Ecology: Article

Local and Regional Geographic Variation in Inducible Defenses

James J. Corbett and Geoffrey C. Trussell*

Department of Marine and Environmental Sciences and Coastal Sustainability Institute,
Northeastern University, Nahant MA 01908 USA

*Contact/Corresponding Author: g.trussell@northeastern.edu

Keywords: *Carcinus maenas*, countergradient variation, inducible defenses, invasive species, *Littorina obtusata*, ocean warming, phenotypic plasticity, predatory crab, regional and local scales, trade-offs

Data Availability Statement: Data (Trussell and Corbett, 2023a-d) are available at the National Science Foundation BCO-DMO data repository (https://www.bco-dmo.org/dataset/911221; https://www.bco-dmo.org/dataset/911365; https://www.bco-dmo.org/dataset/911390; https://www.bco-dmo.org/dataset/911409).

Abstract

Invasive predators can cause substantial evolutionary change in native prey populations. Although invasions by predators typically occur over large scales, their distributions are usually characterized by substantial spatiotemporal heterogeneity that can lead to patchiness in the response of native prey species. Our ability to understand how local variation shapes patterns of inducible defense expression has thus far been limited by insufficient replication of populations within regions. Here, we examined local and regional variation in the inducible defenses of 12 native marine snail (*Littorina obtusata*) populations within two geographic regions in the Gulf of Maine that are characterized by vastly different contact histories with the invasive predatory green crab (*Carcinus maenas*). When exposed in the field to water-borne risk cues from the green crab for 90 days, snails expressed plastic increases in shell thickness that reduce their vulnerability to this shell crushing predator. Despite significant differences in contact history with this invasive predator, snail populations from both regions produced similar levels of shell thickness and shell thickness plasticity in response to risk cues. Such phenotypic similarity emerged even though there were substantial geographic differences in shell thickness of juvenile snails at the beginning of the experiment, and we suggest that it may reflect the effects of warming ocean temperatures and countergradient variation. Consistent with plasticity theory, a trend in our results suggests that southern snail populations, which have a longer contact history with the green crab, paid less in the form of reduced tissue mass for thicker shells than northern populations.

Introduction

Inducible defenses are plastic changes in prey phenotypes in response to predator risk cues that reduce prey vulnerability to predation (Harvell 1990). Such plasticity has been documented in a broad array of taxa and manifests in a variety of forms including changes in morphology, life history, behavior, and chemical defense (Tollrian & Harvell 1999, Appleton & Palmer 1988, Dodson 1989). The existence of inducible defenses implies a trade-off between defended and undefended phenotypes. That is, relative to undefended phenotypes, defended phenotypes reduce vulnerability to predation but are more costly in the absence of predators (Tollrian & Harvell 1999). Otherwise, prey would be expected to express defensive traits constitutively, even in the absence of predation risk (Lively 1986 a,b). Inducible defenses can thus allow individuals to defer the cost of a defended phenotype when predation risk is low while retaining the ability to produce a defense when appropriate cues signal that predation risk is high (Schlichting & Pigliucci 1998, West-Eberhard 2003). Hence, inducible defenses can be particularly effective in environments where predator-prey interactions are unpredictable (Lively 1986 a,b) or occur intermittently, such as along the edges of range shifts or invasions (*sensu* Levins 1968, Baldwin 1896, Trussell & Nicklin 2002).

The frequency of predator-prey interactions likely varies considerably across spatial scales, potentially leading to local and broad scale (regional) geographic variation in the expression of inducible defenses. It is well known that the expression of inducible defenses can vary regionally (Trussell & Smith 2000, Trussell & Nicklin 2002, Edgell et al., 2009, Kishida et al., 2007, Long et al., 2011, Jones & Long 2018, Nunes et al., 2014), but what remains less clear is how regional (≥ 100s of km) patterns in inducible defense expression vary with more local scale (≤ 10s of km) patterns. Attention to scale-dependent patterns in the expression of inducible defenses will help identify the factors shaping local vs. regional patterns of adaptation as well as enhance our understanding of the role that inducible defenses may play in shaping local and regional variation in community structure and dynamics via trait-mediated indirect interactions (Werner & Peacor 2003, Peckarsky et al., 2008, Trussell & Schmitz 2012, Schmitz &Trussell 2016).

Predator invasions often encompass broad geographic ranges, exerting strong selective forces on native prey populations (Strauss et al., 2006, Sax et al., 2007, Trussell & Smith 2000). Native prey populations lacking an evolutionary history with an invasive predator may fail to recognize it as a threat (the naïve prey hypothesis) and thus suffer heavy mortality and even local extinction (Case & Bolger 1991, Cox & Lima 2006, Sih et al., 2010). Yet prey species may rapidly evolve adaptations, including inducible defenses, that mitigate their vulnerability (Carroll et al., 1997, Reznick & Endler 1982, Trussell & Smith 2000, Stuart et al., 2014, Bible et al., 2017). We also know that the inducible defenses of prey from invaded regions can differ substantially from those of prey in uninvaded or recently invaded regions (Nunes et al., 2014, Trussell & Smith 2000, Freeman & Byers 2006), but the strength of inference in many of these studies is limited because of low replication of local populations within different geographic regions (Schmitz & Trussell 2016). Such local variation may be key to the capacity of populations to evolve as invasion progresses and may ultimately couple or decouple local and regional patterns of inducible defense expression. Indeed, while it is convenient to conceptualize invasions as homogenous fronts that sweep across vast geographic ranges, predator invasions are often patchy and chaotic in nature (Petrovskii et al., 2005, Morozov et al., 2006). The assessment of inducible defense expression in multiple populations across local and regional scales is thus essential to more robust predictions of how predator invasions will impact prey populations and natural communities within and across geographic regions (Schmitz & Trussell 2016).

Multiple invasions of the European green crab (*Carcinus maenas*) have been highly disruptive across the globe by impacting commercial shellfisheries (Glude 1955, Lafferty & Kuris 1996), facilitating additional invasions (Green et al., 2011, Grosholz 2005), and altering the dynamics of coastal ecosystems (Grosholz et al., 2000, Trussell et al., 2002, Kimbro et al., 2009). In the southern Gulf of Maine (GOM), green crabs have been common since the early 1900's (Say 1817, Welch 1968, Audet et al., 2003), but only recently (during the past 20 years) have they become well established in the northern GOM (Audet et al., 2003, Edgell & Rochette 2008). The latitudinal progression of the green crab invasion into the GOM provides an excellent system to explore local and regional variation in the expression of inducible defenses. For example, a reciprocal transplant experiment in the field over 20 years ago (Trussell & Smith 2000) found that a northern GOM population of the snail, *Littorina obtusata*, exhibited significantly higher shell thickness plasticity in response to green crab risk cues than a southern population. While these different responses may reflect geographic differences in contact history, the lack of replicate populations in each region substantially limited the strength of inference with respect to regionally-based processes. Such regionally-based processes may be particularly important for species having limited dispersal such as *L. obtusata*, which lacks planktonic dispersal and instead produces egg capsules that undergo direct development. Hence, to explore this issue further, we examined local and regional variation in inducible defenses with a field experiment that exposed juvenile *L. obtusata* from multiple northern and southern GOM populations to the presence or absence of green crab risk cues.

Materials and Methods

We conducted crab surveys in 2019, 2020, and 2021 at eight sites in the Gulf of Maine (GOM) that are sheltered from direct wave action to characterize local and regional variation in green crab abundance (Fig. 1, Appendix S1: Table S1). Four sites were located in the northern Gulf (Quoddy Region) and the other four were located in the southern Gulf (Nahant, MA to Cape Ann, MA). Surveys lasted 1 hour each, began and concluded within 2 hours of low tide, and were conducted approximately every two months at all 8 sites from April through November/early December. Tidbit loggers (Onset Computer Corp.) were also installed underneath the fucoid algal canopy in the mid-intertidal zone at these sites in early 2021 to record seawater temperatures in the two regions.

To assess local and regional variation in shell thickness plasticity, we conducted a field experiment that utilized 12 *L. obtusata* populations in the GOM, with 6 in the northern Gulf and 6 in the southern Gulf. Hence, in addition to the 4 sites surveyed for crab abundance within each region, our experiment included two additional sites to increase site replication and power within each region (Fig. 1, Appendix S1: Table S1). Juvenile *L. obtusata* (5-6mm in length) were also collected from each population in late-April and individually tagged with color-coded paint that was sealed with cyanoacrylate glue. Initial shell length and shell thickness were measured with digital calipers (Trussell 1996) and initial tissue mass was determined using a non-destructive weighing technique (see Palmer 1982).

In mid-May, 2021 snails were placed in replicate chambers and returned to their native sites in the mid-intertidal zone where *L. obtusata* and *C. maenas* co-occur. We placed four snails (hereafter "response snails") into one of two paired chambers (10 x 10 x 7cm, l x w x h) that had mesh windows (mesh size = 3mm) to allow water flow and the transmission of risk cues (Appendix

S1: Figure S1). These response chambers also contained approximately 60g of brown algae (*Ascophyllum nodosum*) to serve as food for response snails. The other chamber (the "stimulus chamber") was perforated with small holes on all sides, which allowed response snails to be exposed to water borne cues signaling either the presence (Crab, + C) or absence (No Crab, – NC) of predation risk. The Crab treatment was created by placing a mature male green crab and 30 adult *L. obtusata* to serve as food for the crab in the stimulus chamber whereas stimulus chambers for the No Crab treatment received just 30 adult *L. obtusata*. These paired response-stimulus chambers were housed within a larger plastic chamber (14 x 14 x 16 cm) that also had mesh windows (mesh size = 3mm) to permit water flow. At each site, replicate chambers (n = 4 for each treatment) were anchored beneath the fucoid canopy and Tidbit loggers (Onset Computer Corp.) placed within a subset of chambers recorded seawater temperature at 5-minute intervals. The food supply for crabs was replaced approximately every 21 days while fucoid algae that served as food for the response snails was replaced at day 42. Replicate chambers remained in the field for 90 days before their return to the Northeastern University Marine Science Center (Nahant, Massachusetts) for final measurements of shell length, shell thickness, and tissue mass in response snails.

Statistical Analyses – Mean annual crab abundance was analyzed with a nested ANOVA that considered Region as a fixed effect and Site as a random effect nested within Region. Seawater temperature data (monthly mean values coinciding with high tide) collected in 2021 prior to and during the experiment, and across both time periods, were analyzed in two ways. For data across both time periods (i.e., overall temperature), we conducted an ANOVA that considered Region as a fixed effect and Site as a random effect nested within Region. For temperature data involving the time periods before and during the experiment, we conducted a two-factor ANOVA that considered Region and Period as fixed effects, and Site as a random effect nested within Region. We also conducted analyses (linear regression, ANCOVA) to explore the relationship between shell thickness and seawater temperature. Initial mean shell thickness for each site was regressed as a function of mean water temperature at each respective site prior to response snail collection (early-March through late-April). Only four sites from each region were included in this analysis because pre-experiment temperature data were not available for all sites. For final shell thickness data on response snails from the field experiment, we first conducted separate ANCOVAs for the northern and southern GOM that considered Risk as a fixed effect. In this analysis the mean shell thickness for each site served as the response variable and mean seawater temperature at each site during the experiment served as the covariate. These separate analyses were necessary because divergent seawater temperatures in the northern and southern GOM did not overlap, thus violating a key premise of ANCOVA. For both regions, these analyses revealed no effect of risk cues on the relationship between mean shell thickness and seawater temperature (see Results). We therefore combined the risk treatment data for each region and performed separate linear regressions for each region with mean shell thickness as the response variable and mean seawater temperature as the covariate.

Initial trait values for shell thickness were analyzed with a two-factor ANCOVA that considered Region (North, South) as a fixed effect, Site as a random effect nested within Region and initial shell length as the covariate. Trade-offs in the form of reduced tissue mass are driven by architectural constraints on the internal volume of the shell and these constraints are directly shaped by shell thickness (Palmer 1981, Trussell 2000). Because we wanted to determine how investment into shell thickening influenced variation in tissue mass, our analysis of initial tissue mass used the same ANCOVA model as above but with initial mean shell thickness as the covariate. Final mean shell thickness and final tissue mass were analyzed with a three-factor ANCOVA that considered Region (North, South) and Risk Treatment (Crab, No-Crab) as fixed effects, Site as a random effect nested within Region, and replicate chambers as a random effect nested within each treatment combination. For these analyses, final shell length was used as the covariate for shell thickness and final mean shell thickness was used as the covariate for final tissue mass. All analyses were conducted using JMP Software (Version 15.0, SAS Institute, Cary, NC).

Results

Regional variation in crab density varied among years (Region*Year: $F_{2,12} = 4.48$, $P = 0.0352$) but overall crab density was consistently more than two times higher in the northern versus southern GOM (Region: $F_{1,6} = 22.49$, $P = 0.0032$, Fig. 2). Site explained 16.9% of the variation in crab density ($\sigma^2 = 55.6$, Wald $P = 0.1292$). By contrast, mean (± SE) water temperature from early March to early August was substantially higher in the southern (11.50 ± 0.26 °C) than in the northern (7.91 ± 0.26 °C) GOM (Region: $F_{1,6} = 97.90$, $P < 0.0001$, Fig. 3). Site explained 1.1% of the variation in seawater temperature ($\sigma^2 = 0.20$, Wald $P = 0.1821$). To further examine potential water temperature effects on shell thickness, we examined regional differences in water temperature for the time period (approximately 7 weeks) preceding the collection of juvenile snails and during the 90 day experimental period. A significant interaction (Region*Period: $F_{1,6} = 61.62$, $P = 0.0002$) revealed that regional effects (Region: $F_{1,9} = 97.26$, $P < 0.0001$) depended on time period (Period: $F_{1,6} = 1{,}003.60$, $P < 0.0001$, Fig. 3). Site explained 3.1% of the variation in seawater temperature ($\sigma^2 = 0.18$, Wald $P = 0.3800$). Prior to the experiment, mean water temperature differed by 1.49 °C among regions, with temperatures averaging 5.79 (± 0.34) °C in the southern GOM and 4.30 (± 0.34) °C in the northern GOM (Fig. 3). During the experiment, this regional difference increased to 5.35 °C, with temperatures averaging 15.52 (± 0.26) °C in the southern GOM and 10.17 (± 0.26) °C in the northern GOM (Fig. 3).

Comparison of initial juvenile mean shell thickness as a function of water temperature prior to the experiment revealed that shell thickness was positively correlated with water temperature (linear regression, $Y = 0.073X + 0.42$, $R^2 = 0.65$, $F_{1,6} = 10.96$, $P = 0.0162$, Fig. 4a). The relationship between final shell thickness and water temperature during the experiment was not affected by risk cues for northern (Risk: $F_{1,8} = 0.74$, $P = 0.4166$) and southern (Risk: $F_{1,8} = 1.24$, $P = 0.2976$) snails. Analysis of combined data (both risk treatments) for each region revealed no relationship between final shell thickness and seawater temperature during the experiment for northern snails (linear regression, $Y = - 0.03X + 1.52$, $R^2 = 0.10$, $P = 0.3295$, Fig. 4b) but there was a positive trend for southern snails (linear regression, $Y = 0.04X + 0.61$, $R^2 = 0.31$, $P = 0.0604$; Fig. 4c).

Comparison of the initial shell thickness of juvenile snails before their exposure to the presence and absence of risk cues revealed that southern snails were significantly thicker (10.5%) than northern snails (Region: $F_{1,9.9} = 9.04$, $P = 0.0133$, Fig. 5a). Site explained 30.7% of the variation in juvenile shell thickness ($\sigma^2 = 0.002$, Wald $P = 0.0388$). We did not detect any regional differences in the initial tissue mass (Region: $F_{1,10.01} = 0.46$, $P = 0.5108$) of juvenile snails prior to beginning the experiment (Fig. 5b). Site explained 82.1% of the variation in juvenile tissue mass ($\sigma^2 = 1.03 \times 10^{-4}$, Wald $P = 0.0271$).

After 90 days in the field, snails raised in the presence of risk cues produced shells that were significantly thicker than those of snails raised without risk cues (Risk: $F_{1,11.2} = 8.43$, $P = 0.0141$, Fig. 6a) but these responses were rather subtle for both northern (3.3%) and southern (1.7%) snails. The effect of risk cues did not vary between regions (Risk*Region: $F_{1,9.2} = 1.81$, $P = 0.2109$) and the observed regional differences in the initial shell thickness of juvenile snails before beginning the experiment were no longer apparent after 90 days in the field (Region: $F_{1,9.8} = 0.34$, $P = 0.5738$). Site explained 36.1% of the variation in shell thickness ($\sigma^2 = 0.004$, Wald $P = 0.0368$). Variation in final tissue mass after adjusting for investment into final shell thickness revealed that risk cues significantly reduced final tissue mass (Risk: $F_{1,8.3} = 25.56$, $P = 0.0009$). The effect of region (Region: $F_{1,10} = 0.50$, $P = 0.4964$) was not significant but there was a trend (Risk*Region: $F_{1,8.05} = 4.12$, $P = 0.0768$) suggesting that the adverse effect of risk cues on tissue mass was stronger for northern (-16.7%) versus southern (-6.9%) snails (Fig. 6b). Site explained 69.1% of the variation in tissue mass ($\sigma^2 = 2.5 \times 10^{-4}$, Wald $P = 0.0279$

Discussion

It is well established that invasive predators often have strong impacts on recipient ecosystems (Simberloff & Von Holle 1999, Grosholz et al., 2000). Because many invasions occur over broad geographic scales, examining geographic variation in antipredator defenses can provide key insights to the adaptive capacity of native prey species (Kishida et al., 2007, Long et al., 2011, Jones & Long 2018, Nunes et al., 2014). In this study, we compared inducible defense expression in snails from two regions having substantially different contact histories with the invasive predatory green crab. Such regionally-based processes can shape the evolution of genetic and plastic controls on phenotypic variation, but their influence may also depend on the effects of local-scale processes on phenotypes (Kawecki & Ebert 2004, Blanquart et al., 2013, Schmitz & Trussell 2016) such as variation in (1) food availability and snail foraging rates and attendant effects on growth rates (Kemp & Bertness 1984, Trussell 1996, Appleton & Palmer 1988) and (2) the effects of water turbulence on the ability of prey to detect predator risk cues (Smee & Weissburg 2006). Hence, our experiment utilized snails from multiple local populations within each region to explore how inducible defense expression varies with spatial scale and thus invasion history.

Theory predicts that selection will favor the evolution or maintenance of plasticity in prey traits in variable, unpredictable environments such as at the edge of invasive predator fronts and less plasticity in more constant, predictable environments (Levins 1968, Lively 1986a,b, West-Eberhard 2003) such as those that may be created after invasive predators have become fully established. Assuming sufficient genetic variation, plasticity can evolve in response to changing environments and accompanying shifts in selection pressure (Schlichting & Pigliucci 1998, Windig et al., 2004) that favor increased or decreased plasticity (genetic accommodation) or the evolutionary loss of plasticity via genetic assimilation (West-Eberhard 2003).

Edgell et al. (2009) explored these ideas in the laboratory by examining how trans-Atlantic differences in contact history between green crabs and snails may shape snail trait plasticity. Using populations from the United Kingdom (UK), where *L. obtusata* and green crabs have the longest contact history (the UK is part of the native range of green crabs), and the northern (more recent invasion history) and southern (longer invasion history) GOM, they examined geographic variation in plasticity of shell retractability in the presence and absence of green crab risk cues. Shell retractability measures the capacity of snails to retract deep into their protective shell when confronted with predation risk. In the UK, retractability was canalized (not plastic) but plasticity in retractability for snails from the southern and northern GOM was moderate to high, respectively, revealing an inverse relationship between contact history and trait plasticity.

Consistent with theory and the results of earlier empirical work that included two of the populations used in this study (Trussell & Smith 2000, Trussell & Nicklin 2002, Edgell et al.

2009), we predicted that juvenile northern snails would exhibit higher shell thickness plasticity than southern snails despite the relatively recent (~ the last 20 years) increases in green crab density in the northern GOM (Fig. 1) because of their significantly thinner shells prior to their exposure to our experimental treatments (Fig. 5a). Yet, after 90 days of exposure to experimental treatments in the field, we found that both mean shell thickness in the absence of risk cues and shell thickness plasticity in response to green crab risk cues did not vary across these two regions or across sites within each region (Fig. 6a). For example, the average degree of shell thickness plasticity was 3.3% for northern snails and 1.7% for southern snails. Thus, despite their substantially different contact histories, reaction norms for the shell thickness of southern and northern snails have evolved similar degrees of plasticity and have done so rather rapidly. As noted above, two of the populations (West Quoddy, ME and Lobster Cove, MA) in this study were used in earlier work in the late 90's and early 2000's, which found that shell thickness plasticity was significantly higher for northern than for southern snails (Trussell & Smith 2000, Trussell & Nicklin 2002).

These results are especially intriguing given the substantial differences in the shell thickness of snails at the beginning of the experiment. On average, juvenile southern snails were ~11.5% thicker than northern snails, but this difference disappeared after 90 days of growth in the field, regardless of risk treatment. Thus, other factors were clearly influencing the trajectory of shell thickening as snails grew. One possibility is that northern snails raised in the absence of risk (No Crab, – C) were able to produce shells of similar thickness to their southern counterparts because they responded to naturally occurring risk cues associated with the significantly higher ambient green crab density in the northern GOM. However, if such naturally occurring risk cues were operating additively with our experimental application of risk, then one might also expect shell thickening in the presence of experimental risk (Crab, + C) to be significantly greater in northern versus southern snails. We did not observe this pattern, and therefore suggest that the effects of naturally occurring cues were either minimal or that our experimental application of risk cues rendered natural cues unimportant by exceeding some threshold risk level above which additional cue did not matter.

We also know that water temperature can also influence broad-scale patterns of shell thickening (Trussell 2000, Trussell & Smith 2000) and other aspects of snail shell morphology, with colder waters limiting calcification rates or increasing the dissolution of deposited shell material (Vermeij 1978, Graus 1974). In this study we found that initial shell thickness in juvenile snails was associated with the seawater temperatures that they experienced prior to their collection for the experiment (Fig. 4a). This pattern was driven by thinner shells in the colder waters of the northern GOM and thicker shells in the warmer waters of the southern GOM. Yet, we did not detect any relationship between shell thickness and seawater temperature during the experiment (Figs. 4b,c). This result was surprising because, as noted above, northern snails in both risk treatments were able to achieve shell thicknesses similar to those of southern snails after 90 days in the field. We suggest that the ability of northern snails to catch up to their southern counterparts may also reflect a countergradient response (Conover & Schultz 1995, Trussell 2000) that is driven by recent, dramatic ocean warming in the GOM (Mills et al., 2013, Pershing et al., 2021), and the effects of such warming may be particularly strong during the growing season. For example, water temperature data collected during experiments in the late 1990's (Trussell & Smith 2000) recorded seawater temperatures of 14.61 °C and 8.47 °C during the growing season for the southern and northern GOM, respectively. In this study, temperatures over the same general time period were 15.52 °C and 10.17 °C for the southern and northern

GOM, respectively. Hence, we hypothesize that the 1.7 °C increase in water temperature in the northern GOM may have been sufficient to trigger a countergradient response that allowed northern snails to overcome their thinner shells as juveniles and this response may have matched or superseded that driven by risk cues.

A central tenet of plasticity theory is that plastic traits should be accompanied by trade-offs, otherwise selection will favor the evolution of fixed traits (Levins 1968, Van Tienderen 1991, West-Eberhard 2003). For many marine mollusks, including *L. obtusata*, the costs paid for thicker shells often come in the form of reduced tissue mass and/or tissue mass growth because a thicker shell constrains the internal habitable volume available to support tissue mass for a shell of a given size and shape (Palmer 1981, 1992, Kemp & Bertness 1984). Such architectural constraints may have a direct bearing on fitness because snail fecundity is often a positive function of tissue mass (Spight & Emlen 1976). Despite the substantial differences in the shell thickness of juvenile snails from both regions prior to the exposure to risk treatments (Fig. 4a), we did not detect expected differences in initial tissue mass (Fig. 4b). The absence of a trade-off in juvenile snails may reflect regional differences in the influence of other factors on tissue mass such as water temperature or may simply be due to snails being too early in ontogeny for the trade-off to manifest (Relyea & Hoverman 2003).

We also examined the effect of risk cues on the final tissue mass of snails after adjusting for their investment in shell thickness (Fig. 6b). Risk cues adversely affected tissue mass and the marginally significant interaction suggests that this effect was more substantial for northern snails. Based on theory, we predicted that southern snails would pay less in terms of reduced tissue mass because natural selection has had more time to optimize the trade-off (Murren et al., 2015, DeWitt et al., 1998, 1999). Although further studies are needed, our results are consistent with the view that the longer contact history between green crabs and snails in the southern GOM has allowed natural selection to reduce the impact of this trade-off. Future work examining temporal transitions in shell thickness plasticity and associated trade-offs in tissue mass will further illuminate how selection is shaping spatiotemporal variation in shell thickness plasticity as the green crab invasion continues to unfold.

Given the significant ecological change wrought by invasive predators globally (Ruiz et al., 1997, Molnar et al., 2008), continued attention to invasion scenarios will enhance our understanding of how inducible defenses may mitigate the consequences of invasive predators for native prey populations and communities (Trussell & Schmitz 2012, Schmitz & Trussell 2016). Moreover, because invasive predators often represent a potent agent of selection, theaters of biological invasion should provide key insights into rapid evolutionary change, including the evolution of phenotypic plasticity, and how patterns of adaptation unfold across local and broad geographic scales (Mooney & Cleland 2001, Strauss et al., 2006, Scoville & Pfrender 2010).

Acknowledgments

This paper was submitted as part of a Ph.D. from Northeastern University's Department of Marine and Environmental Sciences. The authors would like to thank Ron Etter, Tarik Gouhier, Ted Grosholz, Randall Hughes, Katie Lotterhos, Eric Sanford, and an anonymous reviewer for their many helpful comments that improved the manuscript. Special thanks to Jackson Cabot and Danielle Whalen for their assistance in the field. This work was supported by a grant from the U.S. National Science Foundation (OCE-2017626) and the Doherty Professorship in Marine and Environmental Science to G.C. Trussell. This is Contribution No. 430 from the Coastal Sustainability Institute/Marine Science Center.

Author Contributions: JJC and GCT designed the experiment, performed fieldwork and measurements, and wrote the manuscript. JJC conducted the statistical analyses.

Conflict of Interest Statement: The authors declare no conflicts of interest.

Literature Cited

Appleton, R.D., and A.R. Palmer. 1988. "Water-borne stimuli released by predatory crabs and damaged prey induce more predator-resistant shells in a marine gastropod." *Proceedings of the National Academy of Sciences USA* 85: 4387-4391.

Audet, D., D.S. Davis, G. Miron, M. Moriyasu, K. Benhalima, and R. Campbell. 2003. "Geographical expansion of a nonindigenous crab, *Carcinus maenas* (L.), along the Nova Scotian shore into the southeastern Gulf of St. Lawrence, Canada." *Journal of Shellfish Research* 22: 255-262.

Baldwin, J.M. 1896. "A new factor in evolution." *American Naturalist* 30: 441-451.

Bible, J.M., K.R. Griffith, and E.S. Sanford. 2017. "Inducible defenses in Olympia oysters in response to an invasive predator". *Oecologia* 183:809-819.

Blanquart, F., O. Kaltz, S.L. Nuismer, and S. Gandon. 2013. "A practical guide to measuring local adaptation." *Ecology Letters* 16: 1195-1205.

Carroll, S.P., H. Dingle, and S.P. Klassen. 1997. "Genetic differentiation of fitness-associated traits among rapidly evolving populations of the soapberry bug." *Evolution* 51: 1182-1188.

Case, T.J., and D.T. Bolger. 1991. "The role of introduced species in shaping the distribution and abundance of island reptiles." *Evolutionary Ecology* 5: 272-290.

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

Cox, J.G., and S.L. Lima. 2006. "Naiveté and an aquatic–terrestrial dichotomy in the effects of introduced predators." *Trends in Ecology & Evolution* 21: 674-680.

DeWitt, T.J., A.Sih, and J.A. Hucko. 1999. "Trait compensation and cospecialization in a freshwater snail: size, shape, and antipredator behaviour". *Animal Behaviour* 58: 397-407.

DeWitt, T.J., A. Sih, and D.S. Wilson. 1998. "Costs and limits of phenotypic plasticity." *Trends in Ecology & Evolution* 13: 77-81.

Dodson, S. 1989. "Predator-induced reaction norms." *Bioscience* 39: 447-452.

Edgell, T.C., and R. Rochette. 2008. "Differential snail predation by an exotic crab and the geography of shell-claw covariance in the northwest Atlantic". *Evolution* 62:1216-1228.

Edgell, T. C., B.R. Lynch, G.C. Trussell, and A.R. Palmer. 2009. "Experimental evidence for the rapid evolution of behavioral canalization in natural populations." *American Naturalist* 174: 434-440.

Freeman, A.S., and J.E. Byers. 2006. "Divergent induced responses to an invasive predator in marine mussel populations." *Science* 313: 831-833.

Graus, R.R., 1974. "Latitudinal trends in the shell characteristics of marine gastropods." *Lethaia* 7: 303-314.

Glude, J.B. 1955. "The effects of temperature and predators on the abundance of the soft-shell clam, *Mya arenaria*, in New England." *Transactions of the American Fisheries Society* 84: 13-26.

Green, P.T., D.J. O'Dowd, K.L. Abbott, M. Jeffery, K. Retallick, and R. MacNally. 2011. "Invasional meltdown: invader–invader mutualism facilitates a secondary invasion." *Ecology* 92: 1758-1768.

Grosholz, E.D., G.M. Ruiz, C.A. Dean, K.A. Shirley, J.L. Maron, and P.G. Connors. 2000. "The impacts of a nonindigenous marine predator in a California bay." *Ecology* 81: 1206-1224.

Grosholz, E.D. 2005. "Recent biological invasion may hasten invasional meltdown by accelerating historical introductions." *Proceedings of the National Academy of Sciences USA* 102: 1088-1091.

Harvell, C.D. 1990. "The ecology and evolution of inducible defenses." *The Quarterly Review of Biology* 65: 323-340.

Jones, E. and J.D. Long. 2018. "Geographic variation in the sensitivity of an herbivore-induced seaweed defense." *Ecology* 99: 1748-1758.

Kawecki, T., and D. Ebert. 2004. "Conceptual issues in local adaptation." *Ecology Letters* 7: 1225-1241.

Kemp, P., and M.D. Bertness. 1984. "Snail shape and growth rates: evidence for plastic allometry in *Littorina littorea*." *Proceedings of the National Academy of Sciences USA* 81: 811-813.

Kimbro, D.L., E.D. Grosholz, A.J. Baukus, N.J. Nesbitt, N.M. Travis, S. Attoe, and C. Coleman-Hulbert. 2009. "Invasive species cause large-scale loss of native California oyster habitat by disrupting trophic cascades." *Oecologia* 160: 563-575.

Kishida, O., G.C. Trussell, and K. Nishimura. 2007. "Geographic variation in a predator-induced defense and its genetic basis." *Ecology* 88: 1948-1954.

Lafferty, K.D., and A.M. Kuris. 1996. "Biological control of marine pests." *Ecology* 77: 1989-2000.

Levins, R. 1968. Evolution in changing environments: some theoretical explorations. *Princeton University Press*, Princeton, NJ.

Lively, C.M. 1986a. "Predator-induced shell dimorphism in the acorn barnacle *Chthamalus anisopoma*." *Evolution* 40: 232-242.

Lively, C.M. 1986b. "Competition, comparative life histories, and maintenance of shell dimorphism in a barnacle." *Ecology* 67: 858-864.

Long, J.D., J.L. Mitchell, and E.E. Sotka. 2011. "Local consumers induce resistance differentially between *Spartina* populations in the field." *Ecology* 92: 180-188.

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: 191-195.

Molnar, J.L., R.L. Gamboa, C. Revenga, and M.D. Spalding. 2008. "Assessing the global threat of invasive species to marine biodiversity". *Frontiers in Ecology and the Environment* 6:485-492.

Mooney, H.A., and E.E. Cleland. 2001. "The evolutionary impact of invasive species". *Proceedings of the National Academy of Sciences USA* 98: 5446-5451.

Morozov, A., S. Petrovskii, and B.L. Li. 2006. "Spatiotemporal complexity of patchy invasion in a predator-prey system with the Allee effect." *Journal of Theoretical Biology* 238: 18-35.

Murren, C.J., J.R. Auld, H. Callahan, C.K. Ghalambor, C.A. Handelsman, M.A. Heskel, J.G. Kingsolver, H.J. Maclean, J. Masel, H. Maughan, and D.W. Pfennig. 2015. "Constraints on the evolution of phenotypic plasticity: limits and costs of phenotype and plasticity." *Heredity* 115: 293-301.

Nunes, A.L., G. Orizaola, A. Laurila, and R. Rebelo. 2014. "Rapid evolution of constitutive and inducible defenses against an invasive predator." *Ecology* 95: 1520-1530.

Palmer, A.R. 1981. “Do carbonate skeletons limit the rate of body growth?” *Nature* 292: 150-152.

Palmer, A.R. 1982. “Growth in marine gastropods. A non-destructive technique for independently measuring shell and body weight.” *Malacologia* 23: 63-74.

Palmer, A.R. 1992. “Calcification in marine molluscs: how costly is it?” *Proceedings of the National Academy of Sciences USA* 89: 1379-1382.

Peckarsky, B.L., P.A. Abrams, D.I. Bolnick, L.M. Dill, J.H. Grabowski, B. Luttbeg, J.L. Orrock, S.D. Peacor, E.L. Preisser, O.J. Schmitz, and G.C. Trussell. 2008. “Revisiting the classics: considering nonconsumptive effects in textbook examples of predator–prey interactions.” *Ecology* 89: 2416-2425.

Pershing, A.J., M.A. Alexander, D.C. Brady, D. Brickman, E.N. Curchitser, A.W. Diamond, L. McClenachan, K.E. Mills, O.C. Nichols, D.E. Pendleton, N.R. Record, J.D. Scott, M.D. Staudinger, and Y. Wang. 2021. “Climate impacts on the Gulf of Maine ecosystem: A review of observed and expected changes in 2050 from rising temperatures.” *Elementa Science of the Anthropocene* 9, doi:https://doi.org/10.1525/elementa.2020.00076.

Petrovskii, S., A. Morozov, and B.L. Li. 2005. “Regimes of biological invasion in a predator-prey system with the Allee effect.” *Bulletin of Mathematical Biology* 67: 637-661.

Relyea, R.A. and J.T. Hoverman. 2003. “The impact of larval predators and competitors on the morphology and fitness of juvenile treefrogs.” *Oecologia* 134: 596-604.

Reznick, D., and J.A. Endler. 1982. “The impact of predation on life history evolution in Trinidadian guppies (*Poecilia reticulata*).” *Evolution* 36: 160-177.

Ruiz, G.M., J.T. Carlton, E.D. Grosholz, and A.H. Hines. 1997. “Global invasions of marine and estuarine habitats by non-indigenous species: mechanisms, extent, and consequences”. *American Zoologist* 37:621-632.

SAS Institute. 2019. JMP Pro, v15.0.0. SAS Institute, Cary, North Carolina, USA.

Sax, D.F., J.J. Stachowicz, J.H. Brown, J.F. Bruno, M.N. Dawson, S.D. Gaines, R.K. Grosberg, A. Hastings, R.D. Holt, M.M. Mayfield, and M.I. O’Connor. 2007. “Ecological and evolutionary insights from species invasions.” *Trends in Ecology & Evolution* 22: 465-471.

Say, T. 1817. “An account of the Crustacea of the United States.” *Journal of the Academy of Natural Sciences Philadelphia* 1: 56-57.

Schlichting, C.D., and M. Pigliucci. 1998. Phenotypic evolution: a reaction norm perspective. *Sinauer Associates,* Sunderland, MA.

Schmitz, O.J. and G.C. Trussell. 2016. “Multiple stressors, state-dependence and predation risk-foraging trade-offs: toward a modern concept of trait-mediated indirect effects in communities and ecosystems.” *Current Opinion in Behavioral Sciences* 12: 6-11.

Scoville, A.G., and M.E. Pfrender. 2010. “Phenotypic plasticity facilitates recurrent rapid adaptation to introduced predators”. *Proceedings of the National Academy of Sciences USA* 107: 4260-4263.

Sih, A., D.I. Bolnick, B. Luttbeg, J.L. Orrock, S.D. Peacor, L.M. Pintor, E. Preisser, J.S. Rehage, and J.R. Vonesh. 2010. “Predator–prey naïveté, antipredator behavior, and the ecology of predator invasions.” *Oikos* 119: 610-621.

Simberloff, D. and B. Von Holle. 1999. “Positive interactions of nonindigenous species: invasional meltdown?” *Biological Invasions* 1: 21-32.

Smee, D.L., and M.J Weissburg. 2006. “Clamming up: environmental forces diminish the perceptive ability of bivalve prey”. *Ecology* 87: 1587-1598.

Spight, T.M., and J. Emlen. 1976. “Clutch sizes of two marine snails with a changing food supply.” *Ecology* 57: 1162-1178.

Strauss, S.Y., J.A. Lau, and S.P. Carroll. 2006. “Evolutionary responses of natives to introduced species: what do introductions tell us about natural communities?” *Ecology letters* 9: 357-374.

Stuart, Y.E., T.S. Campbell, P.A. Hohenlohe, R.G. Reynolds, L.J. Revell, and J.B. Losos. 2014. “Rapid evolution of a native species following invasion by a congener.” *Science* 346: 463-466.

Tollrian, R.I., and C.D. Harvell. 1999. The Ecology and Evolution of Inducible Defenses. *Princeton University Press*, Princeton, NJ.

Trussell, G. C., Corbett, J. J. 2023a. *Littorina obtusata* shell length, shell thickness, and tissue mass measured during a field experiment conducted at 12 sites in the Gulf of Maine from April to August 2021. Biological and Chemical Oceanography Data Management Office (BCO-DMO). (Version 1) Version Date 2023-10-11. doi:10.26008/1912/bco-dmo.911221.1

Trussell, G. C., Corbett, J. J. 2023b. Green crab (*Carcinus maenas*) density at rocky intertidal sites determined at 8 sites in the Gulf of Maine from April 2019 to December 2021. Biological and Chemical Oceanography Data Management Office (BCO-DMO). (Version 1) Version Date 2023-10-12. doi:10.26008/1912/bco-dmo.911365.1

Trussell, G. C., Corbett, J. J. 2023c. Seawater temperatures at high tide at study sites in the Gulf of Maine prior to, during, and after an outplant experiment that was conducted from April to

August 2021. Biological and Chemical Oceanography Data Management Office (BCO-DMO). (Version 1) Version Date 2023-10-12. doi:10.26008/1912/bco-dmo.911390.1

Trussell, G. C., Corbett, J. J. 2023d. Seawater temperatures at study sites in the Gulf of Maine prior to and during an outplant experiment that was conducted from April to August 2021. Biological and Chemical Oceanography Data Management Office (BCO-DMO). (Version 1) Version Date 2023-10-12. doi:10.26008/1912/bco-dmo.911409.1

Trussell, G.C., 2000. "Phenotypic clines, plasticity, and morphological trade-offs in an intertidal snail." *Evolution* 54: 151-166.

Trussell, G.C. 1996. "Phenotypic plasticity in an intertidal snail: the role of a common crab predator." *Evolution* 50: 448-454.

Trussell, G.C., and O.J. Schmitz. 2012. Species functional traits, trophic control and the ecosystem consequences of adaptive foraging in the middle of food chains. Pages 324-338 *in* Ohgushi, T., O.J. Schmitz, and R.D. Holt, editors. *Trait-mediated Indirect Interactions*. Cambridge University Press, Cambridge U.K.

Trussell, G.C., and M.O. Nicklin. 2002. "Cue sensitivity, inducible defense, and trade-offs in a marine snail." *Ecology* 83: 1635-1647.

Trussell, G.C., P.J. Ewanchuk, and M.D. Bertness. 2002. "Field evidence of trait-mediated indirect interactions in a rocky intertidal food web." *Ecology Letters* 5: 241-245.

Trussell, G.C., and L.D. Smith. 2000. "Induced defenses in response to an invading crab predator: an explanation of historical and geographic phenotypic change." *Proceedings of the National Academy of Sciences USA* 97: 2123-2127.

Van Tienderen, P.H. 1991. “Evolution of generalists and specialists in spatially heterogeneous environments.” *Evolution* 45: 1317-1331.

Vermeij, G.J. 1978. Biogeography and Adaptation: Patterns of Marine Life. *Harvard University Press*, Cambridge, MA.

Welch, W.R. 1968. “Changes in abundance of the green crab *Carcinus maenas* in relation to recent temperature changes.” *Fishery Bulletin* 67: 337-345.

West-Eberhard, M.J. 2003. Developmental plasticity and evolution. *Oxford University Press*, Oxford, U.K.

Werner, E.E., and S.D. Peacor. 2003. “A review of trait-mediated indirect interactions in ecological communities.” *Ecology* 84: 1083-1100.

Windig, J.J., C.G.F. de Kovel, and G. de Jong. 2004. Genetics and Mechanisms of Plasticity. Pages 31-49 *in* T. J. Dewitt and S. M. Scheiner, editors. *Phenotypic plasticity*. Oxford University Press, Oxford, U.K.

Figure Legends

Figure 1: Map of study sites in the northern and southern Gulf of Maine. Southern Sites (Massachusetts): Nahant (1), Doliber Cove (2), Lobster Cove (3), Stage Fort Park (4), Loblolly Cove (5), Hodgkins Cove (6); Northern Sites (Maine): Jasper Beach (7), Cutler (8), Moose Cove (9), Hamilton Cove (10), Carrying Place Cove (11), West Quoddy (12). Asterisks denote sites where crab surveys were conducted and pre-experiment seawater temperatures were recorded. See Appendix S1: Table S1 for more details on site locations and their role in the study.

Figure 2: Mean (±SE) crab (*Carcinus maenas*) density on sheltered shores (N = 4 per region per year) in the northern and southern Gulf of Maine in 2019, 2020, and 2021. See Appendix S1: Table S1 for sites used for crab surveys.

Figure 3: Mean (±SE) seawater temperatures on sheltered shores in the northern and southern Gulf of Maine prior to and during the experiment in 2021. See Appendix S1: Table S1 for sites used for temperature monitoring.

Figure 4: The relationship between (a) shell thickness of juvenile snails (*Littorina obtusata*) and seawater temperature prior to beginning the experiment, and the relationship between final shell thickness of snails and seawater temperature after the 90 day field experiment for (b) northern and (c) southern populations. Note that for the southern population (c) there was a positive trend between shell thickness and seawater temperature (P = 0.0604, see Results for more details).

Figure 5: Mean (± SE) (a) shell thickness and (b) tissue mass of juvenile snails (*Littorina obtusata*) from sheltered shores in the northern and southern Gulf of Maine prior to the experiment. Large symbols denote regional means (± SE).

Figure 6: Mean (± SE) (a) shell thickness and (b) tissue mass of snails (*Littorina obtusata*) from sheltered shores in the northern and southern Gulf of Maine after 90 days of exposure to the presence (+C) and absence (–C) of green crab (*Carcinus maenas*) risk cues. Large symbols denote regional means (± SE) for each risk treatment.

Figure 1.

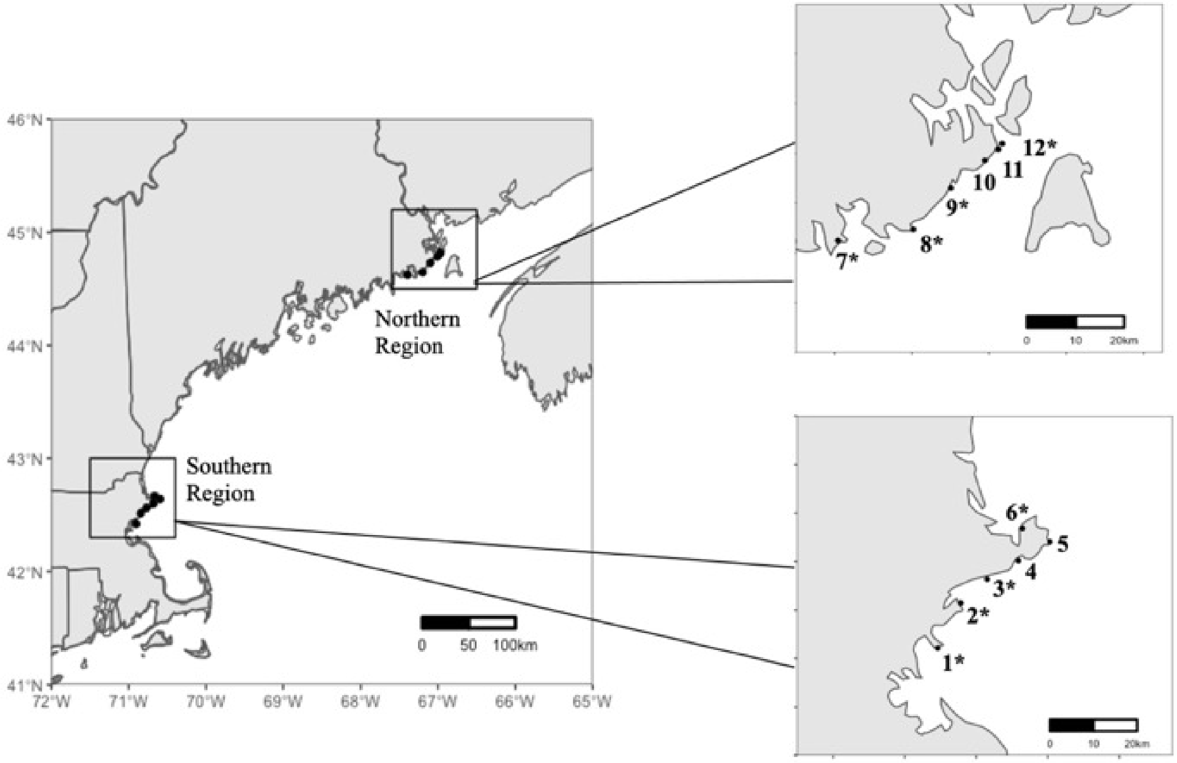
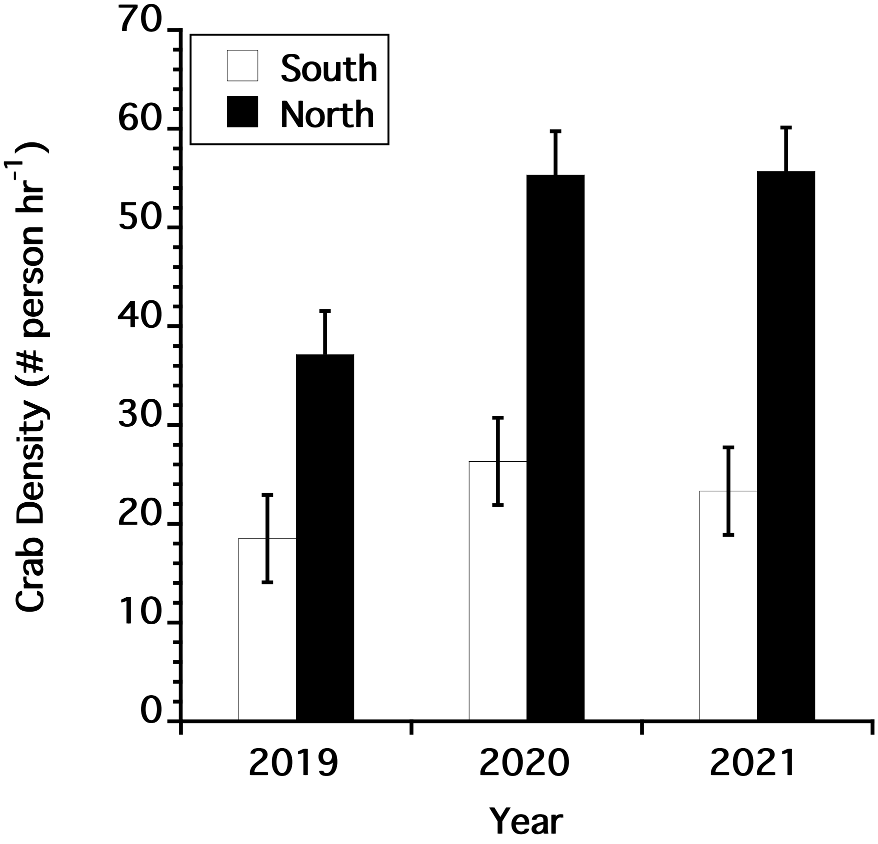
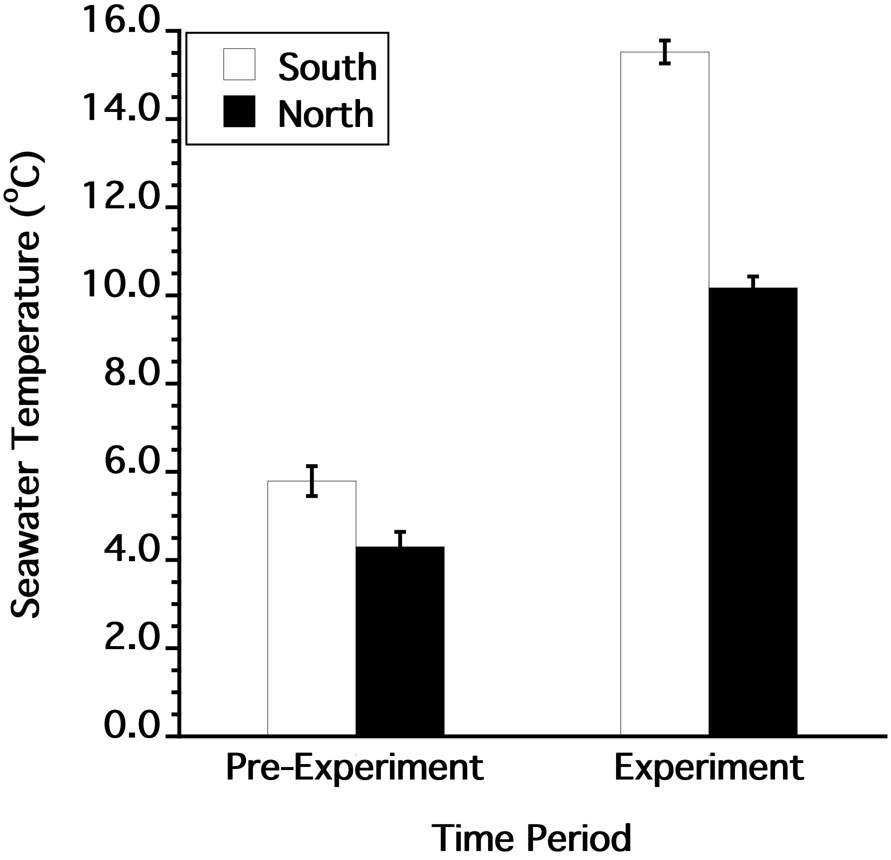

46°N
45°N
44°N
43°N
42°N
41°N
72°W
71°W
70°W
69°W
68°W
67°W
66°W
65°W
Northern Region
Southern Region
0 50 100km
12*
11
10
9*
8*
7*
0 10 20km
6*
5
4
3*
2*
1*
0 10 20km

Figure 2.



70
60
50
40
30
20
10
0
Crab Density (# person hr^{-1})
South
North
2019
2020
2021
Year

Figure 3.



16.0
14.0
12.0
10.0
8.0
6.0
4.0
2.0
0.0
Seawater Temperature (°C)
☐ South
■ North
Pre-Experiment
Experiment
Time Period

Figure 4.

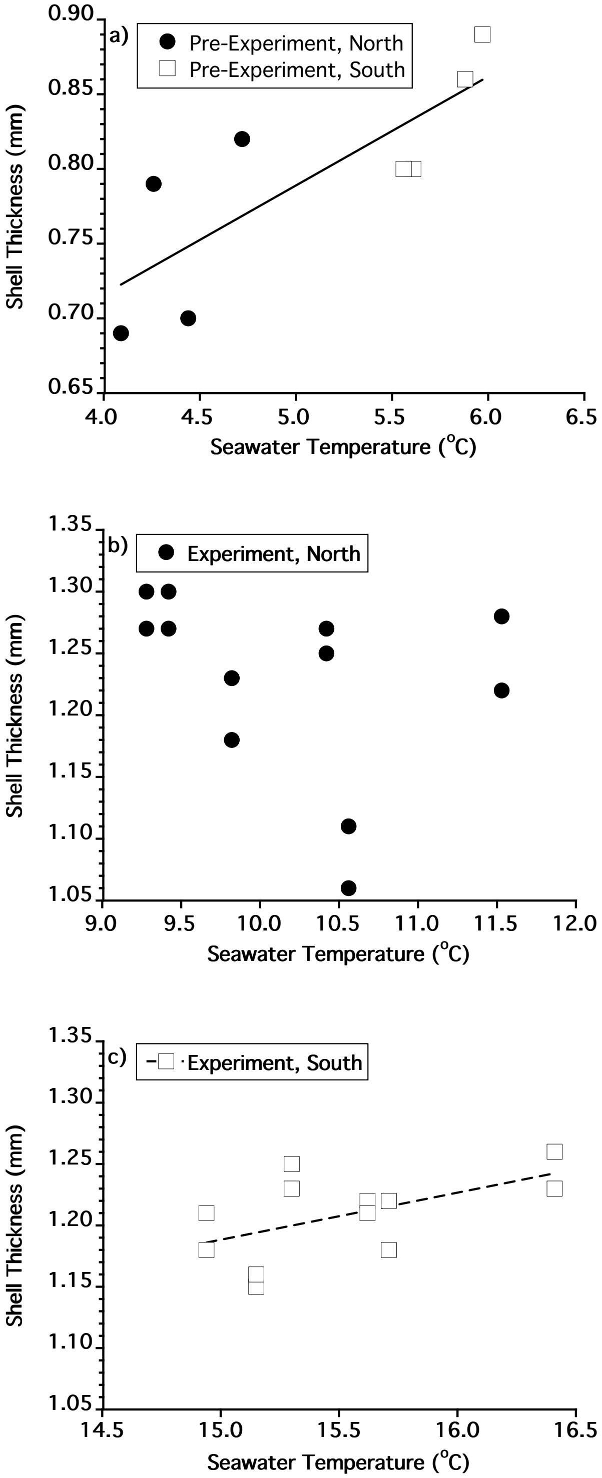

a)
Pre-Experiment, North
Pre-Experiment, South
Shell Thickness (mm)
0.90
0.85
0.80
0.75
0.70
0.65
4.0
4.5
5.0
5.5
6.0
6.5
Seawater Temperature (^{o}C)
b)
Experiment, North
Shell Thickness (mm)
1.35
1.30
1.25
1.20
1.15
1.10
1.05
9.0
9.5
10.0
10.5
11.0
11.5
12.0
Seawater Temperature (^{o}C)
c)
Experiment, South
Shell Thickness (mm)
1.35
1.30
1.25
1.20
1.15
1.10
1.05
14.5
15.0
15.5
16.0
16.5
Seawater Temperature (^{o}C)

Figure 5.

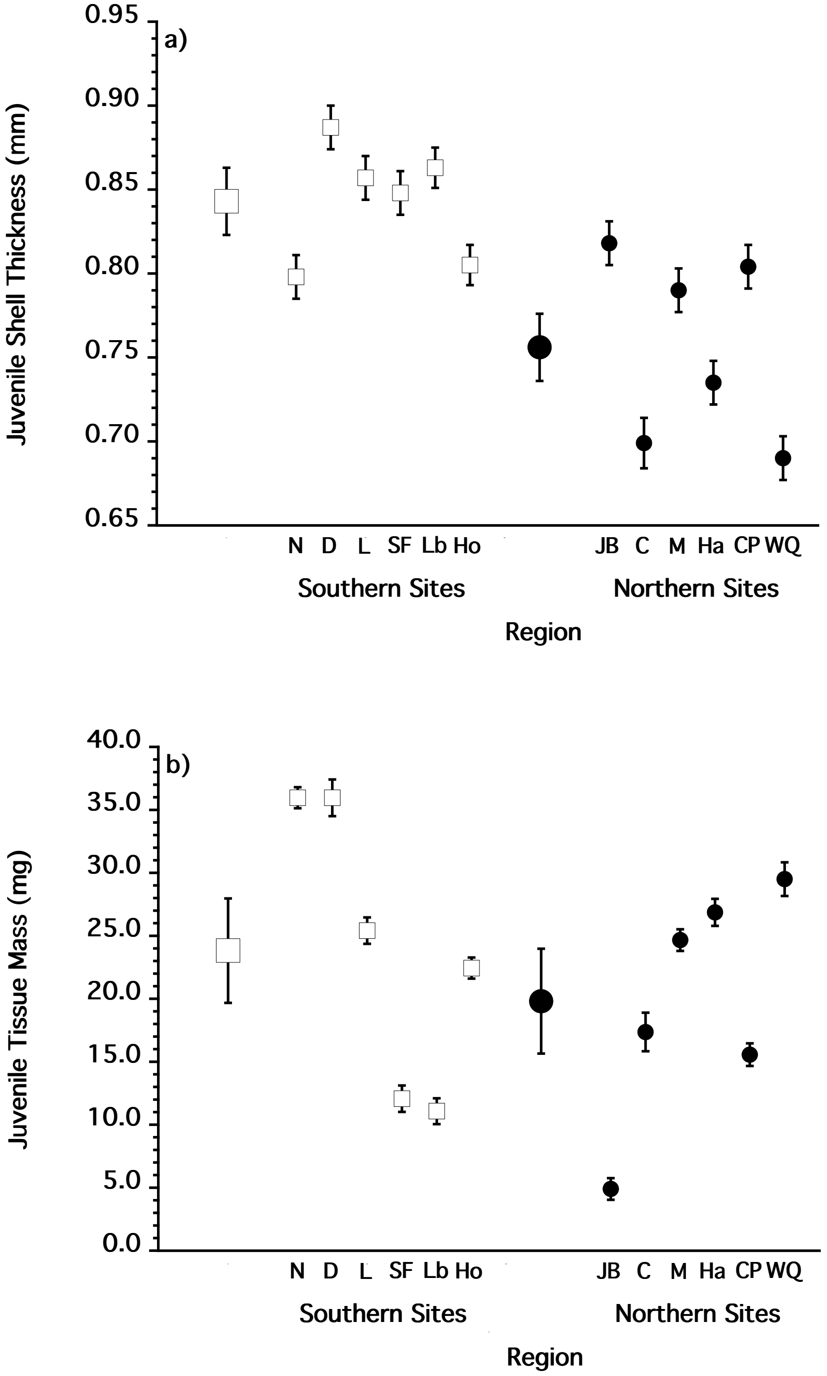
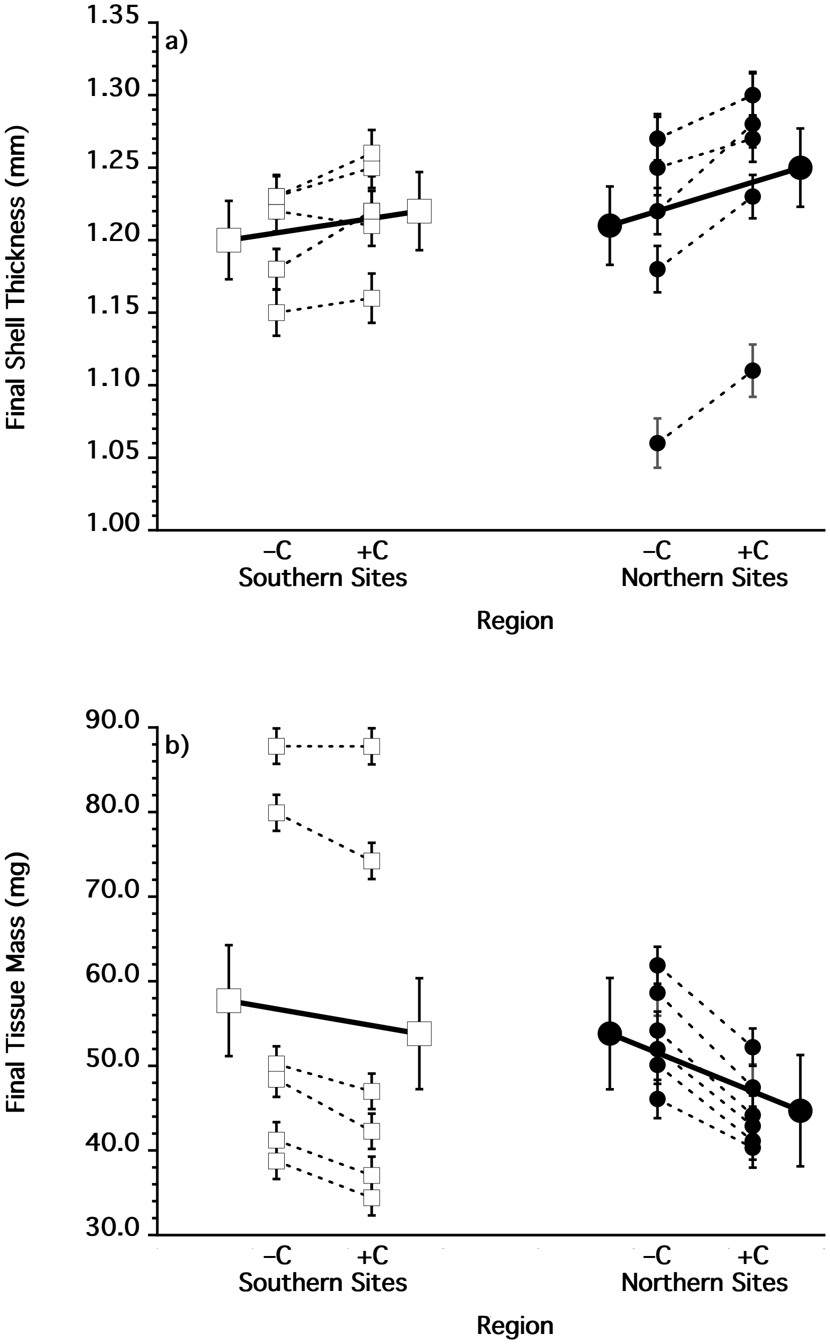

a)
0.95
0.90
0.85
0.80
0.75
0.70
0.65
Juvenile Shell Thickness (mm)
N D L SF Lb Ho
Southern Sites
JB C M Ha CP WQ
Northern Sites
Region
b)
40.0
35.0
30.0
25.0
20.0
15.0
10.0
5.0
0.0
Juvenile Tissue Mass (mg)
N D L SF Lb Ho
Southern Sites
JB C M Ha CP WQ
Northern Sites
Region

Figure 6.


a)
1.35
1.30
1.25
1.20
1.15
1.10
1.05
1.00
Final Shell Thickness (mm)
–C
+C
Southern Sites
–C
+C
Northern Sites
Region
b)
90.0
80.0
70.0
60.0
50.0
40.0
30.0
Final Tissue Mass (mg)
–C
+C
Southern Sites
–C
+C
Northern Sites
Region