw no effect of either temperature or pCO_2 on swimming speed.

Although feeding rate was only measured in EGoM postlarvae, we found no significant effects of temperature or pCO_2 treatments on feeding. This result was in contrast with Waller *et al*. (2016) who found an increase in feeding activity at elevated pCO_2. Previous studies on terrestrial arthropods have noted that higher consumption rates correlate with higher metabolism in insects (de Valpine and Harte, 2001; Rall *et al*., 2010). However, when metabolic rates rise more quickly than consumption rates, the resulting ingestion inefficiency can cause reduced fitness, as in the urchin *Lytechinus variegatus* (Lemoine & Burkepile, 2012). Although we did not see a change in feeding behavior in our experiment, it is possible that increasing stressors can lead to a reduced ingestion efficiency for larvae from the EGoM population.

2.4.4 Conclusion

Within this study, end-century projected temperature elicited a greater response in more of the measured variables than end-century-projected pCO_2. These results support previous studies examining the joint effects of elevated temperature and pCO_2 on lobster larvae (Small *et al*., 2015; Waller *et al*., 2017). The effects, however, differed notably between larvae originating from the different thermal regimes of eastern Gulf of Maine and southern New England. One consistent trend was a strong difference between the populations in larval stages I and II across measured parameters. Larvae from EGoM had a higher proportion carbon relative to dry weight, and consumed more oxygen per unit body weight than those from SNE. Within these two stages,

SNE appeared to be more responsive to the temperature increase, resulting in larvae that were longer and heavier, and had higher mass-specific OCRs at elevated temperature. Larvae from the EGoM, on the other hand, only exhibited a temperature effect on development. We found no evidence that larvae from this cooler regime were adversely affected by the warmer temperature treatments.

For several response variables, patterns discovered in Stage I-II larvae were not evident in Stage III. The molt from stage II to III represents a considerable bottleneck in laboratory studies of this species, due presumably to a high physiological stress of molting and an opportunity for cannibalism (MacKenzie, 1988; Small *et al.,* 2015; Waller *et al*., 2016). In our experiments, differences between populations observed in previous stages often disappeared at this stage, and the low numbers of surviving stage III larvae may have exacerbated our ability to statistically resolve treatment effects.

Lastly, climate change studies have demonstrated that for some ectotherms, the combination of environmental stressors can exhibit significant interactive effects while single stressor effects go undetected (Werberg *et al*., 2012). However, this study did not show consistent interactive effects, though EGoM larvae exhibited interactive effects more often under the combined exposure to the most extreme treatment of 19°C and 1200 ppm.

The effects of projected climate change are complex, and manifested by differences in a species-specific, stage-specific, and possibly population-specific scale. Understanding these relationships is critical in order to understand and predict the effects of warming and acidification on this economically and culturally valuable species.

CHAPTER 3

GENE REGULATORY RESPONSE IN POSTLARVAE

3.1 Introduction

The impact of ocean acidification (OA) and warming (OW), as single and joint stressors, varies across the diversity of marine invertebrates (Kurihara, 2008; Fabry *et al.,* 2008; Wittmann & Portner, 2013; Kroeker *et al*., 2013). Some species have a capacity to tolerate acid-base disturbances (Melzner *et al.,* 2009, Harms *et al*., 2014). Crustaceans, as a group, have been shown to respond to effects of OA though changes in acid-base homeostasis, mortality, growth rates and metabolism (Pane & Barry, 2007; Walther, *et al*., 2010; Whiteley, 2011; Schiffer *et al*., 2012; Zittier *et al*., 2013; Long, *et al.,* 2013; Small *et al*., 2015; Waller *et al.,* 2017). These responses are highly interdependent, and compensating for adverse effects of stressful environments involves added energy consumption.

The larval stages of the American lobster (*Homarus americanus*) are of particular concern as important biological bottlenecks for recruitment to the benthic population (Incze *et al*., 2007). Not only do they occupy the upper water column, where temperature and pH can fluctuate quickly, but they also undergo a rapid succession of physiologically demanding molts within these conditions (Factor, 1995). Results from the previous chapter, as well as physiological studies on larval and early juvenile *Homarus,* suggest a range of responses to end-century acidification and warming (Ries *et al*., 2009; Keppel *et al*., 2012; Agnalt *et al*., 2013; Small *et al*., 2015; Waller *et al*., 2017; Rato *et al*., 2017; Menu-Courey *et al*., 2019). Experimental studies to date suggest that the larval response to warming projected for the end of the 21st century is greater than that for the levels of $p\mathrm{CO_2}$ predicted by that time (Agnalt *et al*., 2013; Small *et al*., 2015; Waller *et al*., 2017). However, this should not be taken to mean there

is no response to changes in *p*CO_2. Recent research with lobster larvae exposed to elevated *p*CO_2 observed that while there was no detectable response in physiological parameters, a number of genes for exoskeleton formation and stress response were differentially expressed (Waller, 2016). The present study provides important insight into how *H. americanus* may be compensating to hypercapnic conditions on through gene regulation. To our knowledge, no study has examined molecular-level compensation within *H. americanus* larval stages to effects of warming and acidification as joint stressors.

Although *H. americanus* is a well-studied organism in biological and genomic research, a fully annotated genome for the species currently does not exist. Transcriptome sequencing has become an important tool to understand molecular responses to environmental stress, particularly among organisms without a complete annotated genome. Next generation (NextGen) sequencing provides a wealth of information that allow physiological investigation within and among organism stages, populations or environmental conditions (Hines *et al*., 2013; Clark & Greenwood, 2016). The advantage this tool confers is the ability to detect molecular compensation for environmental stress that may otherwise go undetected using more traditional physiological studies (Gracey, 2007). As genetic techniques for analysis increase in complexity and efficiency, transcriptomic analyses have become important metrics for quantifying the expression of stress-related genes (Evans & Hoffman, 2012). This approach enables one to examine a broad range of genetic responses to environmental change on early life stages (Todgham & Hofmann, 2009; Harms *et al*., 2014).

In the present study, we evaluated the joint effects of elevated *p*CO_2 and warming associated with end-century projected oceanic conditions within the Gulf of Maine (IPCC, 2013) on regulatory gene response in postlarval *H. americanus* . To our knowledge, this is the

first report of the impact of changes in pCO_2 and temperature, as joint stressors, on gene expression in the postlarval stage of this species.

3.2 Methods

Seven ovigerous female American lobsters (*Homarus americanus*) were collected from the coastal waters of Rhode Island in summer 2016 by the Rhode Island Department of Environmental Management's Fisheries Section. Egg bearing females were held at the University of Maine's Darling Marine Center, Walpole, ME, in aerated, 300 L hatching tanks at ~15°C until hatching. Upon hatching, stage I larvae were transported to Bigelow Laboratory of Ocean Sciences and distributed randomly in 18 20-L buckets, pre-equilibrated to the experimental treatments. Each bucket was stocked with 250 larvae, resulting in a starting density of 12.5 larvae/L.

Larval rearing tanks were maintained in a full factorial design with two temperatures and two levels of pCO_2. Temperatures represented average present-day summer sea surface temperature for the Gulf of Maine (16°C), and the 3°C predicted increase for the end of the century (19°C). All tanks were held in a temperature controlled room at 16°C and the elevated temperature treatments (19°C) were achieved using Hydor submersible aquarium heaters.

Two pCO_2 treatments (400ppm and 1200 ppm) were achieved by mixing CO_2 with CO_2-stripped, compressed air to create predetermined concentrations of gasses(Waller *et al.* 2017).The two pCO_2 concentrations represented the ambient atmospheric concentration (400ppm), and an elevated pCO_2 concentration to generate pH values representative of end-century projected estuarine and coastal regions (1200ppm) (IPCC, 2013; Gledhill *et al*., 2015; Table 1). Each of the six treatment combinations were maintained in triplicate.

Salinity, temperature and pH were monitored daily. Salinity was measured using an Oakton SALT meter and pH and temperature were measured using a Thermo Orion 3-star benchtop pH probe, calibrated using NIST buffers. Spec pH and alkalinity was measured weekly to validate daily readings. The pH(tot) was measured spectrophotometrically (Hitachi U-310 dual-beam, Company, location) using the pH sensitive indicator dye m-cresol purple (Sigma-Aldrich) following SOP (standard operating procedure 6b: Dickson, 2007; Table 3.1).

Table 3.1. Water Chemistry parameters during course of experiment. All parameters list average value and SD through experimental period.

Treatment	Temperature (°C)	Salinity (ppt)	pH	ΩCa	ΩAr
400ppm 16C	16.6 ± 0.5	30.3 ± 0.8	7.94 ± 0.06	2.34 ± 0.10	1.45 ± 0.06
750ppm 16C	17.1 ± 0.4	29.8 ± 0.4	7.75 ± 0.03	1.68 ± 0.12	1.05 ± 0.07
1200ppm 16C	17.0 ± 0.4	30.0 ± 0.8	7.56 ± 0.01	1.16 ± 0.025	0.72 ± 0.02
400ppm 19C	18.7 ± 0.4	30.2 ± 0.9	7.89 ± 0.03	2.44 ± 0.25	1.52 ± 0.16
750ppm 19C	19.3 ± 1.0	30.2 ± 1.0	7.81 ± 0.04	2.23 ± 0.23	1.38 ± 0.14
1200ppm 19C	19.5 ± 1.0	29.9 ± 0.7	7.63 ± 0.01	1.42 ± 0.004	0.88 ± 0.002

Tanks were monitored daily for presence of postlarvae. Postlarvae were separated into individual containers and maintained at treatment levels for 48 hours. After this period, animals were starved for 24 hours to flush residual *Artemia salina* genetic material out of their digestive tract. Post larvae were rinsed thrice in UV-sterilized 0.2 µm FSW and placed in sterile cryotubes with 3mL RNAlater (Ambion, USA). All samples were kept at -80°C for long term storage.

RNA was extracted following the method of Clark *et al.* (2013) and RNA-seq performed on a total of 11 animals drawn from the four treatment combinations (n=3 in all treatments except the ambient 16°C, 400ppm treatment, where n=2). Sample RNA was processed and read at Genome Quebec (Montreal, PQ, Quebec). RNA was prepared for a DNA-library construction using TruSeq Stranded Total RNA Prep kit (Illumina). Quality was assessed and paired-end Sequences read using Illumina 2000. Raw sequence reads were

uploaded onto the Galaxy web platform and analyzed on the public server at usegalaxy.org (Afgan *et al*., 2016). Quality assessment of raw reads was done using FastQC (Blakenberg *et al*., 2010, Galaxy Version 1.0.0) and trimmed adapter sequences were removed using Trim Galore! (Galaxy Version 0.4.3.1). A minimum of three trimmed sequences were concatenated from each treatment. A *de novo* transcriptome was constructed using Trinity (Langmead *et al*., 2009, Galaxy Version 0.0.1). A mapped file of the reads to the transcripts was generated using HISAT2 and a count file for each sample using StringTie. The file counts were merged using StringTie Merge and normalized using Featurecounts. Differential expression of genes between treatments was analyzed using both DESeq2 and edgeR.

Differentially expressed genes were uploaded onto Blast2Go and assigned gene names using Blastx, functional terms were assigned with Gene Ontology, enzyme numbers were assigned with KEGG and domain information assigned with Interpro (Blast2GO 4.0.2, Ashburner *et al*., 2000; Gotz *et al*., 2008; Cock *et al*., 2013).

3.3 Results

RNA transcripts were compared from *H. americanus* postlarvae raised in the ambient treatment (400 ppm 16°C) to those raised at the same pCO_2 but at elevated temperature (400 ppm 19°C), to those raised at elevated pCO_2 but ambient temperature (1200 ppm 16°C), and those raised under elevated levels for both stressors (1200 ppm 19°C). Using the DESeq2 differential expression software, we identified 1,108 unannotated genes that were differentially expressed across treatments (Figure 3.1).

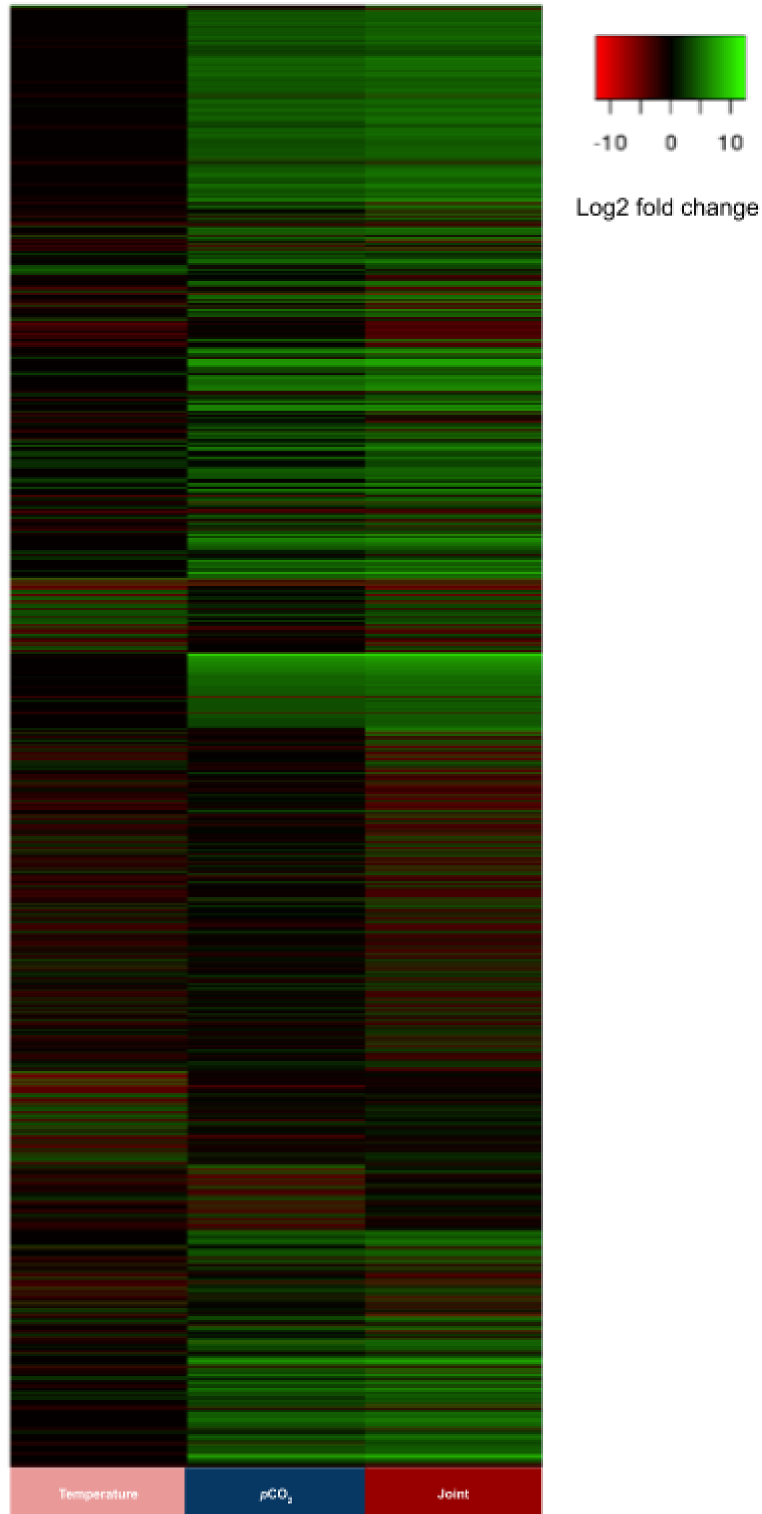
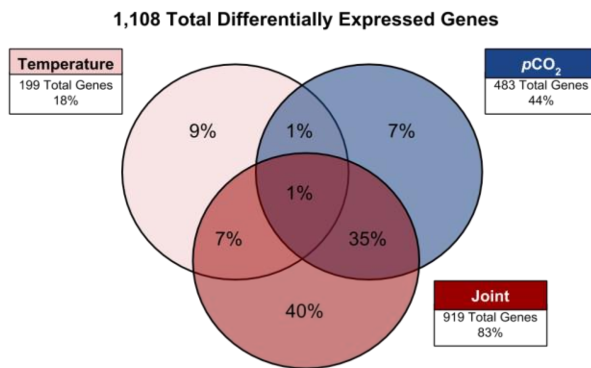


Figure 3.1. Heat map depicting expression of 1,108 differentially expressed genes relative to ambient treatment of 400ppm, 16°C. Colors represent absolute value of log2 fold change. Red represents a downregulation relative to the ambient, whereas green represents upregulation.

We found gene expression changes in postlarvae to be most responsive to the joint treatments of elevated pCO_2 and temperature together, compared to elevated levels of either treatment alone. Out of the 1,108 unannotated, differentially expressed genes, elevated temperature alone induced the differential expression of 199 genes (18%) relative to the ambient conditions; elevated pCO_2 alone induced differential expression of 483 genes (44%), and 919 genes (83%) were differentially expressed when both stressors were present (Figure 3.2a).

a



b

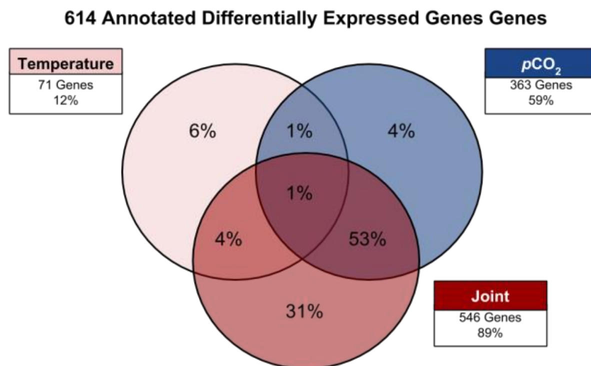


Figure 3.2. Percent of total (a) and annotated (b) differentially expressed genes as quantified by DESeq2 analysis within elevated temperature treatment (400 ppm 19°C), elevated pCO_2 treatment (1200ppm 16°C), and joint temperature and pCO_2 treatment (1200ppm 19°C). All differential expression is relative to ambient treatment (400ppm 16°C).

Functional annotation was possible for 55% of the differentially expressed genes using Blast2GO software (Figure 3.2b). As with the unannotated genes, we found the majority of

differentially expressed annotated genes in the joint treatment of high temperature and high *p*CO_2 (89%) compared to only 59% in the treatment with only elevated *p*CO_2 and 12% with only elevated temperature (Figure 3.2). Overall, differentially expressed genes were predominantly upregulated in the elevated *p*CO_2 treatment (1200ppm 16°C) and joint elevated temperature and *p*CO_2 treatment (1200ppm 19°C; Figure 3.3).

Analysis by edgeR provided little overlap in differentially expressed genes with DESeq2 analysis. We found 7% of genes to be shared between both analyses for the elevated temperature treatment (Figure 3.4a). There were no differentially expressed genes within the *p*CO_2 treatment using edgeR analysis (Figure 3.4b). Conversely, there were 30% of the differentially expressed DESeq2 identified genes shared with the edgeR analysis within the joint-stressor treatment (Figure 3.4c).

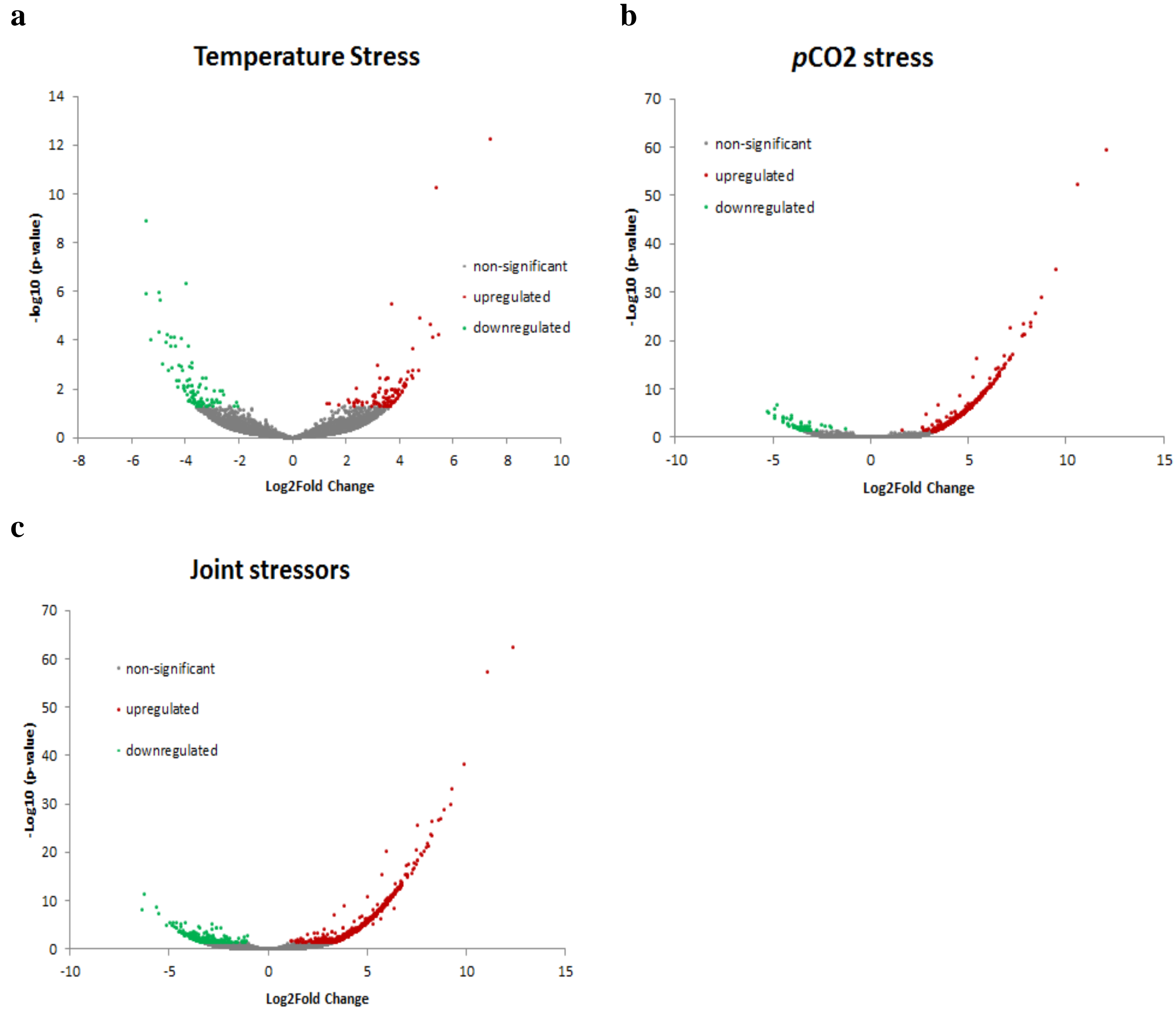
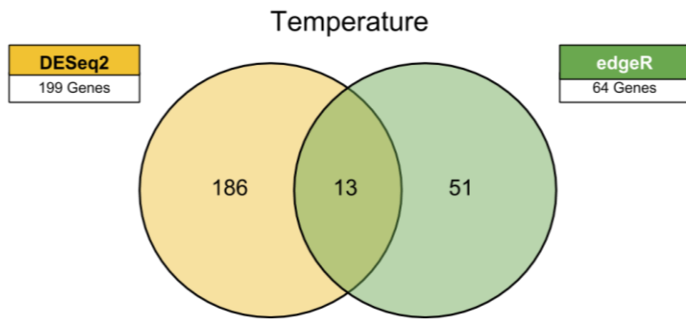
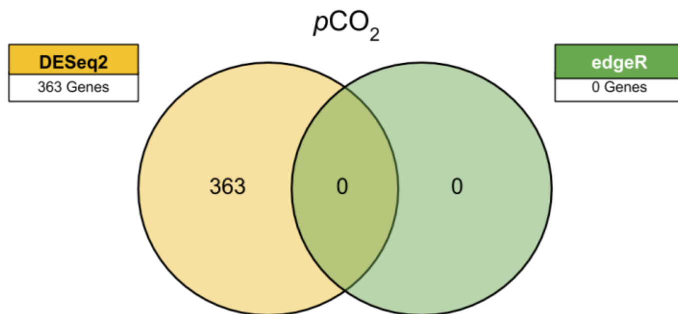


Figure 3.3. MA plots exhibiting the strength of significance (-Log10(p-values)) of particular genes against the Log2 fold change of each gene across samples of treatments of increased temperature (a), increased *p*CO_2 (b), and increased temperature and *p*CO_2 (c), relative to the ambient condition. Values in red represent statistically significant upregulated and green represent downregulated genes in treatment samples relative to ambient treatment.

a



b



c

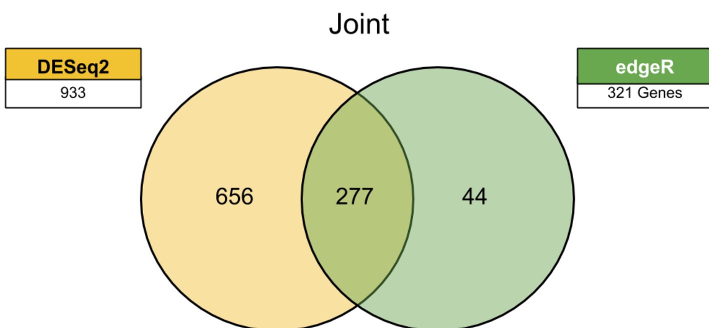


Figure 3.4. Comparison of number of annotated, differentially expressed genes identified by DESeq2 and edgeR analysis within elevated temperature (a), elevated pCO_2 (b), and joint elevated temperature and pCO_2 treatments (c).

We selected several genes of interest for further analysis given their functional importance: heat shock proteins (2 genes), cuticle proteins (13), cuticle protein binding molecules (CBM) (2), chitin binding protein (3), chitinase (4), calcification-associated peptides (2), arthrodial cuticle proteins (4) (Figure 4).

In treatments where only temperature was elevated, DESeq2 analysis showed down-regulation of several cuticle proteins, chitin-binding proteins, and calcification-associated peptides (Figure 3.5a). The edgeR results were in agreement with a downregulation of arthrodial cuticle protein 16.3, but an upregulation of arthrodial cuticle protein 9.3. The two methods gave contradictory results for cuticle protein AM1159, whereby DESeq2 indicated downregulation while edgeR indicated upregulation.

In treatments where only pCO_2 was elevated, the DESeq2 analysis showed significant upregulation for cuticle protein binding molecules, chitinase and chitinase-like proteins. The edgeR analysis for this treatment resulted in no differential regulation for any of these genes. (Figure 3.5b). In treatments where both temperature and pCO_2 were jointly elevated, both analytical methods indicated downregulation of cuticle proteins, chitin binding proteins and calcification-associated peptides, and upregulation of cuticle protein binding molecules, chitinase, and chitinase-like proteins (Figure 3.5c).

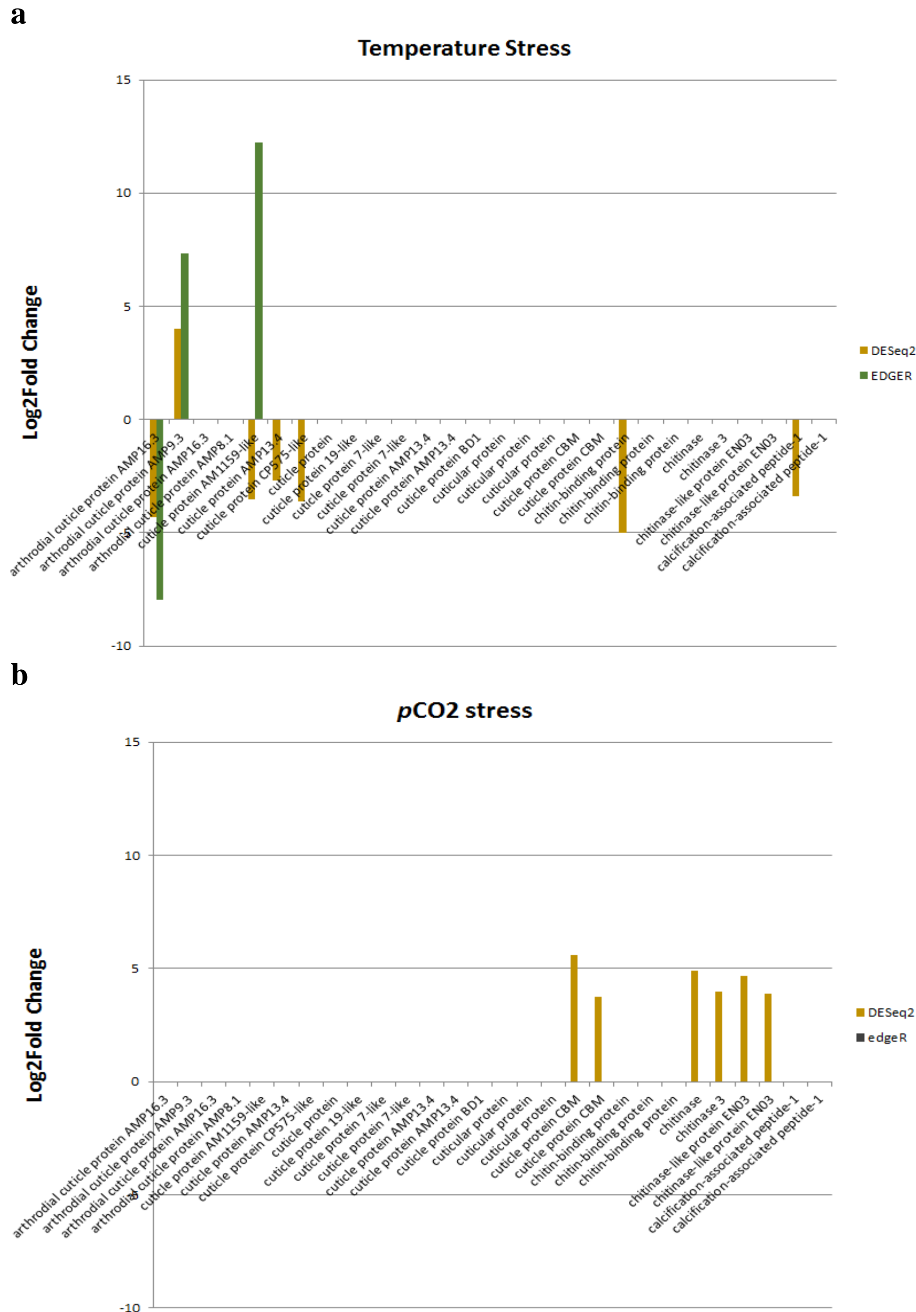


Figure 3.5. Response of Genes of Interest of carapace formation to elevated temperature and *p*CO_2. Log2Fold change of 28 GOI's within postlarvae reared at elevated temperature treatment (a), elevated *p*CO_2 treatment (b), and joint elevated temperature & *p*CO_2 treatment (c). All results are relative to expression represented in ambient treatment.

c

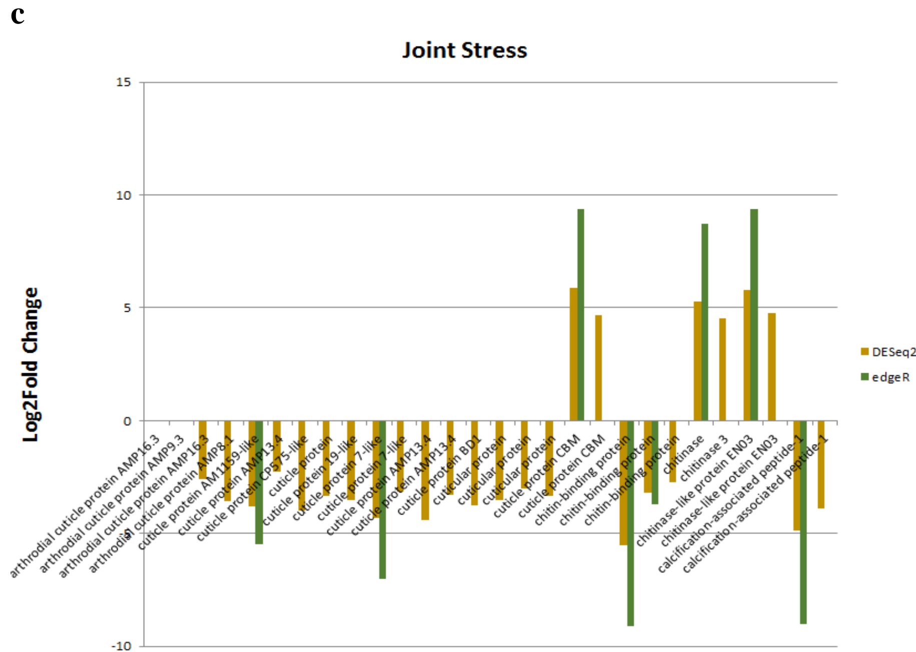


Figure 3.5. continued.

In addition, we examined the differential expression of several genes related to immune response and function (Clark & Greenwood, 2016): Heat shock proteins (3), Hemocyanin subunits (27), Mannose binding proteins (5), Crustin (2), C-type lectin (2), Glutathione S-transferase (2), Octopamine receptor (1) (Figure 5). In the elevated temperature treatment, according to the DESeq method, postlarvae downregulated HSP 83, and upregulated one gene for hemocyanin subunit 1. EdgeR detected no differential expression in these genes (Figure 3.6a). In the elevated pCO_2 treatment, DESeq2 analysis indicated postlarvae upregulated HSP70, 15 genes related to hemocyanin and its subunits, mannose binding units, crustin, C-type lectin, and glutathione S-transferase. Again, edgeR detected no change in these genes (Figure 5b). In the joint-stressor condition, DESeq2 indicated postlarvae upregulated 28 genes related to hemocyanin and its subunits, octopamine receptor, including all the genes differentially regulated within the high pCO_2 only treatment; according to the edgeR analysis, 11 genes within this category were also differentially expressed (Figure 5c).

a

Temperature Stress

b

pCO2 Stress

Figure 3.6. Response of Genes of Interest of immune response and function to elevated temperature and pCO_2. .Log2Fold change of 42 GOI's within postlarvae reared at raised temperature treatment (a), raised pCO_2 treatment (b), and joint raised temperature & pCO_2 treatment (c). All results are relative to expression represented in ambient treatment.

c

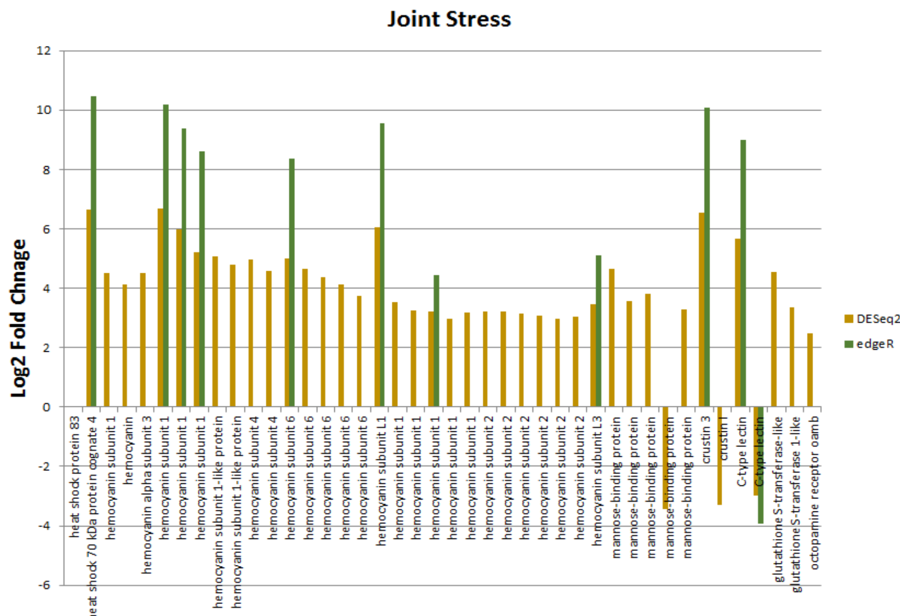


Figure 3.6. continued

3.4 Discussion

These results reveal an interactive effect of end-century-predicted pCO_2 and temperature on genes controlling the developmental processes and immune functioning of postlarval *Homarus americanus*. Postlarvae differentially expressed more genes under high pCO_2 than temperature, and showed an even greater response when they were exposed to both stressors simultaneously. These findings contrast previous studies that found physiological indicators were more responsive to end-century levels temperature than pCO_2 (Small *et al*., 2015; Waller *et al*., 2017). These results indicate a strong compensatory response at the gene transcription level in response to pCO_2 stress.

The effects of elevated pCO_2 and temperature on the transcription of genes associated with cuticle formation and calcification were stressor-dependent. Elevated temperature resulted in downregulation of cuticle proteins and a calcification associated peptide. Calcification-

associated proteins act as acidic protein sites for nucleation of $CaCO_3$ during larval biomineralization (Addadi & Weiner, 1985; Faircloth & Shafer, 2007). A downregulation may indicate a temperature cost to calcification, though fewer genes were differentially regulated than in other treatments. Warming has been shown to reduce shell integrity in *Mytilus edulis* when food limited, an effect the study attributed to a reallocation of energy away from biomineralization in order to address temperature-related increases in maintenance requirements (MacKenzie *et al*., 2014).

In elevated $p\mathrm{CO}_2$, postlarval lobster upregulated chitinase and cuticle proteins associated with binding, both of which are important components of shell reshaping and development. We saw this trend despite the fact that these genes have previously been shown to be downregulated in lobsters when exposed to other stressors such as the organochlorine pesticide endosulfan (Bauer *et al*., 2013) and $p\mathrm{CO}_2$ raised to 750 ppm (Waller, 2016). However, postlarvae upregulate chitinase in later stages relative to earlier larval stages as a mechanism to support increased chitin maintenance and synthesis (Cohen, 2010; Hines *et al*., 2013). Juvenile lobsters have been shown to increase shell calcification under hypercapnic conditions, though that effect might come at the expense of growth and reproduction (Ries *et al*., 2009; Whiteley, 2011). The transcription data presented here support the hypothesis that larvae allocate more energy to shaping and maintaining shell integrity under acidified conditions.

The combination of end-century temperature and $p\mathrm{CO}_2$ stressors appeared to have additive effects on exoskeleton forming genes, with additional cuticle protein downregulation. These findings mirror other studies that have examined skeletal formation in marine invertebrates when exposed to heightened temperature and acidity. Downregulation of calcification-related genes was reported for pearl oysters (*Pinctada fucata*) after being exposed

to end-century conditions (Liu *et al*., 2012). However, larval *Strongylocentrotus purpuratus* exhibit inhibited skeletal growth under elevated pCO_2, but not under elevated temperatures (Padilla-Gaillimo *et al.,* 2013). However, bivalves and urchins appear to have lower capacity to compensate for these stressors than some crustaceans (Kurihara, 2008; Wood *et al*., 2008).

Heat shock proteins were down-regulated in lobster larvae under elevated temperature alone, but were upregulated when exposed to elevated $pCO_{2,}$ and especially when pCO_2 and temperature were increased together. Virtually all organisms use heat shock proteins (HSP) as a mode to alleviate physiologically stressful conditions (Evans & Hoffman, 2012). HSPs are molecular chaperones that are upregulated after exposure to stressful conditions as a mechanisms to protect proteins from being denatured or improperly folded (Flaherty *et al*, 1990; Kiang and Tsokos, 1998; Alberts *et al*., 2015). Thus, HSPs can modify an organism's thermal sensitivity, and be used as important stress biological markers (Tedeschi *et al*., 2015). When exposed to higher temperatures, *H. americanus* postlarvae downregulated HSP production. When reared in high pCO_2, or joint stressor conditions, HSP70 was the highest upregulated gene from the GOI's, suggesting a level of compensation by *H. americanus* for an environmentally stressful condition. This type of compensation has been evaluated as an energetic trade-off in the prawn *Machrobrachium rosenbergii* at the cost of reproductive output (Mohammed et al., 2017). Similarly, Waller (2016) also found HSP 70 upregulation when postlarvae from the central Maine coast were exposed to higher pCO_2 of 750 ppm, In addition, Liu *et al.* (2012) found upregulation of HSP70 under joint stress from elevated temperature and pCO_2 in pearl oysters (*Pinctada fucata*). Therefore HSP 70 may be a broadly relevant biological marker for monitoring stress under both temperature and $pCO_{2.}$

Although temperature stress caused the upregulation of a single hemocyanin subunit, overall the genes of interest related to immune functioning and response were more differentially expressed under elevated pCO_2 either alone or together with warming. When larvae were exposed to 1200 ppm pCO_2 at ambient temperatures, we observed the upregulation of 15 genes related to hemocyanin, mannose binding proteins, crustin, c-type lectin, all of which play roles in antigen recognition and/or defense (Clark & Greenwood, 2016). These same genes were differentially expressed in greater numbers in the joint-stressor treatment, indicating an overall higher energy input to immune function when both stressors are present. This could have implications on the *H. americanus* antigen defense systems in future oceanic conditions.

Crustaceans, as a group, have shown relative resistance to end-century ocean acidification as a single stressor compared to other calcifiers in traditional physiological studies (Whiteley, 2011). This may be the result of a heightened capacity for ionoregulation (Whitely, 2011; Weinburg *et al.*, 2012; Gledhill *et al.,* 2015). For postlarval lobster we found a clear effect of increased pCO_2 on gene expression regulation. It is possible crustaceans have molecular mechanisms for compensation to these stressors that maintain physiological homeostasis and therefore exhibit little or no response in measured behavioral or morphometric endpoints, but are apparent with gene expression analysis. Our results highlight the importance of molecular approaches in addition to whole-organism physiological processes to understanding response and identifying threats of ocean changes to species and populations.

Despite the need for these types of experiments, it is important to underscore the limitations inherent in short term exposures such as ours. Lacking is an understanding of how fast populations may be able to adapt to changing conditions over a lifetime or multiple generations. A comparison of gene regulation between sub-populations along environmental

gradients may elucidate differences between populations under contrasting thermal regimes. A comparative approach may lend new insights into local adaptation in this species.

This is the first study to examine how the joint stressors of a warming and acidifying ocean may affect gene expression of American lobster larvae. Our results indicate that the larval response to heighted acidity is more dramatic than indicated in physiological and morphometric studies conducted thus far.

REFERENCES

Addadi, L., Weiner, S., 1985. Interactions between acidic proteins and crystals: stereochemical requirements in biomineralization. Proc. Natl. Acad. Sci. U.S.A. 82, 4110-4114.

Agnalt, A.L., Grefsrud, E.S., Farestveit, E., Larsen, M., and Keulder, F. 2013. Deformities in larvae and juvenile European lobster (*Homarus gammarus*) exposed to lower pH at two different temperatures. Biogeosciences. 10:7883-7895.

Aiken, D.E., Waddy, S.L., 1976. Controlling growth and reproduction in the American lobster. Proc. 7th Annu. Meet. World Aquacult. Soc., 415–430.

Alberts, B., Johnson, A., Lewis, J., Morgan, D., Raff, M., Roberts, K., Walter, P. 2015. *Molecular Biology of the Cell.* Sixth edition. Garland Science. New York, NY. pp. 355-357.

Anger, K. 2001. Ecology and behavior. In: *The Biology of Decapod Crustacean Larvae*, pp. 261-314. Ed. by K.Anger. AA Balkema publishers, Lisse. Pp.262

Anger, K. 1987. The DO threshold: a critical point in the larval development of decapod crustaceans. Journal of Experimental Marine Biology and Ecology 108:15-30.

Annis, E.R., 2005. Temperature effects on the vertical distribution of lobster postlarvae (*Homarus americanus*). Limnol. Oceanogr. 5, 1972-1982.

Anthony, K.R.N., Kline, D.I., Diaz-Pulido, G., Dove, S. & Hoegh-Guldberg, O. 2008. Ocean acidification causes bleaching and productivity loss in coral reef builders. Proceedings of the National Academy of Sciences of the United States of America 105, 17442-17446.

Arnberg, M., Calosi, P., Spicer, J. I., Tandberg, A. H. S., Nilsen, M., Westerlund, S., and Bechmann, R. K. 2013. Elevated temperature elicits greater effects than decreased pH on the development, feeding and metabolism of northern shrimp (Pandalus borealis) larvae. Marine biology, 160: 2037-2048.

Arnold, K.E., Findlay, H.S., Spicer, J.I, Daniels, C.L., and Boothroyd, D. 2009. Effects of CO_2-related acidification on aspects of larval development of the European lobster, *Homarus gammarus* (L.). Biogeosciences, 6: 1747-1754.

Bailey, A., Thor, P., Browman, H. I., Fields, D. M., Runge, J., Vermont, A., *et al.* 2016. Early life stages of the Arctic copepod Calanus glacialis are unaffected by increased seawater pCO2. ICES Journal of Marine Science: Journal du Conseil, fsw066.

Balch, W.M., Drapeau, D.T., Bowler, B.C., Huntington, T.G. 2012. Step-changes in the physical, chemical and biological characteristics of the Gulf of Maine, as documented by the GNATS time series. *Marine Ecology Progress Series,* 450, 11-35.

Bauer, M., Greenwood, S.J., Clark, K.F., Jackman, P., and Fairchild, W. 2013. Analysis of gene expression in *Homarus americanus* larvae exposed to sublethal concentrations of endosulfan during metamorphosis. Comparative Biochemistry and Physiology Part D: Genomics and Proteomics, 8:300-308.

Beal, B.F. and Chapman, S.R. 2001. Methods for mass rearing stages I-IV larvae of the American lobster, Homarus americanus H. Milne Edwards, 1837, in static systems. Journal of Shellfish Research, 20:337-346.

Benestan, L., Gosselin, T., Perrier, C., Sainte-Marie, B., Rochette, R., & Bernatchez, L. (2015). RAD genotyping reveals fine-scale genetic structuring and provides powerful population assignment in a widely distributed marine species, the american lobster (homarus americanus). *Molecular Ecology, 24*(13), 3299-3315. doi:10.1111/mec.13245

Benestan, L., Quinn, B. K., Maaroufi, H., Laporte, M., Clark, F. K., Greenwood, S. J., . . . Bernatchez, L. (2016). Seascape genomics provides evidence for thermal adaptation and current-mediated population structure in american lobster (homarus americanus). *Molecular Ecology, 25*(20), 5073-5092. doi:10.1111/mec.13811

Blunden, J., Arndt, D.S. 2014. State of the climate in 2013. *Bulletin of the American Meterological Society*. 95:7

Botsford, L.W., Rauch, H.E., Shleser, R.A., 1974. Optimal temperature control of a lobster plant. IEEE T. Automat. Contr. AC-19, 541–542.

Browman, H. I. 2016. Applying organized skepticism to ocean acidification research. ICES Journal of Marine Science: Journal du Conseil, 73: 529-536.

Byrne, M., 2011. Impact of ocean warming and ocean acidification on invertebrate life history stages: vulnerabilities and potential for persistence in a changing ocean. Oceanogr. Mar. Biol. 49, 1-42.

Byrne, M., and Przeslawski, R. 2013. Multistressor impacts of warming and acidification of the ocean on marine invertebrates' life histories. Integrative and Comparative Biology, 53: 582-596.

Caputi, N., de Lestang, S., Frusher, S., Wahle, R.A. 2013. The impact of climate change on exploited lobster stocks. B.F. Phillips (Ed.), Lobsters: Biology, Management, Aquaculture and Fisheries (2nd ed.), John Wiley and Sons Ltd, Oxford, UK. pp. 84-112.

Clark, K. F., Acorn, A. R., and Greenwood, S. J. 2013. Differential expression of American lobster (Homarus americanus) immune related genes during infection of Aerococcus viridans var. homari, the causative agent of Gaffkemia. Journal of Invertebrate pathology, 112: 192-202.

Clark, K. F., and Greenwood, S. J. 2016. Next-Generation Sequencing and the Crustacean Immune System: The Need for Alternatives in Immune Gene Annotation. Integrative and comparative biology: icw023.

Conover, D.O., Schultz, E.T. 1995. Phenotypic similarity and the evolutionary significance of countergradient variation. *Trends in Ecology and Evolution,* 10: 248-252.

de Valpine, P., Harte, J. 2001. Plant responses to experimental warming in a montane meadow. Ecology 82:637-648.

Dickson, A. G., Sabine, C. L., and Christian, J. R. 2007. Guide to best practices for ocean CO2 measurements. PICES Special Publication, 3:191.

Ennis, G.P. 1975. Observation on hatching and larval release in the lobster *Homarus americanus. J. Fish. Res. Board Can.* 32: 2210-2213.

Ennis, G. P. 1995. Larval and postlarval ecology. In: *Biology of the lobster: Homarus americanus* (Factor, J.R.). Academic Press, INC. San Diego, CA. pp. 23-46.

Evans, T.G. & Hofmann, G.E. (2012) Defining the limits of physiological plasticity: how gene expression can assess and predict the consequences of ocean change. *Philosophical Transactions of the Royal Society B-Biological Sciences*, 367, 1733–1745.

Fabry, V.J., Seibel, B.A., Feely, R.A., Orr, J.C. 2008. Impacts of ocean acidification on marine fauna and ecosystem processes. ICES Journal of Marine Science, 65: 414-432.

Fabry, V. J., McClinton, J.B., Mathis, J.T. and Grebmeier, J.M. 2009. Ocean acidification at high latitudes: The bellweather. Oceanography, 22: 160-171

Factor, J.R., 1995. Introduction, anatomy and life history. In: *Biology of the lobster: Homarus americanus.* Ed. by Factor, J. R. Academic Press, INC. San Diego, CA. pp. 1-12.

Faircloth, L.M., Shafer, T.H. 2007. Differential expression of eight transcripts that their role in the cuticle of the blue crab, *Callinectes sapidus*. Comparative Biochemistry and Physiology 146: 370-383.

Fields, D.M., Sherma, S.D., Browman, H.I., Browne, T.Q. Skiftesvik, A.B. 2012. Light primes the escape response of the Calaniod copepod, *Calanus finmarchicus*. PLos ONE. 7.6: pe39594.

Flaherty, K.M., DeLuca-Flaherty, C., and McKay, D.B. 1990. Three-dimensional structure of the ATPase fragment of a 70K heat-shock cognate protein. Nature, 346: 623-628.

Gibbin, E.M., Chakravarti, L., Jarrold, M.C., Christen, F., Turpin, V., N'Siala, G.M., Blier, P., Calosi, P. 2016. Can multi-generational exposure to ocean warming and acidification lead to the adaptation of life history and physiology in a marine metazoan? Journal of Experimental Biology. 220, 551-563.

Gledhill, D. K., White, M. M., Salisbury, J. E., Thomas, H., Mlsna, I., Libman, M., Mook, B. *et al.* 2015. Ocean and coastal acidification off New England and Nova Scotia. Oceanography, 28: 182-197.

Gracey, AY. 2007. Interpeting physiological response to environmental change through gene expression profiling. J Exp Biol, 210:1584-1592.

Guo, L., Perrie, W., Long, Z., Chasse, J., Zhang, Y., Huang, A., 2013. Dynamic downscaling over the Gulf of St. Lawrence using the canadian regional climate model. Atmos. Ocean 51, 265-283.

Haarr, M.L., Sainte-Marie, B., Comeau, M., Tremblay, M.J., Rochette, R. 2018. Female American lobster(*Homarus americanus*) size-at-maturity declined in Canada during the 20th and early 21st centuries. Canadian Journal of Fisheries and Aquatic Sciences. 75(6): 908-924.

Harms, L., Frickenhaus, S., Schiffer, M., Mark, F.C., Storch, D., Held, C., Pörtner, H-O, Lucassen, M. 2014. Gene expression in profiling in gills of the great spider crab *Hyas araneus* in response to ocean acidification and warming. BMC Genomics 15:789.

Hartnoll, R.G., 1982. Growth. In: Abele, L.G. (ed.), The Biology of the Crustacea: Embryology, Morphology and Genetics, vol 2. Academic Press Inc, New York, pp. 111-196.

Helmuth, B. 2009. From cells to coastlines: how can we use physiology to forecast the impacts of climate change? Journal of Experimental Biology, 212: 753-760.

Hines, D.J., Clark K.F., and Greenwood, S.J. 2014. Global gene expression profiling of *Homarus americanus* (Crustacea) larval stages during development and metamorphosis. Invertebrate Reproduction & Development, 58: 97-107.

Hudon, C. & Fradette, P. 1988. Planktonic growth of larval lobster (*Homarus americanus)* off Iles de la Madeleine (Quebec), Gulf of St. Lawrence. *Can. J. Fish Aquat. Sci.* 45:868-878.

Hüning, A.K., Melzer, F., Thomsen, J., Gutowska, M.A., Krämer, L., Frickenhaus, S., *et al*. 2013. Impacts of seawater acidification on mantle gene expression patterns of the Baltic Sea blue mussel: implications for shell formation and energy metabolism. Marine biology, 160:1845-1861.

Incze, L., Naimie, C.E., 2000. Modelling the transport of lobster (*Homarus americanus*) larvae and postlarvae in the Gulf of Maine. Fish. Oceanogr. 9, 99-113.

Incze, L., Xue, H., Wolff, N., Xu, D., Wilson, C., Steneck, R., *et al.* 2010. Connectivity of lobster (Homarus americanus) populations in the coastal Gulf of Maine: part II. Coupled biophysical dynamics. Fisheries Oceanography, 19: 1-20.

IPCC, 2013: Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Ed. by T.F. Stocker, D. Quin, G.K. Plattner, M. Tignor, S.K. Allen, J. Boschung, A. Nauels, Y. Xia, V. Bex and P.M. Midgley. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA, 1535 pp.

Kelly, M.W., Grosberg, R.K., Sanford, E. 2013. Trade-offs, geography and limits of thermal adaptation in a tide pool copepod. *The American Naturalist,* 181: 846-854

Kenchington, E.L., Harding, G.C., Jones, M.W., & Prodohl, P.A. 2009. Pleistocene glaciation events shape genetic structure across the range of American lobster, *Homarus americanus*. *Molecular Ecology.* 18(8): 1654-1667.

Keppel, E.A., Scrosati, R.A., and Courtenay, S.C. 2012. Ocean acidification decreases growth and development in American lobster (*Homarus americanus*) larvae. Journal of Northwest Atlantic Fishery Science, 44: 61-66.

Kiang, J. G., and Tsokos, G. C. 1998. Heat shock protein 70 kDa: molecular biology, biochemistry, and physiology. Pharmacology & therapeutics, 80:183-201.

Koch, M., Bowes, G., Ross, C., Zhang, X.H. 2013. Climate change and ocean acidification effects on seagrasses and marine macroalgae. Glob Chang Biol. 19:103-132

Kroeker, K.J., Kordas, R.L., Crim, R.N. and Singh, G.G. 2010. Meta-analysis reveals negative yet variable effects of ocean acidification on marine organisms. Ecology Letters, 13: 1419-1434.

Kroeker, K.J., Kordas, R.L., Crim, R., Hendrks, I.E., Ramajo, L., Singh, G.S., Duarte, C.M., Gattuso, J.P. 2013. Impacts of ocean acidification on marine organisms: quantifying sensitivities and interaction with warming. Global Change Biology. 2013. 19(6): 1884-1896.

Kunkel, J.G., Nagel, W., Jercinovic, M.J. 2012. Mineral fine structure of the American lobster cuticle. *Journal of Shellfish Research.* 31(2), 515-526.

Kurihara, H., Kato, S., Ishimatsu, A. 2007. Effects of increased seawater pCO_2 on early development of the oyster *Crassostrea gigas*. Aquatic Biology 1: 91-98.

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

Lawton, P., Lavalli, K.L., 1995. Postlarval, juvenile, adolescent and adult ecology. In: Factor, J.R. (Ed.), Biology of the lobster *Homarus americanus*. Academic Press Inc, New York, pp. 47-88.

Le Bris, A., Pershing, A.J., Gaudette, J., Pugh, T.L., Reardon, K.M. 2017. Multi-scale quantification of the effects of temperature on the size of maturity in the American lobster (*Homarus americanus)*. Fisheries Research. 186: 397-406.

Le Bris, A., Mills, K.E., Wahle, R.A., Chen, Y. Alexander, M.A., Allyn, A.J., Schuetz, J.G., Scott, J.D., Pershing, A.J. 2018. Climate vulnerability and resilience in the most valuable North American fishery. PNAS 115(8):201711122

Lemoine, N.P., Burkepile, D.E. 2012. Temperature-induced mismatches between consumption and metabolism reduce consumer fitness. Ecology 93(11): 2483-2489.

Lewis, E., Wallace, D., Allison, L.J. 1998. Program developed for CO_2 system calculations. Carbon Dioxide Information Analysis Center, managed by Lockheed Martin Energy Research Corporation for the US Department of Energy, Oak Ridge, Tennessee. 38 pp.

Liu W, Huang X, Lin J, He M (2012) Seawater Acidification and Elevated Temperature Affect Gene Expression Patterns of the Pearl Oyster *Pinctada fucata*. PLoS ONE 7(3): e33679. https://doi.org/10.1371/journal.pone.0033679

Liu, X., Patsavas, M. C., and Byrne, R. H. 2011. Purification and characterization of meta-cresol purple for spectrophotometric seawater pH measurements. Environmental science & technology, 45: 4862-4868.

Long, Z., Perrie, W., Chasse, J., Brickman, D., Guo, L., Drozdowski, Al, Hu, H., 2015. Impacts of climate change in the Gulf of St. Lawrence. Atmos. Ocean, 15.

Long, W. C., Swiney, K. M., Harris, C., Page, H. N., and Foy, R. J. 2013. Effects of ocean acidification on juvenile red king crab (Paralithodes camtschaticus) and Tanner crab (Chionoecetes bairdi) growth, condition, calcification, and survival. PLoS One, 8: e60959.

Longhurst, A. 1998. Ecological geography of the sea. Academic Press: San Diego.

MacKenzie, B.R. 1988 Assessment of temperature effects on interrelationships between stage durations, mortality and growth in laboratory-reared *Homarus americanus* Milne Edwards larvae. *J. Exp. Mar. Biol. Ecol.* 116:87-98.

MacKenzie, C.L., Ormondroyd, G.A., Curling, S.F., Ball, R.J., Whiteley, N.M., Malham, S.K. 2014. PLoS One, 9: e86764

Maine DMR (Department of Marine Resources). 2017. Website: www.maine.gov/dmr/commerciallandings Referenced 02, May 2018

Martin, S., Gattuso, J.P. 2009. Response of Mediterranean coralline algae to ocean acidification and elevated temperature. Glob Chang Biol 15:2089-2100

Melzner, F., Gutowska, M.A., Langenbuch, M., Duport, S., Lucassen, M., Thorndyke, M.C., Bleich, M., Pörtner, H-O. 2009. Physiological basis for high CO_2 tolerance in marine ectothermic animals: pre-adaptation through lifestyle and ontogeny? Biogeosciences. 6:1-19.

Menu-Courey, K., Noisette, F., Piedalue, S., Daoud, D., Blair, T., Blier, P.U., Azetsu-Scott, K., Calosi, P. Energy metabolism and survival of the juvenile recruits of the American lobster (*Homarus americanus)* exposed to a gradient of elevated seawater pCO_2. Marine Environmental Research 143:111-123.

Metzger, R., Sartoris, F.J., Langenbuch, M., & Portner, H.O. 2007. Influence of elevated CO_2 concentrations on the thermal tolerance of the edible crab *Cancer pagurus*. Journal of Thermal Biology, 32: 144-151.

Miller, J. J., Maher, M., Bohaboy, E., Friedman, C. S., and McElhany, P. 2016. Exposure to low pH reduces survival and delays development in early life stages of Dungeness crab (Cancer magister). Marine Biology, 163: 1-11.

Mills, K.E., A.J. Pershing, C.J. Brown, Y. Chen, F.-S. Chiang, D.S. Holland, S. Lehuta, J.A. Nye, J.C. Sun, A.C. Thomas, and R.A. Wahle. 2013. Fisheries management in a changing climate: Lessons from the 2012 ocean heat wave in the Northwest Atlantic. Oceanography 26(2):191–195.

Mohamed, A., Arshad, A., Sung, Y.Y., Jasmani, S. 2017. Effects of thermal stress on Hsp70 gene expression and female reproductive performance of giant freshwater prawn, *Macrobrachium rosenbergii*. *Aquaculture Research.* 49(1): 135-150.

Morris, M. J., Gust, G., and Torres, J. J. 1985. Propulsion efficiency and cost of transport for copepods: a hydromechanical model of crustacean swimming. Marine Biology, 86: 283-295.

Padilla-Gamino, J.L., Kelly, M.W., Evans, T.G., Hofmann, G.E. 2013. Temperature and CO_2 additively regulate physiology, morphology and genomic responses of larval sea urchins, *Strongylocentrotus purpuratus*. Preceedings of the Royal Society.

Pane, E.F., Barry, J.P. Extracellular acid-base regulation during short-term hypercapnia is effective in a shallow-water crab, but ineffective in a deep-sea crab. Mar Ecol Prog Ser, 334: 1-9.

Pershing, A.J., Alexander, M.A., Hernandez, C.M., Kerr, L.A., Le Bris, A., Mills, K.E., Nye, J.A., Record, N.R., Scannell, H.A., Scott, J.D., Sherwood, G.D., Thomas, A.C. 2015. Slow adaptation in the face of rapid warming leads to collapse of the Gulf of Maine cod fishery. Science 350(6262), 809-812.

Pörtner, H. O., and Farrell, A. P. 2008. Physiology and climate change. Science, 322: 690-692.

Pörtner, H. O., and Peck, M. A. 2010. Climate change effects on fishes and fisheries: towards a cause- and- effect understanding. Journal of fish biology, 77:1745-1779.

Quinn, B. K., Rochette, R., Ouellet, P., and Sainte-Marie, B. 2013. Effect of temperature on development rate of larvae from cold-water American lobster (Homarus americanus). Journal of Crustacean Biology, 33: 527–536.

Quinn, B.K. 2014. Assessing potential influence of larval development time and drift on large-scale spatial connectivity of american lobster (*Homarus americanus*). MSc Thesis, University of New Brunswick, Saint John, NB, pp. 157.

Quinn, B. K., and Rochette, R. 2015. Potential effect of variation in water temperature on development time of American lobster larvae. ICES Journal of Marine Science, 10: i79–i90.

Quinn, B.K. 2017. Threshold temperatures for performance and survival of American lobster larvae: A review of current knowledge and implications to modeling impacts of climate change. Fisheries research: 186: 383-396.

Rall, B.C., Vucic-Pestic, O., Ehnes, R.B., Emmerson, M., Brose, U. 2010. Temperature, predator-prey interaction strength and population stability. Global Change Biology 16:2145-2157

Rato, L.D., Novais, S.C., Lemos, M.F.L., Leandro, S.M. 2017. *Homarus gammarus* (Crustacea: Decapoda) larvae under an ocean acidification scenario: responses across different levels of biological organization. Comp. Biochem. Physiol. 203:29-38.

Reusch, T. B. 2014. Climate change in the oceans: evolutionary versus phenotypically plastic responses of marine animals and plants. Evolutionary Applications, 7: 104-122.

Reynaud, S., Leclereq, N., Romaine-Lioud, S., Ferrier-Pages, C., Jaubert, J., and Gattuso, J. P.: (2003). Interacting effects of CO_2 partial pressure and temperature on photosynthesis and calcification in a scelractinian coral, *Glob. Change Biol., 9,* 1660-1668.

Ries, J.B., Cohen, A.L., and McCorkle, D.C. 2009. Marine calcifiers exhibit mixed responses to CO_2-induced ocean acidification. Geology, 37: 1131-1134

Robert, D., Castonguay, M., Fortier, L., 2007. Early growth and recruitment in Atlantic mackeral *Scomber scombrus*: discriminating the effects of fast growth and selection for fast growth. Mar. Ecol. Prog. Ser. 337, 209-219.

Rodolfo-Metalpa R, Houlbreque F, Tambutte E, Boisson F, Baggini C, Patti FP, Jefree R., Fine, M., Foggo, A., Gattuso, J.P., Hall Spencer J.M. 2011. Coral and mollusc resistance to ocean acidification adversely affected by warming. Nat Clim Chang 1:308-312.

Rodolfo-Metalpa, R., Lombardi, C., Cocito, S., Hall-Spencer, J.M., Gambi, M.C. 2010. Effects of ocean acidification and high temperatures on the bryozoan *Myriapoda truncata* at natural CO2 vents. Marine Ecology.

Ross, R.M., Quentin, L.B., and Kirsch, E. 1988. Effect of temperature on developmental times and survival of early life stages of *Euphausia superba*. Journal of Experimental Marine Biology and Ecology, 121: 55-71.

Sanford, E., Holzman, S.B., Haney, R.A., Rand, D.M., Berness, M.D. 2006. Larval tolerance, gene flow and the northern geographic range limit of fiddler crabs. *Ecology*. 87(11), 2882-2894.

Schatzlein F.C. and J.D. Costlow. 1978. Oxygen consumption of larvae of decapod crustaceans, *Emerita talpoida* (Say) and *Libinia emarginata* Leach. Comp. Biochem. Physiol. A. 61: 441-450.

Schiffer, M., Harms, L., Pörtner, H. O., Lucassen, M., Mark, F. C., and Storch, D. 2012. Tolerance of Hyas araneus zoea I larvae to elevated seawater pCO2 despite elevated metabolic costs. Marine Biology, 160: 1943-1953.

Small, D.P., Calosi, P., Boothroyd, D., Widdicombe, S., and Spicer, J.I. 2015. Stage-specific changes in physiological and life-history responses to elevated temperature and $p\mathrm{CO_2}$ during the

larval development of the European lobster *Homarus gammarus* (L.). Physiological and Biochemical Zoology, 88: 494.

Somero, G.N. 2010. The physiology of climate change: how potentials for acclimation and genetic adaptation will determine 'winners' and 'losers'. Journal of Experimental Biology, 213(6): 912-20

Somero, G.N., Lockwood, B., Tomanek, L. 2017. *Biochemical Adaptation.* Sinauer Publishing

Spees, J.L., Chang, S.A., Snyder, M.J., Chang, E.S. 2002. Thermal acclimation and stress in the American lobster, *Homarus americanus*: Equivalent temperature shifts elicit unique gene expression patterns for molecular chaperones and polyubiquitin. Cell Stress and Chaperones 7(1):970106

Swingle, J.S., Daly, B., Hetrick, J. 2013. Temperature effects on larval survival, larval period, and health of hatchery-reared red king crab, *Paralithodes camtschaticus*. Aquaculture, 384: 13-18.

Tedeschi, J. N., Kennington, W. J., Berry, O., Whiting, S., Meekan, M., & Mitchell, N. J. 2015. Increased expression of Hsp70 and Hsp90 mRNA as biomarkers of thermal stress in loggerhead turtle embryos (*Caretta Caretta*). *Journal of Thermal Biology*. 47: 42–50.

Tepolt, C.K., Somero, G. 2014. Master of all trades: Thermal acclimation and adaptation of cardiac function in a broadly distributed marine invasive species, the European green crab, Carcinus maenas. Journal of Experimental Biology: 217(Pt 7): 1129-38.

Todgham, A.E., Hofmann, G.E. 2009. Transcriptomic response of sea urchin larvae *Strongylocentrotus purpuratus* to CO_2-driven seawater acidification. J Exp Biol. 212: 2579-2594.

Waddy, S.L., Aiken, D.E., de Kleijn, D.P.V. 1995. Control of growth and reproduction. In: Factor, J.R. (Ed.), Biology of the Lobster *Homarus Americanus.* Academic Press Inc, New York, pp. 217-266.

Wahle, R. A., Castro, K.M., Tully, O. & Cobb, J.S. 2013. *Homarus*. In: *Lobsters: Biology, Management, Aquaculture & Fisheries* (Phillips, B). Jon Wiley & Sons, Ltd., Sussex, UK. pp 221-258.

Wahle, R.A., C. Brown & K. Hovel. 2013. The geography and body-size dependence of top-down forcing in New England's lobster-groundfish interaction. *Bulletin of Marine Science.* 89: 189–212.

Wahle, R.A., Dellinger, L., Olszewski, S., Jekielek, P., 2015. American lobster nurseries of southern New England receding in the face of climate change. ICES J. Mar. Sci. 72 (Suppl. 1), i69–i78.

Waller, J. D., Wahle, R. A., McVeigh, H., Fields, D. M. 2017. Linking rising pCO2 and temperature to the larval development and physiology of the American lobster (Homarus americanus). *ICES Journal of Marine Science*. doi:10.1093/icesjms/fsw154.

Waller, J. 2016. Linking rising pCO2 and temperature to the larval development, physiology and gene expression of the american lobster (*Homarus americanus*). *Electronic Theses and Dissertations*. 2527. http://digitalcommons.library.umaine.edu/etd/2527

Walther, K., Sartoris, F.J., Bock, C., and Portner, H.O. 2009. Impact of anthropogenic ocean acidification on thermal tolerance of the spider crab *Hyas araneus*. Biogeosciences 6: 2207-2215.

Walther, K., Anger, K., Pörtner, H-O. 2010. Effects of ocean acidification and warming on the larval development of the spider crab *Hyas araneus* from different latitudes (54° vs 79°N). Mar. Ecol. Porg. Ser. 417: 159-170.

Walther, K., Sartoris, F.J., Portner, H.O. 2011. Impacts of temperature and acidification on larval calcium incorporation of the spider crab *Hyas araneus* from different latitudes (54° and 79°N). Marine Biology 158:2043-2053.

Weiss, M., Thatje, S., Heilmayer, O., Anger, K., Brey, T., Keller, M. 2009. Influence of temperature on the larval development of the edible crab, *Cancer pagurus.* Journal of the Marine Biological Association of the United Kingdom, 89: 753-759.

Wernberg, T., Smale, D.A., and Thomsen, M.S. 2012. A decade of climate change experiments on marine organisms: procedures, patterns and problems. Global Change Biology, 18: 1491-1498.

Whitely, N.M. 2011. Physiological and ecological responses of crustaceans to ocean acidification. Mar. Ecol. Prog. Ser., 430: 257-271.

Whitman, A.C., Pörtner, H-O. 2013. Sensitivities of extant animal taxa to ocean acidification. Nature climate change. Doi: 10.1038/NCLIMATE1982.

Zittier, Z.M.C, Hirse, T., Pörtner, H-O. 2013. The synergistic effects of increasing temperature and CO_2 levels on activity capacity and acid-base balance in the spider crab *Hyas araneus.* Mar Biol 160(8): 2049-2062.

BIOGRAPHY OF AUTHOR

Maura Niemisto was born in Traverse City, Michigan in 1991. She grew up in Leelanau County and graduated from Glen Lake Community Schools in 2009. She attended the University of Michigan in Ann Arbor for her undergraduate studies, achieving a Bachelor of Science degree in Program in the Environment with minors in International Studies and Ecology & Evolutionary Biology in summer 2013. Upon graduation she moved to the Hudson Valley and worked as an education coordinator onboard the Hudson River Sloop Clearwater, teaching grades 4-12 about estuarine ecology and environmental stewardship. In January 2017, she moved to Wiscasset, Maine to begin her masters work at the University of Maine's Darling Marine Center in Walpole. She studied under the mentorship of co-advisors David Fields and Richard Wahle. Maura is a member of the Association for the sciences of Limnology and Oceanogoraphy. Maura is a candidate for the Master of Science degree in Marine Biology from the University of Maine in May 2019.