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Phenotypic clines in the intertidal snail Littorina obtusata: The role of water temperature and predator effluent as inducers of phenotypic plasticity and associated trade-offs in shell form

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PHENOTYPIC CLINES IN THE INTERTIDAL SNAIL *LITTORINA OBTUSATA*: THE ROLE OF WATER TEMPERATURE AND PREDATOR EFFLUENT AS INDUCERS OF PHENOTYPIC PLASTICITY AND ASSOCIATED TRADE-OFFS IN SHELL FORM

A Dissertation

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The Faculty of the School of Marine Science

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In Partial Fulfillment

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Doctor of Philosophy

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by

Geoffrey Clayton Trussell

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APPROVAL SHEET

This dissertation is submitted in partial fulfillment of
the requirements for the degree of

Doctor of Philosophy

Geoffrey C. Trussell

Approved, December 1998

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DEDICATION

Thomas Wolfe said, "A man's soul is reflected by his friends". I would like to make that a little more specific: "A man's soul is reflected by his family". To my wife Ramsay, and our son, Clayton Gifford Trussell, for making me all that I am, and all that I will be, I offer this Dissertation as a small token of my appreciation, love, and devotion.

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ABSTRACT

My research focused on the importance of predator effluent and water temperature to phenotypic plasticity in shell form in the intertidal snail *Littorina obtusata*. I examined variability in the shell form of 25 *Littorina obtusata* populations from Massachusetts to northern Maine. I chose this scale because the history of one of *L. obtusata's* principal predators, the crab *Carcinus maenas*, has changed dramatically in the past 100 years. Before 1900, *Carcinus* did not occur north of Cape Cod, Massachusetts, but by 1950 it had reached Canadian border. In addition, water temperatures during the growing season average 6-8°C colder at northern versus southern locations.

Shell thickness and mass increased and body mass decreased with increasing latitude. To test whether these patterns may reflect plasticity in response to predator effluent, snails from two northern and two southern populations were raised in the laboratory with and without *Carcinus* effluent. Snails raised with *Carcinus* produced thicker shells than conspecifics raised without *Carcinus*. In addition, this response was accompanied by reduced body size and body growth.

Another experiment examined whether geographic differences in water temperature induce changes in shell form. Snails reciprocally transplanted between a northern (Maine) and southern (Massachusetts) exhibited substantial plasticity in shell form. Southern snails transplanted north produced significantly thinner, lighter, shells than snails raised at their native shore, while northern snails transplanted south produced thicker, heavier shells than snails raised at the native shore. In addition, snails producing thicker, heavier shells exhibited reduced body mass and growth. Although patterns in final phenotypes exhibited cogradient variation, growth in both shell thickness and mass exhibited countergradient gradient variation. Most examples of countergradient variation are associated with temperature differences suggesting that differences in water temperature are responsible for this pattern.

A third experiment involved reciprocally transplanting snails between the same two sites (temperature effect) and raising them with and without *Carcinus* effluent. In general, *Carcinus* effluent and warmer water temperatures induced thicker, heavier shells and reduced body mass and growth. Overall, it appears that predator effluent and water temperature have similar effects on shell form.

I also examined the effects of flow on growth. Wave-exposed and protected snails were raised in flumes under different flow regimes. There was little difference in all measures of growth between wave-exposed snails raised in high flow and protected snails raised in low flow. Protected snails raised in high flow exhibited reduced growth relative to their controls. In contrast, wave-exposed snails raised under low flow exhibited dramatic increases in growth relative to their controls. These results suggest

that while selection favors a genetic capacity for rapid growth on wave-exposed shores, this ability is constrained by increased flow. By raising snails in a better growth environment (i.e., low flow), the genetic capacity for rapid growth occurred in wave-exposed snails..

My work on phenotypic plasticity encourages a more pluralistic view of phenotypic variation. Moreover, my results suggest that phenotypic plasticity is a ubiquitous strategy in adapting to different environments and that its evolution may be driven by life history trade-offs.

PHENOTYPIC CLINES IN THE INTERTIDAL SNAIL *LITTORINA OBTUSATA*: THE ROLE OF INDUCERS OF PHENOTYPIC PLASTICITY AND ASSOCIATED TRADE-OFFS IN SHELL FORM

CHAPTER 1:

PREDATOR-INDUCED MORPHOLOGICAL TRADE-OFFS IN LATITUDINALLY-SEPARATED POPULATIONS OF *LITTORINA OBTUSATA*

ABSTRACT

Predation by shell crushing predators is thought to be a principal force driving the evolution of gastropod shell form. However, recent evidence suggests that ecophenotypic responses to the risk stimuli associated with predators may also be important. Hence, the close coevolutionary relationship between predators and their gastropod prey may be driven by plastic rather than strictly genetic responses to predators. This study examined geographic variation in the shell form, shell strength, and body size of *Littorina obtusata* populations in the northern and southern Gulf of Maine. The shells of snails from northern populations were thinner, lighter, and weaker than those of southern conspecifics. The green crab (*Carcinus maenas*) has been present in the northern Gulf of Maine for at most 50 years, but has been well established in the southern Gulf of Maine for ~100 years. Hence, geographic differences in *Littorina obtusata* shell form likely reflect, in part, the geographically-based differences in *Carcinus*' role as a selective agent and inducer of plasticity.

A laboratory experiment raising snails from two northern and two southern populations in the presence and absence of *Carcinus* effluent was also conducted to test whether differences in the duration of contact with *Carcinus* influences plastic responses in shell form. When raised in the presence of crabs, snails from all populations produced thicker, heavier shells than conspecifics raised in the absence of crabs. These results support the hypothesis that geographic differences in shell form may reflect geographic differences in the abundance of *Carcinus* and, thus, the concentration of effluent indicating a risk of predation. Interestingly, the magnitude of plastic increases in shell thickness and mass was similar for all populations indicating that reaction norms have evolved similar trajectories within each region. Predator-induced increases in shell thickness and mass were accompanied by significant reductions in body size (defined as wet tissue mass) and body growth. These trade-offs are likely the result of geometric constraints imposed by shell form on body size and may explain the existence of micro- and macrogeographic variation and the evolution of inducible defenses in marine gastropod shell form.

INTRODUCTION

Natural selection via predation by crushing predators is thought to be a principal force driving the evolution of gastropod shell form in ecological and geological time (Vermeij, 1976, 1978, 1981, 1987; Palmer, 1979; West & Cohen, 1996). For example, the fossil record indicates that shells from the post-Paleozoic have better defended morphologies (e.g., low spires, thick shell walls and apertural lips, narrow apertures) and higher frequencies of shell repair (Vermeij et al., 1981) than Paleozoic assemblages (Vermeij, 1978, 1987; Signor & Brett, 1984). These morphological transitions coincided with the diversification of shell crushing predators in the Mesozoic (Vermeij, 1977) and are thought to reflect co-evolution between predators and prey (Vermeij, 1987--check on this).

Biogeographic variation in shell form also reveals more robust shell morphologies in regions where shell crushing predators are more taxonomically diverse and powerful and there has been longer time for co-evolution between predator and prey to occur (Vermeij, 1978, 1987; Vermeij & Veil, 1978). For example, tropical gastropod shells are more robust than those of temperate snails (Vermeij, 1978; Vermeij & Currey, 1980); Indo-West Pacific snails are better defended than Caribbean congeners (Vermeij, 1976); freshwater snails from ancient African rift valley lakes are stronger than snails from nearby, but younger lakes (West et al., 1991). These defensive morphologies are effective; experiments have repeatedly demonstrated the greater resistance of thick-

shelled morphs to crab predation than thin-shelled morphs (Bertness & Cunningham, 1981; Reimchen, 1982; Palmer, 1985a; Seeley, 1986; West & Cohen, 1996).

Rapid and recent (within the last 100 years) morphological transitions to better defended shells in two intertidal gastropods after the biogeographic range expansion of the crab, *Carcinus maenas*, are thought to reflect powerful examples of natural selection in action (see Vermeij, 1982; Seeley, 1986). Although thought to be native to the northeastern Atlantic, *Carcinus* was introduced to the U.S. Mid-Atlantic in the early 1800's from Europe (Cohen et al., 1995). Around 1900, *Carcinus* spread north of Cape Cod, Massachusetts into the Gulf of Maine. Increased water temperatures are thought to have facilitated this range expansion (Welch, 1968; Lazzari, 1997; Figure 1). *Carcinus* continued moving north reaching Portland, Maine in the early 1900's, mid-coastal Maine by the 1930's, and northern Maine and the Bay of Fundy by the 1950's (Scattergood, 1952; Welch, 1968; Figure 2). The northern extent *of Carcinus* is apparently limited by cold water; following a decline in water temperatures in the late 1950's, *Carcinus* populations in the Bay of Fundy decreased (Welch, 1968). Presently, *Carcinus* is abundant at some sheltered sites in the northern Gulf of Maine, but crab populations in this region are generally small or ephemeral compared to the well-established and dense populations on protected shores in the southern Gulf of Maine (G. Trussell & L.D. Smith, pers. obs.). Even if *Carcinus* populations in the northern Gulf of Maine are currently stable, it is clear that snails in this region have been cohabiting with *Carcinus* for ~50

years while snails in Massachusetts have been cohabiting with *Carcinus* for ~100 years. Hence, there is a historical gradient in the presence of *Carcinus* in the Gulf of Maine that corresponds to latitude, in its role as a selective agent (Vermeij, 1982; Seeley, 1986) and as an inducer of phenotypic plasticity (Palmer, 1990; Trussell, 1996).

Seeley (1986) argued that morphological shifts to more defended shells in *Littorina obtusata* that coincided with the *Carcinus* range expansion in the 1900's reflected evidence of natural selection driven by *Carcinus*. Museum specimens collected in New England before 1900 were high-spired and thin-shelled. Comparison of these snails with collections from the same three regions in the mid-1980's revealed that pre-1900 snails were thinner and higher-spired than recent specimens. In addition, experiments with thin shelled, high-spired morphs from northern Maine revealed that these morphs were more vulnerable to predation than thick-shelled, low-spired morphs.

Although Seeley's (1986) results support natural selection by shell crushing predators as an explanation for evolutionary and biogeographic patterns in shell form, recent experiments have shown that gastropods can modify their shell form in response to effluent emanating from predators (Appleton & Palmer, 1988; Crowl & Covich, 1990; Palmer, 1990; Trussell, 1996). For example, Appleton & Palmer (1988) demonstrated that the scent of crabs and damaged conspecifics can induce the development of larger apertural teeth in the gastropod *Nucella lamellosa*. And Trussell (1996) found that *Littorina obtusata* from the southern Gulf of Maine develop thicker shells when raised in

the presence of effluent associated with *Carcinus* feeding on conspecifics. The taxonomic and geographic diversity of this response (e.g., marine gastropod *Nucella lamellosa* in the northeast Pacific [Appleton & Palmer, 1988]; marine gastropod *Nucella lapillus* in the British Isles [Palmer, 1990]; marine gastropod *Littorina obtusata* and marine mussel *Mytilus edulis* in the Gulf of Maine [Trussell, 1996; Leonard et al., in press]; and freshwater gastropod *Physella virgata virgata* [Crowl & Covich, 1990]) indicate that it is a general phenomenon. The discovery of plasticity in gastropod shell form has invited reinterpretation of views that recent or fossil transitions in shell form are evidence of rapid selection (Vermeij, 1982; Seeley, 1986) or speciation (Williamson, 1981; Palmer, 1985). Clearly, there is a close co-evolutionary relationship between predators and their gastropod prey (Vermeij, 1987), but this relationship may also be driven by plastic, rather than strictly genetic, responses to predators.

My previous work (Trussell, 1996) suggests that the shifts in *Littorina obtusata* shell form documented by Seeley (1986) may reflect phenotypic plasticity rather than strict micro-evolutionary change but my conclusions were limited because I worked with populations from the southern Gulf of Maine. These populations have been in contact with *Carcinus* for a longer time (~80-100 years) than populations near the Maine-Canadian border (at most 50 years). Geographic differences in the historical exposure of *Littorina obtusata* to *Carcinus* may result in the evolution of different shell morphologies and reaction norms in each region. Here I examine these possibilities by measuring

variation in the shell thickness and breaking force of two *Littorina obtusata* populations in northern Maine and two populations in Massachusetts. In addition, snails from all populations were raised in the presence and absence of *Carcinus* effluent to provide (1) a better understanding of how predator-induced reaction norms evolve in broadly separated populations and (2) a more complete test of the hypothesis that historical transitions in *Littorina obtusata* shell form may reflect phenotypic plasticity. I also address the potential life history trade-offs associated with the production of thicker shells by (1) examining geographic differences in body mass and (2) whether predator-induced increases in shell thickness are accompanied by reductions in body mass and body growth.

MATERIALS AND METHODS

Geographic Differences in Shell Thickness and Mass, Shell Breaking Force, and Body Mass

Geographic differences in shell thickness were examined by collecting approximately 100-150 snails from 0.25 m2 quadrats tossed haphazardly in the mid-intertidal zone at each study site (Figure 2). Collections were made at two sheltered sites in Lubec, Maine (Johnson Bay and Quoddy Head) and two sheltered sites in Manchester, Massachusetts (Lobster Cove and Manchester Harbor). From each sample, 50 snails were randomly chosen for measurement of shell thickness, shell mass, shell breaking force, and body mass. An attempt was made to maximize the size range for each sample.

Measurements of shell thickness and shell length were made as described in Trussell (1996) with digital calipers (±0.01 mm). After shell measurements, I determined the maximum force required to crush each snail's shell with an Instron Dynamic Testing machine (±0.1 N; Model 4301). These tests were not meant to simulate crab predation but to provide a relative measure of shell breaking force, which should influence vulnerability to crab predation (Vermeij & Currey, 1980). Before testing, live snails were kept submerged in running seawater for 24 hours. Snails were then placed aperture down on a stationary platen and their shells crushed by lowering a steel platen onto the shell at a rate of 10 mm/minute. Shells were loaded until they were crushed; a loud "cracking' noise reliably indicated failure of the shell. After testing, soft tissue was separated from shell fragments and both were dried at 60oC for 48 hours before weighing on an analytical balance. Shell mass was measured (±0.001 g) on a Mettler PG503 and soft tissue mass, which served as a measure of body mass, was measured (±0.0001) on a Mettler AE100.

Non-Destructive Estimates of Shell Mass and Body Mass

Because I wanted to examine variation in body mass (defined by tissue mass) and body growth of snails raised in the presence and absence of *Carcinus*, it was necessary to make non-destructive estimates of shell and body mass. Following the methods of Palmer (1982), I generated regressions of actual shell mass (Y) on measurements of shell mass while submerged in seawater (X; hereafter, submerged mass) for each population.

To do so, 50 snails spanning the available size range were collected from the four populations described above. In the laboratory, shell length was measured with digital calipers (±0.01 mm). Submerged mass was measured while snails were submerged in seawater (±0.001 g). Snails were then allowed to dry on toweling for approximately 30 minutes. To remove extra-visceral water trapped inside the shell, snails were forced into their shell with absorbent tissue before weighing in air (hereafter, total mass [±0.001 g]). After total mass measurements, snails were carefully crushed and tissue separated from the shell. Both tissue and shell material were dried at 60oC for 48 hours before weighing to determine the actual mass of each variable.

Regressions of actual shell mass (Y) on submerged mass for snails from each population yielded highly significant R^2 values (Quoddy Head--$Y = 1.561X - 0.002$, $R^2 = 0.9991$; Johnson Bay--$Y = 1.545X - 0.001$, $R^2 = 0.9952$; Lobster Cove--$Y = 1.582X + 0.002$, $R^2 = 0.9999$; Manchester Harbor--$Y = 1.589X + 0.002$, $R^2 = 0.9999$), indicating that submerged mass is a reliable indicator of actual shell mass (Palmer, 1982). By using the respective regression equations for each population, I estimated actual initial shell mass from initial submerged mass for snails collected for the plasticity experiment (described below). To calculate initial body mass, I subtracted the estimate of initial actual shell mass from the initial total mass of snails when weighed in air (Palmer, 1982).

Experimental Test of Predator-induced Phenotypic Plasticity

I made additional collections of approximately 250 juvenile snails from each northern and southern population for a lab experiment to assess the effect of crab effluent on shell morphology and growth. All snails were individually labeled with waterproof markers (Trussell, 1997) and measured for shell length, shell thickness, submerged mass, and total mass. Measurements of growth in body mass and shell mass were calculated by subtracting initial from final values.

Once initial measurements were completed, 30 snails (hereafter, "experimental snails") from each population were randomly assigned to 6 of 24 replicate rectangular chambers (32 cm x 24 cm x 15 cm). Hence, each chamber contained 30 snails from a single population and there were a total of 6 chambers for each of the 4 populations. Plastic mesh panels on the sides, top and bottom of each chamber permitted water flow. Within each chamber I also placed an airstone and 55 g wet mass of the alga *Ascophyllum nodosum* to serve as food. Four of these chambers, one for each population, were then randomly assigned to each of six large seatables. Three seatables were used for the "Crab" treatment, and the remaining three were used as a control ("No-Crab"). The "Crab" treatment was created by placing a small perforated plastic tub (13.5 cm dia x 5.5 cm ht) containing a single crab (*Carcinus maenas*) and 20 conspecific snails (hereafter, "stimulus snails") on top of each of the four chambers housing experimental snails. For the control ("No-Crab"), I placed identical perforated tubs containing only stimulus snails

on top of each of the four chambers housing experimental snails. This design contained 3 replicates for each population nested within each treatment (4 populations x 3 replicates x 2 treatments = 24 chambers).

Every two weeks, I added a new set of 20 stimulus snails to the perforated tubs in each treatment and replaced the food supply for experimental snails with fresh *Ascophyllum*. This experiment was conducted for ~115 days from early May until late August, 1997 when final measurements of shell length and thickness and estimates of shell mass and body mass were made as described above.

Non-destructive estimates of body and shell mass were calculated as described above except that new regressions of final actual shell mass (Y) as a function of final submerged mass were determined for each experimental group. I generated these new regressions because experimental treatments can change the relationship between submerged mass and actual shell mass. New regressions were generated with 25-30 snails randomly sampled from each experimental group by crushing the shell with a C-clamp and separating soft tissue and shell material. Both shell material and tissue were dried in an oven at 60°C for 48 hours and then weighed on a Mettler PG503 (±0.001 g). These new regressions were then applied to each experimental group to estimate actual shell mass and body mass a described above. Like the initial regressions, the new regressions indicated that submerged mass is a reliable indicator of actual shell mass (all R^2 0.999).

Statistical Analyses

A two-factor analysis of covariance (ANCOVA) with populations (random) nested within each geographic region (fixed) was conducted to test for geographic differences in shell thickness and mass, shell breaking force, and body mass. Shell length was used as the covariate in all analyses and all slopes were homogeneous. To analyze final trait values from the predator-induced plasticity experiment, I conducted a three-factor ANCOVA, with treatment (Crab and No-Crab) and geographic location (Maine and Massachusetts) as fixed effects, source population (Johnson Bay, Quoddy Head, Lobster Cove, Manchester Harbor) as a random effect nested within each geographic location, and replicate chambers as a random nested effect. For the analysis on final shell thickness I adjusted for the effects of shell size by using shell length as the covariate. Because I was interested in how effluent treatment influenced allocation to body versus shell mass, I used the ratio of body mass to shell mass with shell length as the covariate. Analysis of growth utilized the ratio of body mass growth to shell mass growth because I wanted to determine how the allocation between body and shell mass changed due to experimental treatment. Initial body mass was used as the covariate in this analysis. Because all analyses employed ANCOVA techniques to adjust for the potential effects of either final or initial size on response variables, discussions regarding statistical differences due to source populations or experimental treatments are based on least squares adjusted means generated by ANCOVA.

All analyses were conducted using JMP software (Version 3.2.1, SAS Institute Inc, Cary, North Carolina) on a PowerMac 7500/100 and data were log(10) transformed when necessary to meet the assumptions of ANCOVA. Because both source population and replicates were declared random nested effects in all models, JMP used the Satterthwaite approximation to calculate F-ratios and their respective degrees of freedom.

RESULTS

Geographic Differences in Shell Thickness and Mass, Shell Breaking Force, and Body Mass

ANCOVA revealed that the shells of snails from both southern populations were thicker (Figure 3a) and heavier (Figure 4a) than those of snails from both northern populations (Table 1). These differences in shell form translated into differences in shell breaking force; southern snails required significantly greater forces to crush than northern snails (Table 1; Figure 3b). Geographic differences in body mass revealed a trend different than that for shell traits; body mass of northern snails was significantly greater than that of southern snails (Table 1; Figure 4b).

Phenotypic Plasticity in Shell Thickness, Body/Shell Mass and Body/Shell Mass Growth

ANCOVA revealed that snails from both populations from each geographic location produced thicker shells when raised in the presence of *Carcinus* feeding on conspecifics (Table 2; Figure 5). The Treatment x Location interaction term was not significant, indicating that snails responded similarly to experimental treatments

regardless of their geographical origin. Induced increases in shell thickness were between 11-13% for southern populations and 17% for both northern populations.

Significant reductions in body mass relative to shell mass were also induced by the presence of crabs. Snails from both northern populations reduced body mass relative to shell mass by 30-36%, while snails from both southern populations reduced body mass relative to shell mass by 20-24%, when raised in the presence of crabs (Table 2; Figure 6a). Interestingly, the response to predator effluent in body mass relative to shell mass for northern populations was significantly greater (33% vs. 22%) than that of southern populations as indicated by a significant Treatment x Location interaction (Table 2).

Differences in final body mass relative to shell mass appear to reflect differential body growth relative to shell mass growth in the absence of predator effluent. Snails from both northern (45-57%) and both southern (40%) populations raised in the absence of *Carcinus* grew significantly more in terms of body mass relative to shell mass than conspecifics raised in the presence of *Carcinus* (Table 2; Figure 6b). The lack of a significant Treatment x Location interaction indicated that responses in body growth were similar for snails from both geographic locations.

DISCUSSION

Adaptive phenotypic plasticity should be favored over fixed strategies when (1) environmental cues are temporally or spatially variable, (2) modification of a trait confers some direct benefit to the organism, such as improved survival, growth, or reproduction, and (3) the cost of producing a fixed phenotype is greater against the local environmental background (Levins, 1968; Lively, 1986a; Stearns, 1989; DeWitt et al., 1998). Among the best documented examples of adaptive phenotypic plasticity are predator-induced responses in prey. This phenomenon is known among diverse taxa, including spine formation in bryozoans (Yoshioka, 1982; Harvell, 1984, 1991), crest enlargement in cladocerans (Grant & Bayly, 1981; Krueger & Dodson, 1981; Dodson, 1989), shell size and shape change in freshwater snails and marine barnacles (Crowl & Covich, 1990; DeWitt, 1996; Lively, 1986b), body deepening in fish (Bronmark & Miner, 1992) byssal thread production and shell thickening in mussels (Cote, 1995; Leonard et al., in press), and shell thickening in marine gastropods (Appleton & Palmer, 1988; Palmer, 1990; Trussell, 1996). Despite the apparent ubiquity of this phenomenon, all of the examples cited above have involved within or among (on a very localized scale) population responses. Although broad scale geographic variation may often reflect genetic differentiation among conspecific populations (Mayr, 1963; Endler, 1977), phenotypic plasticity may also be important. Understanding the role of phenotypic plasticity in

producing biogeographic variation may yield insights into its role in macro-evolutionary processes such as speciation (West-Eberhard, 1989).

This study revealed geographic differences in shell form and shell breaking force that are correlated with the historical presence of *Carcinus* in the Gulf of Maine. In northern Maine, where *Carcinus* has been present for ~50 years, the shells of *Littorina obtusata* are thinner, lighter, and weaker than those of conspecifics in Massachusetts where *Carcinus* has been present for ~100 years. In contrast, northern snails had greater body masses. Although geographic variation in *Littorina obtusata* shell form and body mass may reflect the importance of other factors, such as water temperature (Graus, 1974; Vermeij, 1978; Atkinson, 1994; Trussell, in review), it is likely that *Carcinus*, either as a selective agent (Seeley, 1986) and/or inducer of plasticity (Trussell, 1996), is also important. According to the selection hypothesis, southern snails are thicker, heavier, and stronger because (1) they have been subjected to selection by *Carcinus* for a longer time or (2) the greater abundance of *Carcinus* at southern sites creates higher effluent concentrations that indicate an increased risk of predation.

Although natural selection by *Carcinus* is surely involved, it appears that plastic responses to *Carcinus* effluent contribute to the production of geographic variation in shell form. Snails from both populations in both geographic regions produced thicker shells when raised in the presence of *Carcinus* than conspecifics raised in the absence of *Carcinus*. Interestingly, northern and southern snails exhibited statistically similar

increases in shell thickness suggesting that these reaction norms have evolved similar trajectories within each geographic region. This result is surprising because *Littorina obtusata* develops directly, which should restrict gene flow among such widely separated populations and enhance population differentiation. Such conditions, coupled with the geographically differences in the duration of contact with *Carcinus* in the last 100 years may promote local adaptation and the evolution of different reaction norms in each region.

However, the recent *Carcinus* invasion during this century may be just one similar events in the past. If *Carcinus* has experienced multiple expansions and contractions of its biogeographic range, then the capacity of *Littorina obtusata* to respond to *Carcinus* effluent may have evolved long before this recent invasion and has simply been retained through time. This scenario would explain the retention of the capacity to respond to *Carcinus* in broadly separated populations. However, despite the evolution of responses of similar magnitude (in terms of percent change in shell thickness) in northern and southern snails, northern snails were still thinner than southern snails at the end of the experiment regardless of treatment conditions. Although this difference may reflect differences in shell thickness between northern and southern populations at the beginning of the experiment, the inability of northern snails to produce shells as thick as those of southern snails implies that other environmental stimuli or genetically-based constraints may also be operating. Clearly much remains to be learned about the forces shaping

reaction norms in *Littorina obtusata* shell form. However, my results provide further support of the hypothesis that both macro-scale geographic variation and historical transitions in shell form (sensu Vermeij, 1982; Seeley, 1988) may have a large eco-phenotypic component.

Trade-Offs Associated with Induced Increases in Shell Thickness

A thorough understanding of both the adaptive value of phenotypic plasticity and the constraints on its evolution requires an assessment of accompanying costs or trade-offs (Stearns, 1989, 1992; DeWitt et al., 1998). The evolution of inducible over permanent defensive structures implies that defensive traits are costly to produce; otherwise the same phenotypes would be produced across different environments. The nature of these costs may fall into two broad categories (see DeWitt et al., 1998): (1) "True" or "pure" costs of plasticity (e.g., production costs, maintenance of plasticity machinery, developmental instability) and (2) limits to the benefits of plasticity (e.g., lagged response times, unreliable assessment of the local environment, limited developmental ranges, morphological trade-offs). Costs and limits to plasticity are likely subject to natural selection and instrumental in determining both the adaptive benefits and evolutionary potential of phenotypic plasticity (Schlichting & Pigliucci, 1998).

In the case of predator-induced defenses, there has been an emphasis on the costs directly associated with the production of defensive structures (Harvell, 1986; Lively, 1986c; Tollrian, 1995). However, these costs are not necessarily indicative of costs of

plasticity (DeWitt et al., 1998). For example, in the case of shell thickness, the direct costs associated with producing a thicker shell should be the same for a fixed and a plastic genotype. Only those trait production costs for plastic genotypes in excess of those for fixed genotypes could be considered a true cost of plasticity (DeWitt et al., 1998). Hence, it is possible that trade-offs accompanying the production of thicker shells reflect those costs derived from producing more shell material rather than plasticity in shell thickness per se.

Previous studies of predator-induced changes to better defended shell morphologies have documented life history trade-offs in growth (Appleton & Palmer, 1988), size at maturity (Crowl & Covich, 1990), and fecundity (Lively, 1986). Although the production of more shell material by marine gastropods is expected to be energetically costly (Palmer, 1981), the cost of increased calcification is likely small relative to other metabolic costs, especially in areas where surface seawater is saturated with calcium carbonate (Palmer, 1992). Palmer (1981) concluded that a second, non-energetic cost best explains reduced body mass and body growth in thick-shelled snails. This hypothesis ("skeleton limitation hypothesis) emphasizes the unique geometric constraints that shell form imposes on the snail living inside. Because tissue growth cannot proceed ahead of the protective shell, body mass and growth are partly constrained by the linear rate of shell growth. In addition, there is a maximum rate at which calcification can occur (Palmer, 1992), so the more material that is devoted to

thickening the shell, the less that is available for advancing the shell margin. This limitation is further compounded because snails with a thick-walled shell have less internal volume available for tissue growth than thin-walled morphs of similar size and shape. Kemp & Bertness (1984) documented these patterns in the field. They found that rapidly growing shells in the snail *Littorina littorea* were thinner, more globose, and thus able to accommodate more tissue growth than more slowly growing shells (see also Swan, 1952; Goreau, 1959).

The results of this study indicate that trade-offs due to geometric constraints exist between shell form and body mass. First, geographic patterns in body mass and shell thickness were inversely related; while southern snails had thicker shells they also had reduced body mass relative to northern conspecifics. Second, predator-induced increases in shell thickness for snails from all populations were accompanied by reduced body/shell mass and body/shell growth. Hence, the thicker-walled shells of snails raised with crabs likely limited the internal space available for body growth. The profound implications that predator-induced trade-offs in body size and growth may have to other life history traits (Peters, 1983; Stearns, 1992) may partly explain the existence of micro- and macro-geographic variation in shell form and body mass as well as the evolution of inducible defenses in marine gastropod shell form.

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Table 1. Nested ANCOVA for *Littorina obtusata* from two "Northern" (Lubec, Maine) and two "Southern" (Manchester, Massachusetts) populations USA.

Comparison: log(Shell Thickness) (Y) vs. log(Shell Length) (X): Figure 3a

SOURCE	df	F	p
Location	1, 2	643.58	<0.005
Population{Location}	2, 195	1.62	NS
Slope	2, 193	1.98	NS

Comparison: log(Breaking Force) (Y) vs. log(Shell Length) (X): Figure 3b

SOURCE	df	F	p
Location	1, 2	570.06	< 0.005
Population{Location}	2, 195	0.308	< 0.05
Slope	2, 193	1.92	NS

Comparison: log(Shell Mass) (Y) vs. log (Shell Length) (X): Figure 4a

SOURCE	df	F	p
Location	1, 2	242.02	< 0.01
Population{Location}	2, 195	8.49	< 0.001
Slope	2, 193	06.1	NS

Comparison: log(Body Mass) (Y) vs. log (Shell Length) (X): Figure 4b

SOURCE	df	F	p
Location	1, 2	60.21	< 0.05
Population{Location}	2, 195	14.99	< 0.0001
Slope	2, 193	2.33	NS

Table 2. Nested ANCOVA testing the effect of risk stimuli (Crab vs. No-Crab), geographic location, and source population on shell thickness, body/shell mass and body/shell mass growth of *Littorina obtusata* from two "Northern" (Lubec, Maine) and two "Southern" (Manchester, Massachusetts) populations.

Comparison: Shell Thickness (Y) vs. Shell Length (X): Figure 5

SOURCE	df	F	p
Treatment (Treat)	1, 16	179.98	$p < 0.0001$
Location (Loc)	1, 2	106.95	$p < 0.01$
Population{Loc}	2, 16	4.84	$p < 0.05$
Treat x Loc	1, 16	0.19	NS
Treat x Population{Loc}	2, 661	1.57	NS
Replicate:	16, 646	2.36	$p < 0.01$
Slope:	2, 646	1.66	NS

Comparison: log(Body/Shell Mass) (Y) vs. log(Shell Length) (X): Figure 6a

SOURCE	df	F	p
Treatment (Treat)	1, 4	1, 928.70	$p < 0.0001$
Location (Loc)	1, 2	191.43	$p < 0.001$
Population{Loc}	2, 5	64.82	$p < 0.05$
Treat x Loc	1, 4	63.44	$p < 0.005$
Treat x Population{Loc}	2, 659	2.02	NS
Replicate:	16, 658	0.18	$p < 0.01$
Slope:	2, 658	2.20	NS

Comparison: log(Body/Shell Growth) (Y) vs. log(Initial Body Mass) (X): Figure 6b

SOURCE	df	F	p
Treatment (Treat)	1, 4	268.71	$p < 0.0001$
Location (Loc)	1, 2	214.08	$p < 0.005$
Population{Loc}	2, 4	3.39	NS
Treat x Loc	1, 4	3.33	NS
Treat x Population{Loc}	2, 660	0.16	NS
Replicate:	16, 656	0.87	NS
Slope:	2, 656	0.20	NS

Figure 1. Mean annual sea surface temperature collected in West Boothbay Harbor, Maine over the last 91 years. Water temperature has increased significantly over this time period (Linear Regression: $Y = 0.02\ X - 21.08$; $R^2 = 0.20$; $p < 0.0001$). Redrawn from data in Lazzari (1997).

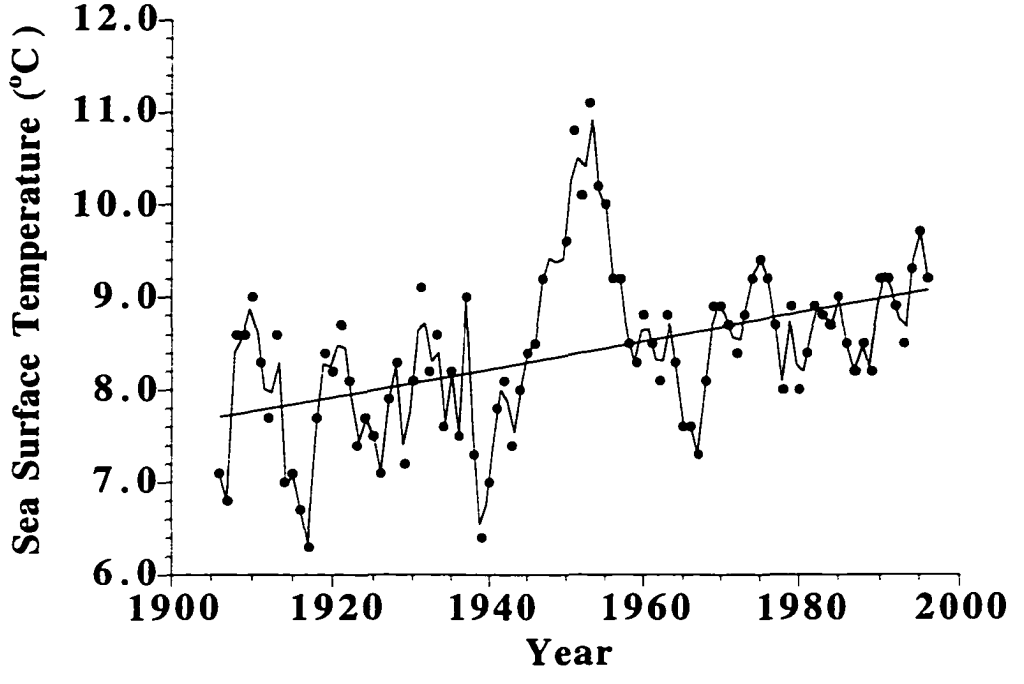


Figure 2. Map of the Gulf of Maine showing the northward progress of *Carcinus maenas*' biogeographic range expansion from 1900 to present day (based on Scattergood (1952) and Vermeij, 1978). Also shown are the locations of the two study areas in the Southern (Manchester, Massachusetts) and Northern (Lubec, Maine) Gulf of Maine.

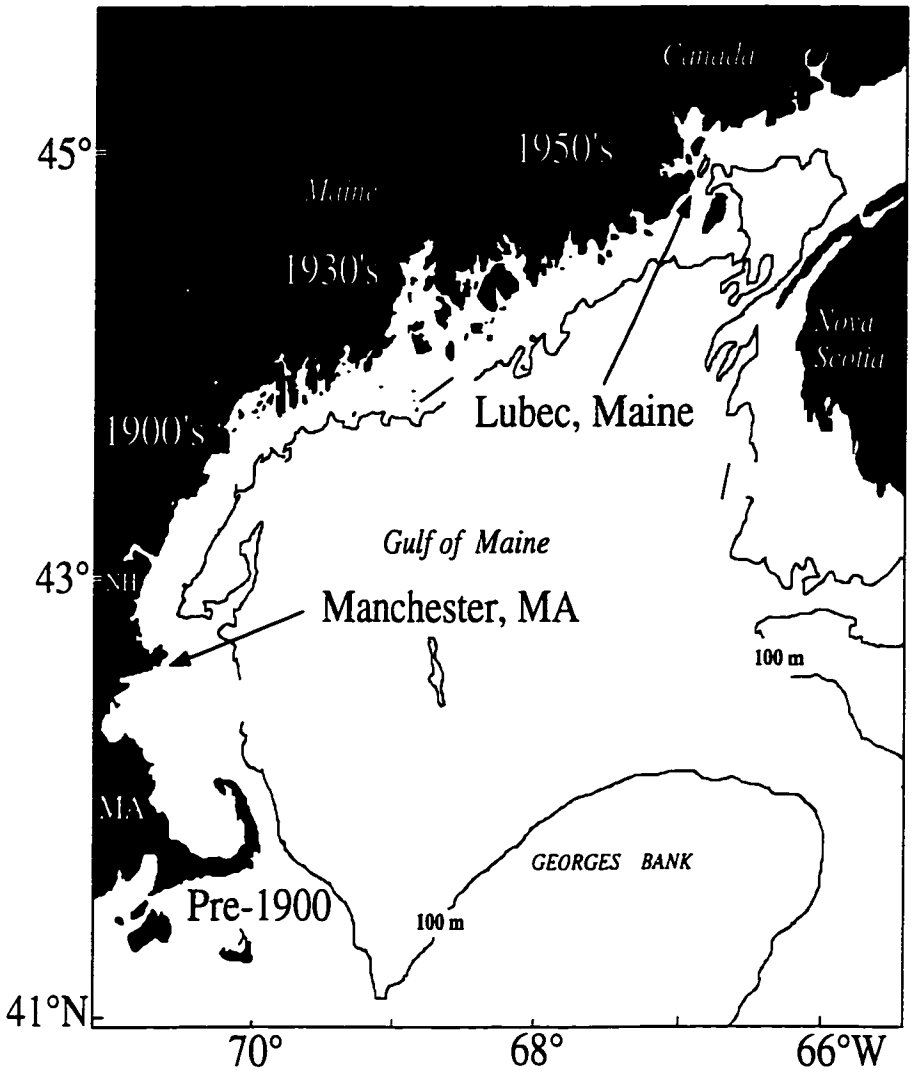
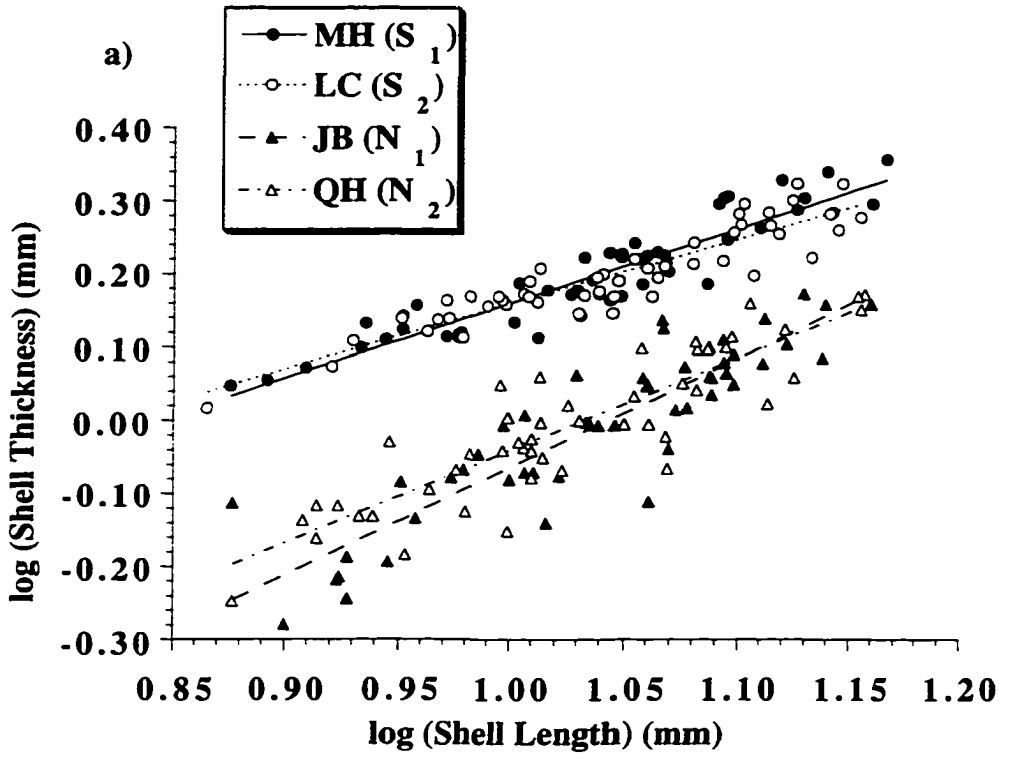


Figure 3. (a) log(Shell Thickness) (Y) as a function of log(Shell Length) (X) for *Littorina obtusata* collected from two southern (S; Manchester, Massachusetts) and two northern (N; Lubec, Maine) populations in the Gulf of Maine. MH = Manchester Harbor ($Y = 1.02X - 0.86$, $R^2 = 0.86$; $p < 0.001$); LC = Lobster Cove ($Y = 0.88X - 0.72$, $R^2 = 0.84$; $p < 0.001$); JB = Johnson Bay ($Y = 1.48x - 1.55$, $R^2 = 0.80$; $p < 0.001$); QH = Quoddy Head ($Y = 1.26X - 1.30$, $R^2 = 0.77$; $p < 0.001$). (b) log(Breaking Force) (Y) vs. log(Shell Length) (X) for the same four populations. MH ($Y = 1.75X + 0.79$, $R^2 = 0.73$; $p < 0.001$); LC ($Y = 1.39X + 1.12$, $R^2 = 0.63$; $p < 0.001$); JB ($Y = 2.92X - 1.23$, $R^2 = 0.69$; $p < 0.001$); QH ($Y = 2.37X - 0.61$, $R^2 = 0.66$; $p < 0.001$). S = South, N = North. See Table 1 for results of ANCOVA.



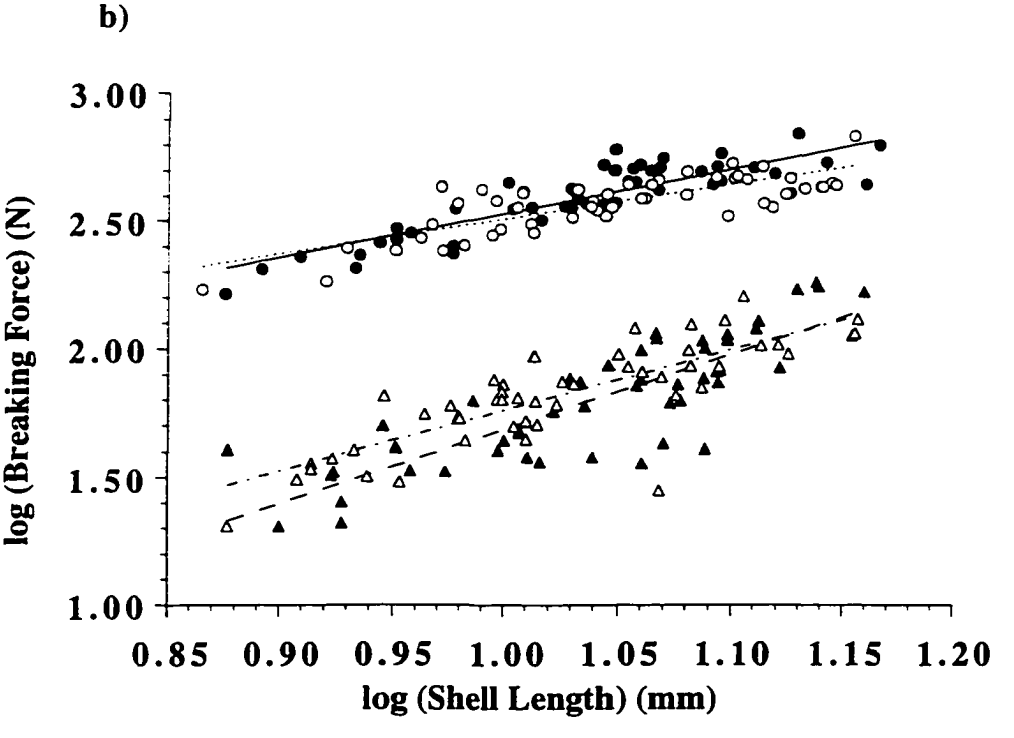
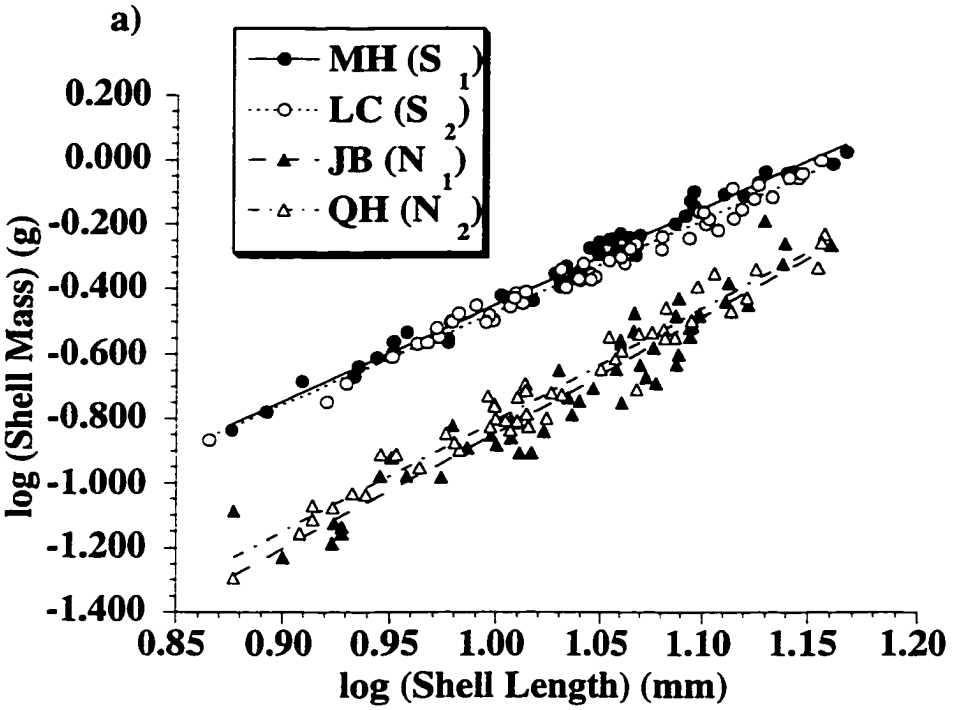


Figure 4. (a) log(Shell mass) (Y) as a function of log shell length (X) for *Littorina obtusata* collected from two southern (S; Manchester, Massachusetts) and two northern (N; Lubec, Maine) populations in the Gulf of Maine. MH = Manchester Harbor ($Y = 2.98X - 3.43$, $R^2 = 0.98$; $p < 0.001$); LC = Lobster Cove ($Y = 2.86X - 3.33$, $R^2 = 0.98$; $p < 0.001$); JB = Johnson Bay ($Y = 3.61X - 4.45$, $R^2 = 0.92$; $p < 0.001$); QH = Quoddy Head ($Y = 3.46X - 4.27$, $R^2 = 0.96$; $p < 0.001$). (b) log(Body Mass) (Y) as a function of log(Shell Length) (X) for the same four populations. MH ($Y = 2.90X - 4.77$, $R^2 = 0.96$; $p < 0.001$), LC ($Y = 3.11X - 4.98$, $R^2 = 0.96$; $p < 0.001$), JB ($Y = 2.56X - 4.16$, $R^2 = 0.93$; $p < 0.001$), QH ($Y = 2.83X - 4.49$, $R^2 = 0.91$; $p < 0.001$). See Table 1 for results of ANCOVA.



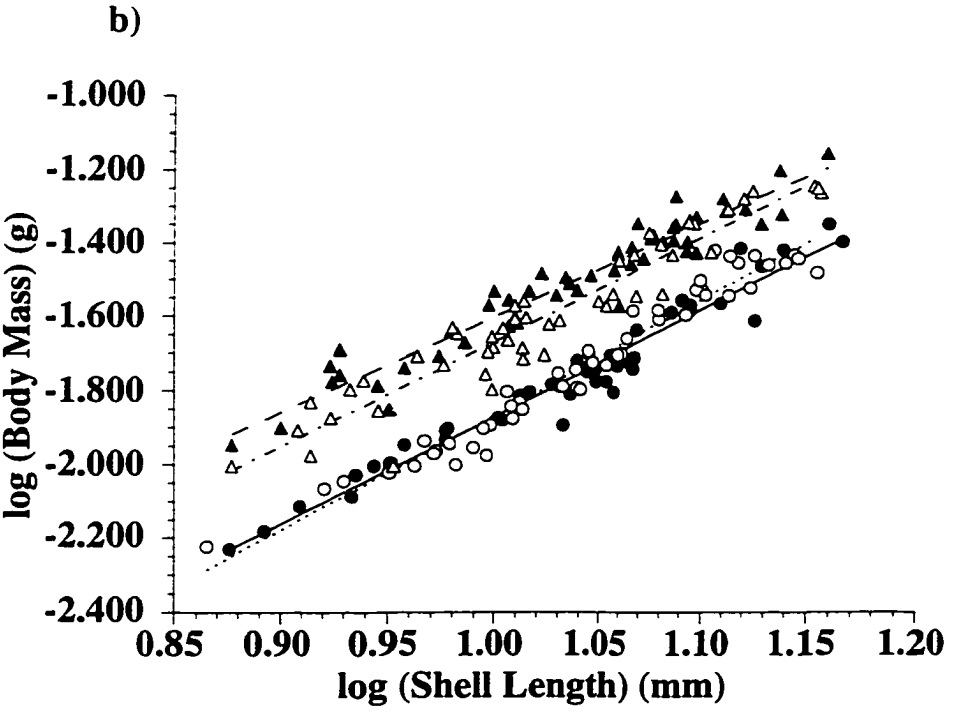


Figure 5. Adjusted mean (±SE) shell thickness from ANCOVA for *Littorina obtusata* from two southern (S; Manchester, Massachusetts) and two northern (N; Lubec, Maine) populations in the Gulf of Maine that were raised in the presence and absence of *Carcinus maenas* for 115 days. Abbreviations as in Figure 3. See Table 2 for results of ANCOVA.

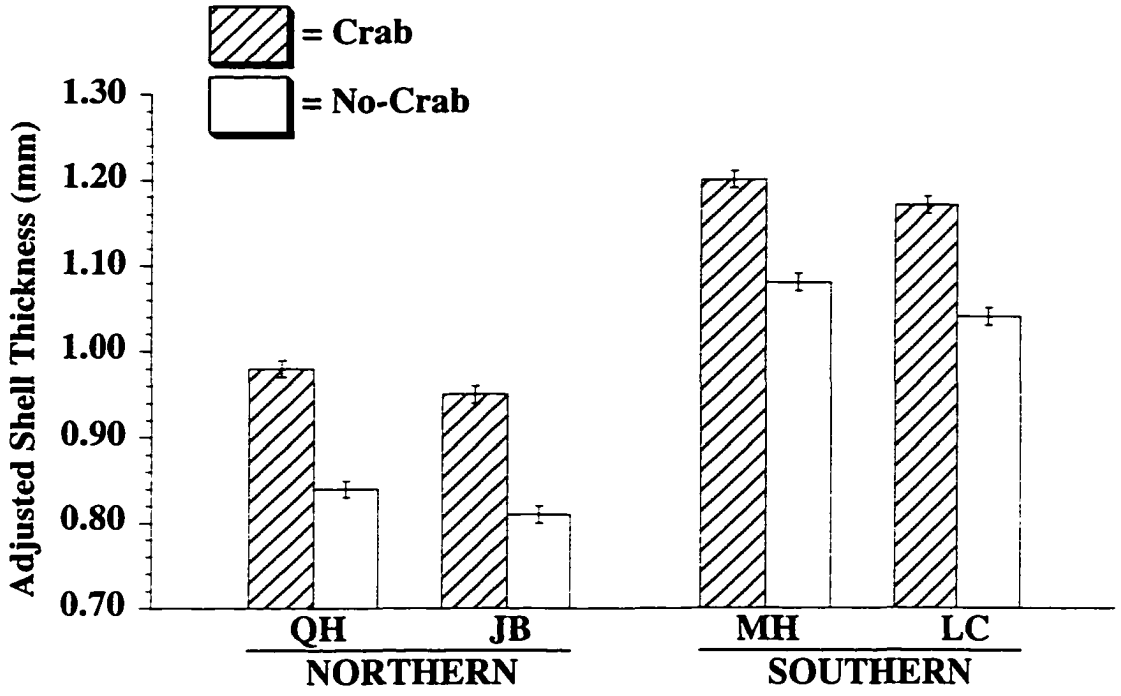
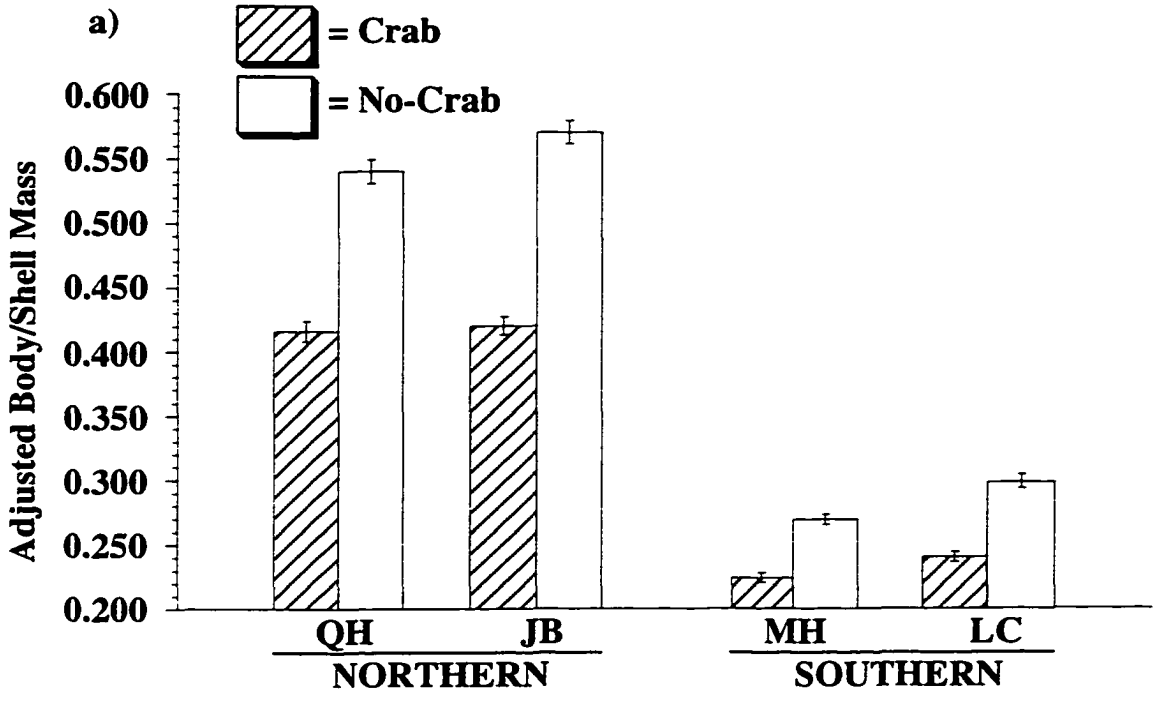
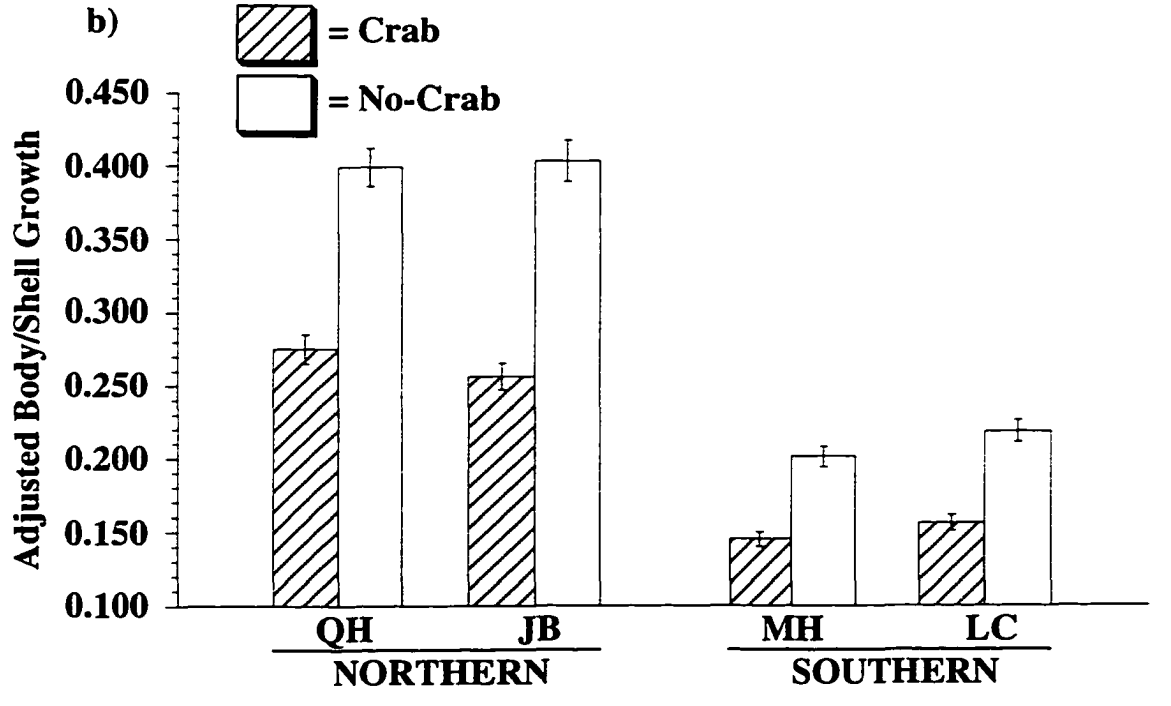


Figure 6. (a) Adjusted mean (±SE) body/shell mass and (b) adjusted mean (±SE) body/shell mass growth from ANCOVA for *Littorina obtusata* from two southern (S; Manchester, Massachusetts) and two northern (N; Lubec, Maine) populations in the Gulf of Maine that were raised in the presence and absence of *Carcinus maenas* for 115 days. Abbreviations as in Figure 3. See Table 2 for results of ANCOVA.





CHAPTER 2:

PHENOTYPIC CLINES, PLASTICITY, AND MORPHOLOGICAL TRADE-OFFS IN AN INTERTIDAL SNAIL

ABSTRACT

Understanding the genetic and environmental basis of phenotypic variation and how these influences covary to produce patterns of phenotypic change on local and broad geographic scales is an important goal of evolutionary ecology. Such information can shed light on how organisms adapt to different and changing environments and how life history trade-offs arise. This approach has been particularly useful in studies on organisms having wide altitudinal and latitudinal distributions. The present study examined biogeographic variation in the shell form, shell strength and body mass of 25 *Littorina obtusata* populations across a latitudinal gradient in the Gulf of Maine. The shell of snails from northern habitats are thinner, weighed less and weaker in compression than those of conspecifics from southern habitats. Latitudinal variation in body mass (defined by tissue mass) follows an opposite pattern, with northern snails exhibiting more body mass than souther snails.

A reciprocal transplant between a northern and southern habitat revealed substantial plasticity in shell form and body mass as well as in several measures of growth. Measures of final shell thickness, shell mass, and body mass yielded cogradient patterns of phenotypic change (i.e., a positive covariance between genetic and environmental influences). Southern snails transplanted to the northern habitat produced thinner, lighter shells and more body mass than controls raised in their native habitat. In contrast, northern snails transplanted to the southern site produced thicker, heavier shells and less body mass than controls raised in their native habitat. Cogradient patterns in final shell form were underlain by countergradient variation (i.e., a negative covariance between genetic and environmental influences) in the respective growth rates. Southern and northern snails raised in the native habitats exhibited remarkably similar growth in shell mass and thickness, but northern snails exhibited dramatic increases in both forms of growth when transplanted to the southern habitat. Cogradient patterns in both final body mass and body growth appear to reflect constraints imposed by cogradient variation in final shell size and thickness and suggest the existence of potential life history trade-offs associated with increased shell production.

Plasticity in shell form, body mass, and their respective measures of growth are likely due two factors that may act in concert. First, water temperatures at the southern site averaged 6.8°C warmer during the course of the 90 day reciprocal transplant experiment. Second, both the duration of historical contact and present day abundance of an invading crab predator (*Carcinus maenas*) is greater in southern Gulf of Maine habitats. Hence, geographic differences in *Carcinus* abundance could also produce the observed plastic shifts in shell form and body mass. Although future experiments are necessary to determine the relative importance of each cue, it is clear that phenotypic

plasticity has an important role in producing biogeographic patterns of phenotypic variation in *Littorina obtusata*.

INTRODUCTION

Understanding the basis (genetic vs. eco-phenotypic) of phenotypic variation across different environments has aided in shaping our views on adaptation, speciation, and geographic variation (Endler, 1977; West-Eberhard, 1989; Schlichting & Pigliucci, 1988). Both narrow and broad scale patterns of phenotypic variation are often viewed as adaptive products of natural selection rather than eco-phenotypic phenomena (Kitching et al., 1966; Endler, 1977, 1986; Futuyma, 1988). However, it is clear that intraspecific phenotypic variation also can reflect phenotypic plasticity: the within-generation response of an organism's genotype to its environment (Via & Lande, 1985; Stearns, 1989; Schlichting & Pigliucci, 1998).

Empirical and theoretical approaches to the evolution of phenotypic plasticity often focus on genotype by environment (G x E) interactions (Via, 1984; Via & Lande, 1985). An emphasis on genetic variation in plasticity provides insight into the potential for it to evolve, but alone this information aids little in understanding micro- and macro-geographic patterns of phenotypic variation. Resolution of this difficulty requires recognition that patterns of phenotypic variation across environments are determined, in part, by whether genetic and environmental influences on phenotypes act in concert or in opposition (Conover & Schultz, 1995). With cogradient variation (CoGV), selection and plasticity act in the same direction on phenotypic values. Hence, the covariance

relationship is positive. In this scenario (also termed 'synergistic' selection, Falconer, (1989)) phenotypic differentiation is promoted in native phenotypes across environmental gradients (N_1 vs. N_2 in Figure 7a) while phenotypes of transplanted organisms converge toward a similar phenotypic value (T_1 vs. T_2 in Figure 7a). In contrast, countergradient (CnGV) variation occurs when selection and plasticity act on phenotypic values act in opposition and the covariance relationship between genetic and environmental influences in thus negative. In this scenario (also termed 'antagonistic selection', Falconer (1989)), little or no phenotypic differentiation occurs in native phenotypes across environments (N_1 vs. N_2 in Figure 7b), while phenotypic values of transplanted organisms diverge from one another (T_1 vs. T_2 in Figure 7b). Appreciation of these covariance relationships aids in understanding the presence (or absence) of clinal variation in phenotypic traits (Levins, 1968, 1969; Berven 1982a,b; Berven et al., 1979) and in interpreting life history tradeoffs across environments (Conover & Schultz, 1995).

Rocky intertidal snails exhibit dramatic morphological variation across environmental gradients on both local and biogeographic scales (Kitching et al., 1966; Phillips et al., 1973; Vermeij, 1978, 1987; Palmer, 1985, 1990; Etter, 1988; Trussell, 1996, 1997; Trussell et al., 1993). Predation by shell-crushing predators is thought to be particularly important in producing geographic and historical variation in gastropod shell form (Vermeij, 1978, 1987). For example, better-defended shell morphologies and higher frequencies of shell repair in post-Paleozoic fossil shells versus Paleozoic

assemblages (Vermeij, 1978, 1987; Vermeij et al., 1981; Signor & Brett, 1984) are thought to reflect the coincident diversification of shell-crushing predators in the Mesozoic (Vermeij, 1977). Biogeographic evidence suggests that gastropod species have more robust shell morphologies in regions (tropical vs. temperate) where shell-crushing predators are more taxonomically diverse, capable of producing greater crushing forces, and there has been a longer time for co-evolution between predator and prey (Vermeij, 1978, 1987; Vermeij & Veil, 1978).

The *Carcinus maenas* Range Expansion and Phenotypic Shifts in *Littorina obtusata*

Transitions in the shell form of two intertidal species (*Nucella lapillus* and *Littorina obtusata*) that occurred after the biogeographic range expansion of the green crab (*Carcinus maenas*) into the Gulf of Maine also support the natural selection hypothesis (see Vermeij, 1982; Seeley, 1986). Although the precise cause for this expansion is unknown, it may have been facilitated by a significant warming trend in mean annual sea surface temperatures over the last 100 years in the Gulf of Maine (Welch, 1968; Lazzari, 1997). Beginning in 1900, the range of *Carcinus* in North America began to expand north of Cape Cod, Massachusetts. *Carcinus* reached Portland, Maine in the early 1900's, mid-coastal Maine by the 1930's, and northern Maine and the Bay of Fundy by the 1950's (Scattergood, 1952; Welch, 1968). Consequently, snails in northern Maine have been cohabiting with *Carcinus* for ~50 years while snails in Massachusetts have been cohabiting with *Carcinus* for ~100 years. Hence, there is a

historical gradient in the presence of *Carcinus* in the Gulf of Maine that corresponds to latitude, in its role as a selective agent (Vermeij, 1982; Seeley, 1986) and as an inducer of phenotypic plasticity (Trussell, 1996).

For example, museum specimens of *Littorina obtusata* collected in the Gulf of Maine before 1900 were thinner and higher-spired than those collected from similar locations in the mid-1980's (Seeley, 1986). Moreover, experiments with thin-shelled, high-spired morphs from northern Maine were more vulnerable to *Carcinus* predation than thick-shelled, low-spired morphs collected elsewhere. Seeley (1986) concluded that the range expansion of *Carcinus* and coincident changes in *Littorina obtusata* shell form were an example of rapid micro-evolutionary change via natural selection.

Recent evidence of phenotypic plasticity in response to predator effluent has changed our thinking about the evolution of shell form (Appleton & Palmer, 1988; Crowl & Covich, 1990; Palmer, 1990; Trussell, 1996). For example, Appleton & Palmer (1988) demonstrated that the scent of crabs and damaged conspecifics induce the development of larger apertural teeth in *Nucella lamellosa*. Similarly, thicker shells in *Littorina obtusata* can be induced by raising snails in the presence of effluents associated with *Carcinus* feeding on conspecifics (Trussell, 1996). Indeed, the taxonomic and geographic diversity of this response indicates that it is a general phenomenon (Appleton & Palmer, 1988; Crowl & Covich, 1990; Palmer, 1990; Trussell, 1996; Leonard et al., in press).

The Environmental Effects of Water Temperature on Gastropod Shell Form

Water temperature also can influence both micro- and macroscopic properties of calcium carbonate ($CaCO_3$) based shells (Lowenstam, 1954a, b; Dodd, 1963, 1964; Kennedy et al., 1969; Graus, 1974; Vermeij, 1978, 1993). Both the deposition and maintenance of shells are expected to be more difficult in colder versus warmer waters because $CaCO_3$ becomes less saturated and more soluble with decreasing water temperature (Malone & Dodd, 1967; Graus, 1974; Clarke, 1983; Vermeij, 1978, 1993). Although there are exceptions to this view (Vermeij, 1993), it is supported by increased calcification indices (the ratio of shell mass to its internal volume) in tropical versus temperate molluscs (Nicol, 1967; Graus, 1974; Vermeij, 1978) and by experimental evidence of increased calcification rates in *Mytilus edulis* at higher temperatures (Malone & Dodd, 1967). In addition, Lowenstam (1954 a, b) and Dodd (1963) found that calcite:aragonite ratios in *Mytilus edulis* increased with latitudinal decreases in water temperature. This latitudinal trend in shell mineralogy may reflect the higher solubility of aragonite (versus calcite) in colder waters (Pytkowicz, 1969). In terms of shell strength, the relative amount of aragonite may be important because calcite is softer, less dense, and tends to break along well defined cleavage planes (Carter, 1980). For gastropods like *Littorina obtusata* that are distributed across a latitudinal temperature gradient in New England (Figure 8), shells in colder waters are expected to be thinner, weaker, and thus more vulnerable to crushing predators than those in warmer waters.

Given the geographic differences in the historical and present day abundances of *Carcinus* and water temperature (Figure 8) in the Gulf of Maine, one objective of this study was to determine whether phenotypic clines in shell traits and body mass (defined as soft tissue mass) exist along a latitudinal gradient. I hypothesized that shell thickness, mass, and strength would decrease, and body mass increase, with increasing latitude. In addition, a reciprocal transplant experiment was conducted in the field between a northern (Lubec, Maine) and southern (Manchester, Massachusetts) population to examine genetic and environmental influences on shell form and body mass and their respective growth. By measuring variation in morphological traits and their respective growth rates, I examined the role of cogradient and countergradient phenomena in producing geographic patterns of phenotypic variation. Finally, data from both experiments were used to address whether there are costs associated with increased shell production.

MATERIALS AND METHODS

Phenotypic Clines in Shell Form, Body Mass, and Shell Breaking Force

To examine geographic variation in several traits across a latitudinal gradient, I sampled 25 populations along the New England coast from Manchester, Massachusetts to Lubec, Maine. All collections were made between November and December (1995-1997) to minimize potential seasonal effects on the traits of interest. From each site, approximately 100 snails were sampled from 0.25 m2 quadrats tossed haphazardly in the

mid-intertidal zone. Fifty snails from each sample were randomly chosen; however, I did attempt to maximize the size range of snails from each population. In the laboratory, I measured shell length and shell thickness with digital calipers (± 0.01 mm). The mean of two measures of shell thickness was used in statistical analyses (see Figure 1 in Trussell, 1996). After measurement of shells, I carefully cracked each shell with a C-clamp and separated shell fragments from soft tissue. Shell material and soft tissue were then oven dried at 60°C for 48 hours before weighing on an analytical balance (±0.001 g; Mettler AE 100).

For seven sites in the Cape Ann region of Massachusetts and six sites in the Quoddy region of Maine, 50 additional snails were collected. These samples were used to measure the maximum force required to crush each snail's shell on an Instron Dynamic Testing machine (±0.01 N; Model 4301). These tests were not meant to simulate crab predation but to provide a relative measure of breaking force, which should influence vulnerability to crab predation (Vermeij & Currey, 1980). Snails were kept submerged in seawater for 24 hours prior to testing and were tested alive. I placed snails aperture down between two steel platens and crushed the shell by lowering the top platen onto each shell at a rate of 10 mm/minute. Shells were loaded until they were crushed; a loud "cracking" noise reliably indicated failure of the shell.

Non-Destructive Estimates of Shell Mass and Body Mass

Because I wanted to document growth in shell and body mass of snails to be used in the reciprocal transplant experiment (described below), it was necessary to make non-destructive estimates of shell and body mass. Following the methods of Palmer (1982), I generated regressions between measurements of actual shell mass (Y) on measurements of shell mass while submerged in seawater (X; hereafter, submerged mass) for each population. To do so, 50 snails spanning the available size range were collected from a sheltered site in Lubec, Maine (Northern) and a sheltered site in Manchester, Massachusetts (Southern). In the laboratory, shell length was measured with digital calipers (±0.01 mm). Submerged mass was determined by weighing snails immersed in seawater (±0.001 g). Snails were then allowed to dry on toweling for approximately 30 minutes. To remove extra-visceral water trapped inside the shell, snails were forced into their shell with absorbent tissue before weighing in air (hereafter, total mass [±0.001 g]). All mass measurements were made on a Mettler (Model PG503) analytical balance. After total mass measurements, snails were carefully crushed and tissue separated from the shell. Both tissue and shell material was dried at 60°C for 48 hours before weighing to determine the actual mass of each variable.

Regressions of actual shell mass on submerged mass for snails from each population yielded highly significant R^2 values (Northern--$Y = 1.561X - 0.0018$, $R^2 = 0.9991$; Southern--$Y = 1.582X + 0.0023$, $R^2 = 0.9999$), indicating that submerged mass is

a reliable predictor of actual shell mass (Palmer, 1982). By inserting measurements of initial submerged mass of snails collected for the reciprocal transplant experiment into the respective regression equations for each population, I was able to estimate initial actual shell mass from initial submerged mass. To calculate initial body mass, I subtracted the estimate of actual shell mass from the total mass of snails when weighed in air (Palmer, 1982).

Reciprocal Transplant Between a Northern and Southern Population

I reciprocally transplanted snails between a northern (Lubec, Maine) and southern (Manchester, Massachusetts) population to test the hypothesis that geographic differences in shell form and body mass (defined by tissue mass) have an eco-phenotypic component. In mid-May 1997, juvenile snails were collected from both populations and individually labeled with waterproof markers (Trussell, 1997). I then measured the shell length and shell thickness of all snails as described above. Following the Palmer (1982) protocol, I also made measurements of submerged mass and total mass in air to obtain estimates of actual shell mass and body mass. Measurements of growth in terms of shell length, shell thickness, shell mass, and body mass were calculated by subtracting initial from final values.

After completion of measurements, 10 snails from each population were placed in 24 separate replicate cylindrical chambers (4 cm h x 15 cm dia) that served as experimental units for statistical analyses. The top and bottom of each chamber were

constructed from plastic mesh (3.75 mm x 2.90 mm) to permit water flow. There were 6 replicate chambers yielding a total of 60 snails for each transplant group: (1) North >> North (NN; Control), (2) North >> South (NS; Transplant), (3) South >> South (SS; Control), and (4) South >> North (SN; Transplant). In the mid-intertidal zone at each site, I anchored chambers to bricks with cable ties. Chambers were haphazardly placed at each site within an area of ~50m2. Although snails were able to feed on the micro-flora that colonized each chamber during the course of the experiment, I also supplemented the food supply by placing 30 g (wet mass) of the alga *Ascophyllum nodosum* in each chamber. Any snails or egg masses on *A. nodosum* fronds were removed before placing the algae in the chambers. I replaced the algae in each chamber every 30 days. Chambers were recovered from the field after 90 days for final measurement of shell length, shell thickness, submerged mass, and total mass in air. Using the same protocol described above, 15-25 snails randomly sampled from each experimental group were sacrificed to generate new regressions to estimate final actual shell mass and final body mass (SS--$Y = 1.568X + 0.0028$, $R^2 = 0.9981$; SN----$Y = 1.582X + 0.0009$, $R^2 = 0.9980$; NS--$Y = 1.607X - 0.0031$, $R^2 = 0.9962$; NN--$Y = 1.548X - 0.0009$, $R^2 = 0.9955$). During the course of the experiment I lost one SN replicate, 3 SS replicates, and 2 NS replicates.

Water temperature was also monitored at each transplant location with HoboTemp dataloggers (Onset Computer Corp., Pocasset, MA). HoboTemps were anchored in the mid-intertidal zone at each site to bricks placed among my experimental

chambers. I programmed HoboTemps to record water temperature each hour for the duration of the experiment. Water temperatures recorded at high tide were used in analyses. Unfortunately, these devices were not available for the first two weeks of the experiment (late May, 1997 to early June, 1997) but based on the trends in Figure 8, I suspect that differences in water temperature between the two regions were even greater than the 7.6 oC average difference recorded for the latter part of June. Water temperature data collected at the same sites in 1998 indicating that water temperatures averaged 6.1 °C colder at the northern site for all of June support this assertion (Trussell, unpublished data).

Statistical Analyses

Clines in phenotypic traits of the 25 sampled populations were examined by first generating size-adjusted least squares means with an analysis of covariance (ANCOVA) model that used shell length as a covariate to adjust for potential size effects on the trait of interest. Thus, I generated separate ANCOVA models for shell thickness, shell mass, and body mass that treated population as a random effect and length as the covariate. The cube roots of shell mass and body mass were used in these analyses to linearize their relationship with shell length. Adjusted least squares means generated by ANCOVA for each trait for each population were then regressed against latitude. Shell strength data were analyzed with a two-way nested ANCOVA that treated geographic region as a fixed effect, sites with each region as a nested random effect, and shell length as the covariate.

Because I was explicitly interested in whether differences in shell strength were due to regional effects rather than among population effects, the F- ratio for the "Region" effect was constructed by dividing its mean square by that for "Population{Region}". All other F-ratios were constructed by using the residual mean square as the denominator mean square.

Data from the reciprocal transplant experiment were analyzed with a two-factor nested analysis of covariance (ANCOVA). Both transplant location and source population were treated as fixed effects and replicates as a random nested effect. Covariates depended on the analysis in question. The effects of shell size on the analysis of shell thickness and body mass were adjusted by using final shell length as the covariate. Because I was interested in the potential energetic consequences of increased shell production in terms of shell mass, this analysis used body mass as the covariate. For analyses of growth (the difference between final and initial measurements) in each of these traits, initial values for the trait in question were used as the covariate. Slopes in all analyses were homogeneous and thus pooled before final analysis. Water temperature data were analyzed with a two-way ANOVA that treated month and site as fixed effects. All analyses were conducted using JMP statistical software (Version 3.2.1 for the MacIntosh, SAS Institute, Cary, North Carolina). Because nested replicates were declared a random effect in all models, JMP used the Satterthwaite approximation to

calculate F-ratios and their respective degrees of freedom. *A priori* post-hoc comparisons on least squares adjusted means were conducted using the linear contrast feature in JMP.

RESULTS

Geographic Differences in Water Temperature, Shell Form, Shell Breaking Force, and Body Mass

Despite significant seasonal variation (ANOVA: $F_{(10, 1156)} = 1{,}435.38$; $p < 0.0001$), water temperatures were also significantly different between geographic regions (ANOVA: $F_{(1,1156)} = 1{,}460.49$; $p < 0.001$; Fig. 8). Geographic differences in water temperature were particularly pronounced during the transplant experiment, with southern waters averaging 6.8°C warmer than northern waters. Although large significant differences in water temperature persisted throughout the summer, temperatures converged in December before diverging in subsequent months.

Regression analyses revealed that both shell mass (Fig. 9a) and shell thickness (Fig. 9b) decreased with increasing latitude. These differences in shell form translated into geographic differences in shell breaking force; snails from the Cape Ann region of Massachusetts required significantly greater force to break than snails from the Quoddy region of Maine (ANCOVA: $F_{(1,11)} = 2{,}183.10$; $p < 0.00001$; Fig. 10).

Regression analysis of body mass as a function of latitude revealed an opposite trend to that found for shell traits; body mass increased with increasing latitude (Fig. 11). Costs to increased shell production in the form of reduced body mass also were suggested

by regression analysis of adjusted body mass of all populations as a function of adjusted shell thickness and adjusted shell mass. For both comparisons body mass decreased significantly with increasing shell mass (Fig. 12a) and increasing shell thickness (Fig. 12b).

Reciprocal Transplant Experiment

Final Phenotypes: Shell Thickness, Shell Mass, and Body Mass

Geographic differences in shell mass and thickness persisted in control groups throughout the experiment; northern snails raised at their native site (NN) weighed less and were thinner than southern snails raised at their native site (SS) (Tables 3a, 4a; Figs. 13a, 14a). However, transplanting between geographic locales had a dramatic effect on the shell mass and thickness of each population. Northern snails transplanted to the southern site (NS) were significantly heavier and thicker than their controls (NN) raised at their native site. In contrast, southern snails transplanted to the northern site (SN) produced lighter and thinner shells than their controls (SS) raised at their native site. A significant Source Population * Transplant Location interaction (Tables 3a, 4a) indicated a significantly greater increase in the shell mass (+64%) and thickness (+31%) of NS snails over their controls than the decrease in these traits for SN snails (-32% for shell mass, -17% for thickness) relative to their controls. Hence, northern snails were more plastic in these traits than southern snails (Table 6).

Interestingly, NS snails and SN snails produced shells of identical mass and thickness (Tables 3a, 4a; Figs. 13a, 14a). Despite large differences in shell mass and thickness of Northern and Southern snails at the beginning of the experiment, the differential thickening of NS snails and differential thinning of SN snails led to a convergence in the shell mass and thickness of these two groups.

Initial differences in body mass between geographic regions persisted in control groups with NN snails maintaining more soft tissue than SS snails (Table 5a; Fig. 15a). Transplanting snails between each location also had a significant effect on body mass, and northern snails were again more plastic (Table 6). Relative to their controls (NN), the body mass of NS snails was significantly smaller (-24%) after the experiment, while the body mass of SN snails was significantly heavier (+12%) than their controls (SS). Although plasticity in body mass occurred, its effects were clearly not as dramatic as those for shell mass and thickness (Table 6), where percentage changes in these traits ranged from 32-64% and 17-31%, respectively. This point is further illustrated by the lack of convergence in body size for NS and SN snails.

Growth Analyses: Shell Thickness, Shell Mass, Shell Length, and Body Mass

Countergradient variation was found in shell mass growth, with no statistical difference between SS and NN snails (Table 3b; Fig. 13b). Comparisons involving transplant groups revealed large differences. For shell mass, SS snails grew 32% more than SN snails, while NS snails grew 64% more than NN snails and 166% more than SN

snails. The significant Pop*Loc interaction indicated that shell mass growth rates in northern snails were significantly more plastic (Table 6).

Countergradient variation in shell thickness growth also was found even though SS snails grew 24% more than NN snails (Table 4b; Fig. 14b). However, the difference in growth between control groups was much smaller than that found between transplant snails and their respective controls; SN snails grew 58% less than SS snails, while NS snails grew 46% more than NN snails. Direct comparison of transplant groups found that NS snails grew 57% more than SN snails. Smaller differences in growth among snails in their native habitats (i.e., NN vs. SS) relative to large differences produced by transplanting (i.e., NS vs. SN) indicate a countergradient pattern in growth.

Variation in body growth followed a cogradient pattern (Table 5b; Fig. 15b) with large differences between snails raised in their native habitats; growth for NN snails was 43% greater than that for SS snails. Although both northern and southern snails raised in their native habitats (NN & SS) exhibited significant differences in body growth relative to their respective transplant groups (NS & SN), growth rates of NS snails were identical to those of SS. The effect of transplanting on northern snail body growth was much more dramatic (-76%) than that revealed for southern snails (+19%; Table 6). Hence, like final body mass, a significant Source Population * Transplant Location interaction indicated that body growth of northern snails was more plastic than that of southern snails (Table 5b; Figure 15b).

The cogradient variation in shell length growth was opposite to that of final shell mass and thickness (Table 4c; Fig. 16). There were large differences in growth rates among snails in their native habitats (NN vs. SS) as well as differences between snails in their native habitats and their respective transplants. Growth rates of NN were 8% greater than those for NS snails, while the growth rates of SN snails were 7% greater than those of SS snails. The lack of a significant Source Population * Transplant Location interaction indicates that northern and southern snails responded similarly to transplant environments.

DISCUSSION

Shell thickness, shell mass and breaking force of New England populations of *Littorina obtusata* all decreased with increasing latitude (Figures 9-10). Phenotypic clines in molluscan shell form are well known in numerous marine species on both micro- (Kitching et al., 1966; Palmer, 1985; Trussell, 1996) and macro-geographic scales (Nicol, 1964, 1967; Phillips et al., 1973; Palmer, 1979; Vermeij, 1977, 1978, 1993). For example, *Littorina littorea* south of Cape Cod, Massachusetts are thicker and stronger than conspecifics found north of Cape Cod (Dudley, 1980), and shell strength is greater for tropical versus temperate species of Thaididae (Vermeij & Currey, 1980). Although the presence of more taxonomically diverse and powerful shell crushing predators may explain the occurrence of more robust, defended prey species in the tropics (Vermeij, 1978, 1987), environmentally induced variation in shell form in response to predator

effluent (*sensu* Appleton & Palmer, 1988; Palmer, 1990; Trussell, 1996) and water temperature (Lowenstam, 1954 a,b; Graus, 1974; Dudley, 1980) also may be important.

Latitudinal Variation in Littorina obtusata Shell Form: The Role of Selection by Carcinus

Geographic variation in the shell form of *Littorina obtusata* may result from geographic differences in the duration of selection (*sensu* Seeley, 1986) imposed by *Carcinus*. If selection by *Carcinus* produced the temporal shifts in *L. obtusata* shell form documented by Seeley (1986), then latitudinal differences in the intensity of selection by *Carcinus*, both presently and historically, also may yield latitudinal clines in shell form. Accordingly, snails from southern latitudes are thicker, heavier (in terms of shell mass), and stronger simply because they have been exposed to natural selection by *Carcinus* for a longer time period (~100 years) than snails from northern latitudes (~50 years). This argument assumes, all else being equal, that similar levels of genetic variation exist across latitudes. Consequently, genetic variation would not act to constrain shell form responses to selection in northern populations had they been subjected to *Carcinus* predation for the same amount of time. I cannot rigorously address this hypothesis with my data. However, in their study of northeast Pacific *Littorina sitkana*, Boulding & Hay (1993) concluded that sufficient additive genetic variance existed for shell form to respond to selection and suggested that their results support Seeley's (1986) conclusion that historical changes in *L. obtusata* shell form reflect evidence of micro-evolutionary change.

Latitudinal Variation in Littorina obtusata Shell Form: The Role of Phenotypic Plasticity

Although selection by *Carcinus* may explain latitudinal and historical variation in *Littorina obtusata* shell form, my reciprocal transplant experiment revealed a cogradient pattern and substantial plasticity in shell form. Relative to their respective controls, the shell mass and shell thickness of southern snails decreased after transplantation to the northern site (SN vs. SS) but increased for northern snails transplanted to the southern site (NS vs. NN). Moreover, plasticity in shell form was so pronounced that transplant groups (SN & NS) produced shells of nearly identical mass and thickness (Figures 13a, 14a).

I am unable to precisely determine the environmental stimuli inducing the plastic shifts in shell form. This difficulty arises because (1) both the duration of historical contact and present day distributions of *Carcinus* and (2) latitudinal gradients in water temperature could explain the observed responses either separately or synergistically. At the northern site, both reduced *Carcinus* abundance and colder water temperatures should promote the development of thinner shells, while increased *Carcinus* abundance and warmer water temperatures in the south should promote thicker shells.

Elsewhere, I have shown that *Littorina obtusata* raised in the presence of *Carcinus* feeding on conspecifics develop thicker and heavier shells than those raised in the absence of *Carcinus*. I documented this response in sheltered and wave-exposed populations in southern Maine (Trussell, 1996) and sheltered populations in northern

(Lubec, Maine) and southern (Manchester, Massachusetts) New England (Trussell, in prep.). Clearly this response to *Carcinus* effluent is typical of New England *L. obtusata*, and similar occurrences in *Nucella lapillus* in the British Isles (Palmer, 1990) and *Mytilus edulis* in the Gulf of Maine (Leonard et al., in press) suggest that it is taxonomically and geographically widespread.

Although the development of heavier, thicker shells by NS snails and lighter, thinner shells by SN snails may reflect geographic differences in *Carcinus* effluent concentrations, I suspect that geographic differences in water temperature are also an important, if not dominating, factor (Lowenstam, 1954 a, b; Graus, 1974; Vermeij, 1978, 1993). Water temperatures differed considerably between the two transplant sites, with water temperatures averaging 6.8°C colder at the northern site during the experiment. Moreover, the plastic shifts in shell form (32-64% for shell mass and 17-31 % for shell thickness; Table 6) were greater than those found in a laboratory study of *Carcinus* induced plasticity in snails from the two populations studied here (4-6% for shell mass, 13-17% for shell thickness; Trussell, in prep.). Laboratory responses to *Carcinus* effluent are expected to be maximal because effluent concentrations in the laboratory were likely greater than those occurring under natural field conditions (Palmer, 1990).

Countergradient Variation in Shell Mass and Shell Thickness Growth

Because growth rate is often a function of temperature (Cossins & Bowler, 1987; Atkinson, 1994), one would expect intraspecific variation in growth rates to decrease with increasing altitude or latitude. However, considerable evidence indicates that countergradient patterns in growth often occur in species having wide altitudinal (Levins, 1968, 1969; Berven 1982a, b; Berven et al., 1979) or latitudinal (Ament, 1979; Dehnel, 1955, 1956) distributions; growth rates in colder environments typical of high altitudes and latitudes are often similar to, or may actually exceed, those of conspecifics in warmer environments (Conover & Schultz, 1995). For some species this pattern may reflect metabolic compensation (*sensu* Levinton, 1983), but this hypothesis does not explain the more rapid growth of some high latitude vs. low latitude conspecifics when raised at warmer temperatures (see Conover & Present, 1990; Conover & Schultz, 1995).

The countergradient pattern I found in shell mass and thickness growth suggests that water temperature strongly influences variation in *Littorina obtusata* shell form. Northern and southern snails raised in their native locations showed similar rates of total shell deposition (shell mass) and thickness deposition (hereafter, defensive deposition; NN vs. SS; Figures 13b, 14b). However, when reared in warmer waters at the southern site, northern transplants showed the highest rates in both forms of growth, outgrowing southern snails in their native environment (NS vs. SS; Figures 13b, 14b). In addition,

relative to their controls, rates for both forms of deposition decreased for southern snails transplanted to the northern site (SN vs. SS; Figures 13b, 14b).

In northern habitats, the critical issue may be shell maintenance rather than defense against crab predation because reduced water temperatures retard shell production by making calcification more difficult and by increasing dissolution of deposited shell material. Given this scenario, selection would be expected to favor increased deposition rates, especially if they are necessary to offset increased dissolution rates and a shorter growing season. Hence, genetic and environmental influences on shell growth in northern habitats act in opposite directions. Despite the negative impact of the environment on deposition rates in northern habitats, genetic differences allow NN snails to maintain deposition rates similar to those of SS snails. Transplanting northern snails to the warmer waters at the southern site allows this genetic potential to become fully realized with NS snails exhibiting dramatic increases in the rates of both forms of deposition.

In southern habitats, genetic and environmental influences on shell growth are expected to act in the same direction. In terms of ecophenotypic responses, both increased water temperature and crab abundance should favor increased deposition rates. Genetic controls for increased deposition rates also would be favored by *Carcinus* imposed selection. However, selection due to water temperature is expected to be weak because the environment is more favorable (vs. northern habitats) to shell deposition and

maintenance. Despite the presumed synergistic effects of selection and plasticity, SS snails still exhibited lower total and defensive deposition rates than NS snails, suggesting that they do not possess the genetic capacity for higher deposition rates. This result begs the question: "Given the presumed adaptive value of better-defended shells in southern habitats, why have higher deposition rates not evolved?" This inability may reflect the evolution of an optimal deposition rate that is closely tied to life history trade-offs in southern habitats (see "Plasticity in Shell Form: Trade-Offs"). Alternatively, the growth of southern snails raised in their native habitat (SS) may simply reflect ontogenetic or architectural constraints arising from their different developmental history (compared to northern snails) in the field before collection for the transplant experiment. In other words, more rapid shell deposition in SS snails may have been limited by the fact that they were already considerably thicker than northern snails at the beginning of the experiment.

The countergradient pattern in total and defensive shell growth suggests that water temperature is an important factor driving shell form in *Littorina obtusata*. However, I should note that the data suggest that *Carcinus* effluents are also modulating shell form. Although SS and NN snails exhibited nearly identical total deposition rates (2% difference), SS snails allocated more of this material to the apertural lip by having defensive deposition rates that were 24% greater than those of NN snails (Table 6).

Plasticity in Shell Form: Trade-Offs

An assessment of the trade-offs associated with phenotypic plasticity is essential if we are to fully understand its adaptive value in changing environments (Stearns, 1989, 1992; Schlichting & Pigliucci, 1998). Because gastropods must live within the shell they construct they provide an ideal system with which to study the potential trade-offs associated with changes in shell form.

Several results from this study suggest that there are costs associated with increased shell production. In field populations, latitudinal gradients in shell form were negatively correlated with gradients in body mass (defined by tissue mass); shell mass and thickness decreased with increasing latitude while body mass increased (Figures 9a-b, 11). Moreover, regression analyses across all populations of body mass against shell mass and thickness revealed strong negative correlations (Figures 12a-b). For NS snails from the reciprocal transplant experiment, both increases in shell mass and thickness and their respective growth rates were accompanied by large decreases in body mass and body growth (Figures 13-15). In contrast, decreases in shell mass and thickness and their respective growth rates in SN snails were accompanied by increases in body size and body growth (Figures 13-15).

Clearly there is a negative relationship between body mass and body growth and the production of more robust shell morphologies. Ultimately, reductions in either growth or body mass could have profound reproductive costs (Peters, 1983). For

example, in their study of plastic responses in *Physella virgata virgata*, Crowl & Covich (1990) found that snails raised in the presence of cues emanating from crayfish feeding on conspecifics were both older and larger at first reproduction than snails raised in the absence of these cues. These life history shifts likely resulted because of the increased energetic investment required to produce, or the constraints associated with producing, a larger shell.

Palmer (1981) focused on two potential costs associated with the production of thicker shells. The first involved an energetic cost associated with shell deposition and maintenance. Although Palmer (1992) experimentally demonstrated a cost to calcification, he concluded that in areas where surface seawater is saturated with $CaCO_3$, the cost is small relative to other metabolic costs and the production of the organic component of the shell. However, in colder waters, where $CaCO_3$ saturation is lower and dissolution rates are higher, energetic costs may be significant. For example, rough calculations based on the relationship between the solubility product of $CaCO_3$ and water temperature (Sverdrup et al., 1942; p. 206) indicate that the $CaCO_3$ solubility product over the course of the reciprocal transplant, was 9.5-13.9% greater at the northern study site.

Palmer (1981) concluded that geometric, rather than energetic, constraints (termed the "skeleton-limitation" hypothesis) best explains reduced body mass and growth in thick-shelled snails. Because body growth cannot proceed ahead of the

protective shell, body mass and growth are constrained by the linear rate of shell growth. Because there is a maximum rate at which calcification can occur (Palmer, 1992), the more shell material devoted to thickening the shell, the less available for advancing the shell margin. This limitation is compounded further because snails with thick-walled shells have less internal habitable volume available for body growth than thin-walled morphs of similar size and shape. Kemp & Bertness (1984) documented this relationship by showing that rapidly growing shells in the snail *Littorina littorea* were thinner and more globose and thus able to accommodate more body growth than more slowly growing snails (see also Swan, 1952; Goreau, 1959).

Growth patterns detected in the reciprocal transplant were consistent the skeleton-limitation hypothesis. While NN and SS snails exhibited similar rates of total deposition, SS snails devoted more of this material to lip thickness but NN snails channeled it into growth in terms of shell length. Hence, the rapidly growing (in terms of shell length), thinner shells of NN snails have more internal volume available for body growth (Figure 15a-b). This pattern is also evident when comparing snails raised in their native environments with their respective transplant groups (i.e., NN vs. NS and SS vs. SN). For example, slowly growing (in terms of shell length) NS snails versus rapidly growing NN snails exhibited increased total and defensive deposition rates but reduced body mass and reduced body growth (Figures 13b, 14b, 15a-b).

Bergmann Variation in Body Size: A Product of Constraints Imposed by Shell Form?

Both the causes and adaptive value of Bergmann clines (Ray, 1960) in body size (increased body size with increasing latitude) are actively debated (McNab, 1971; Geist, 1987, 1988; Paterson, 1988; Atkinson, 1994; Atkinson & Sibly, 1997). Much of this debate has focused on the inadequacy of adaptive arguments based on surface to volume ratios (Geist, 1987, 1988; Paterson, 1988) and whether Bergman clines reflect genetic or ecophenotypic phenomena. However, mounting evidence suggests that growth at reduced temperatures often leads to increased body size (Atkinson, 1994) and that this phenomenon may be the unavoidable product of the relationship between cell size and temperature (Van Voorhies, 1996); that is, the tendency for the development of larger cell sizes at reduced temperatures (Partridge et al. 1994; Partridge & French, 1996; Van Voorhies, 1996).

Although selection may contribute to the latitudinal increases in body size I found for Gulf of Maine *Littorina obtusata*, the reciprocal transplant experiment indicates that ecophenotypic responses are also involved, and these are likely due to geographic differences in water temperature. Snails from both populations raised in the colder waters of the northern site (NN and SN) produced more body mass and exhibited increased rates of body growth relative to individuals from both populations raised in the warmer waters of the southern site (NS and SS, respectively; Figures 15a-b).

Although these results are consistent with the expected effects of reduced water temperature on growth in marine gastropods (Atkinson, 1994), they were inversely correlated with plastic changes in shell form. That is, those snails producing thicker, heavier shells tended to have reduced body mass and body growth (Figures 13a, 14a, 15a-b). Given the well-known constraints imposed by shell form on gastropod body mass and growth (Palmer, 1981; Kemp & Bertness, 1984), it is possible that latitudinal variation in body mass may reflect latitudinally based differences in shell form. Because latitudinal variation in shell form is likely influenced by water temperature (Graus, 1974; Vermeij, 1978), care must be exercised in identifying the reasons for latitudinal increases in the body mass of shelled gastropods. Hence, Bergmann variation in *Littorina obtusata* body mass and in other gastropods may reflect their unique architectural constraints and the effects of reduced water temperature on shell form, in addition to temperature dependent responses in cell number (James et al., 1995) and cell size (Van Voorhies, 1996).

Geographic Variation in Plasticity

The presence of significant Population x Location interactions in all ANCOVA analyses indicate among-population genetic variation in plasticity (Tables 3-5). Genetically based geographic variation in plasticity indicates that reaction norms for the traits measured have evolved different trajectories in each region. Interestingly, these analyses yielded the consistent feature of greater plasticity in northern snails regardless of the trait measured (Table 6).

By definition, adaptive phenotypic plasticity must have a genetic basis and there must be genetic variation in plasticity for it to evolve. There is debate, however, as to whether phenotypic plasticity is a target (Scheiner, 1993) or by-product (Via, 1993, 1994) of selection and a lack of consensus on the relative importance of each remains (Via et al., 1995; Schlichting & Pigliucci, 1998). Assuming that selection by *Carcinus* is shaping reaction norms in *Littorina obtusata* shell form (i.e., plasticity is a target of selection), one would expect reduced plasticity to evolve in southern populations given their longer historical contact with *Carcinus* and the present predictability of *Carcinus* in the southern Gulf of Maine. The evolution of reduced plasticity in southern populations may be especially rapid if there are genetically based limits and costs to plasticity (sensu DeWitt et al., 1998). Clearly, southern populations still retain plasticity in shell form and body mass suggesting that sufficient spatial and/or temporal variation in selection pressures remain to favor plastic responses.

In contrast, the increased plasticity exhibited by northern snails may reflect historically weak selection by *Carcinus* and the present unpredictability of *Carcinus* abundance. In addition, increased developmental sensitivity to differences in water temperature may have evolved in northern populations due to the comparably shorter growing season (Conover & Present, 1990; Conover & Schultz, 1995). If selection favors higher growth for snails at northern latitudes, then placing these snails in better growth conditions may also produce a greater plastic response, and these responses may

override those tied to *Carcinus*. Future experiments that simultaneously address the role of plasticity induced by *Carcinus* effluent and water temperature may clarify the mechanisms underlying biogeographic variation in shell form and body mass as well as biogeographic variation in the magnitude of plastic responses.

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Table 3. Nested ANCOVA for *Littorina obtusata* reciprocally transplanted between a Northern and Southern site. (a) Shell mass (Y) vs. Body mass (X), and (b) Shell mass growth (Y) vs. Initial shell mass (X). Inequality signs between group labels indicate the direction of significant differences in adjusted means (all p 0.01).

(a)

SOURCE	df	F	p	Multiple Comparison
Location (Loc)	1, 28	729.95	<0.0001	SS > SN = NS > NN
Population (Pop)	1, 108	292.38	<0.0001	
Pop X Loc	1, 16	118.14	<0.0001	
Rep{Pop, Loc}	14, 155	0.72	0.7503	
Slope	1, 154	1.58	0.2108	

(b)

SOURCE	df	F	p	Multiple Comparison
Location (Loc)	1, 14	85.59	<0.0001	NS > SS = NN > SN
Population (Pop)	1, 30	52.32	<0.0001	
Pop X Loc	1, 14	10.63	<0.01	
Rep{Pop, Loc}	14, 155	2.24	<0.01	
Slope	1, 154	0.47	0.4956	

Table 4. Nested ANCOVA for *Littorina obtusata* reciprocally transplanted between a Northern and Southern site. (a) Shell thickness (Y) vs. Shell length (X), (b) Shell thickness growth (Y) vs. Initial shell thickness (X), and (c) Shell length growth (Y) vs. Initial shell length. Inequality signs between group labels indicate the direction of significant differences in adjusted means (all p 0.01).

(a)

SOURCE	df	F	p	Multiple Comparison
Location (Loc)	1, 14	284.45	<0.0001	SS > SN = NS > NN
Population (Pop)	1, 22	287.73	<0.0001	
Pop X Loc	1, 14	24.36	<0.001	
Rep{Pop, Loc}	14, 155	1.73	0.0546	
Slope	1, 154	0.45	0.5036	

(b)

SOURCE	df	F	p	Multiple Comparison
Location (Loc)	1, 14	211.58	<0.0001	NS > SS > NN > SN
Population (Pop)	1, 119	21.27	<0.0001	
Pop X Loc	1, 14	18.30	<0.001	
Rep{Pop, Loc}	14, 155	1.64	0.0744	
Slope	1, 154	0.04	0.8385	

(c)

SOURCE	df	F	p	Multiple Comparison
Location (Loc)	1, 14	5.04	<0.05	NN > NS > SN > SS
Population (Pop)	1, 15	35.79	<0.0001	
Pop X Loc	1, 14	0.10	0.7515	
Rep{Pop, Loc}	14, 155	2.61	<0.005	
Slope	1, 154	0.17	0.6848	

Table 5. Nested ANCOVA for *Littorina obtusata* reciprocally transplanted between a Northern and Southern site. (a) Body mass (Y) vs. Shell length (X), and (b) Body mass growth (Y) vs. Initial body mass. Inequality signs between group labels indicate the direction of significant differences in adjusted means (all p 0.01).

(a)

SOURCE	df	F	p	Multiple Comparison
Location (Loc)	1, 14	393.44	<0.0001	NN > NS > SN >SS
Population (Pop)	1, 26	759.18	<0.0001	
Pop X Loc	1, 14	35.34	<0.0001	
Rep{Pop, Loc}	14, 155	1.17	0.3031	
Slope	1, 154	0.02	0.8845	

(b)

SOURCE	df	F	p	Multiple Comparison
Location (Loc)	1, 14	34.43	<0.0001	NN > SN > SS, NS
Population (Pop)	1, 37	5.40	<0.05	
Pop X Loc	1, 14	9.24	<0.01	
Rep{Pop, Loc}	14, 155	3.06	<0.0005	
Slope	1, 154	0.23	0.6325	

Table 6. Summary of changes in morphological traits and growth of *Littorina obtusata*. The direction of the difference (+ or -) is based on the first group relative to the second. For example, for shell mass SS snails were 166% heavier than NN snails, while NN snails were 64% lighter than NS snails. Values in bold refer to comparisons between control groups and comparisons between control groups and their respective transplant groups.

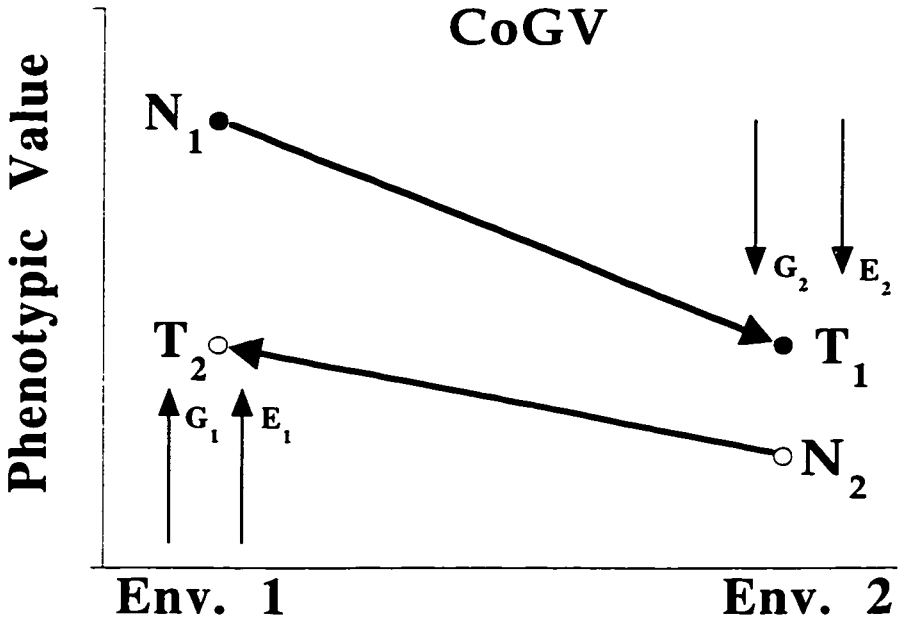
Comparison	Shell Mass	Shell Mass Growth
SS vs. NN	**+166%**	**+2%**
SS vs. SN	**+32%**	**+42%**
NN vs. NS	**-64%**	**-39%**
SS vs. NS	+30%	-37%
NN vs. SN	-64%	+39%
SN vs. NS	-1%	-56%

Comparison	Shell Thickness	Thickness Growth	Shell Length Growth
SS vs. NN	**+74%**	**+24%**	**-25%**
SS vs. SN	**+17%**	**+58%**	**-7%**
NN vs. NS	**-31%**	**-46%**	**+8%**
SS vs. NS	+20%	-33%	-19%
NN vs. SN	-33%	+27%	+24%
SN vs. NS	+3%	-57%	-13%

Comparison	Body Mass	Body Mass Growth
SS vs. NN	**-38%**	**-43%**
SS vs. SN	**-12%**	**-19%**
NN vs. NS	**+24%**	**+76%**
SS vs. NS	-22%	0%
NN vs. SN	+40%	+42%
SN vs. NS	-11%	+24%

Figure 7. (a) Phenotypic variation consistent with cogradient variation (CoGV). Note the large difference in phenotypic values of phenotypes in their native environments (N_1 and N_2) and the shift of their respective transplants (T_1 and T_2) towards the phenotypic values of native phenotypes. (b) Phenotypic variation consistent with countergradient variation (CnGV). Note the similarity in phenotypic values of phenotypes in their native environments (N_1 and N_2) and the divergence in the phenotypic values of their respective transplant phenotypes (T_1 and T_2). Arrows with G and E refer to the direction of genetic and environmental influences on phenotypes within their respective environments. See text for further explanation (Adapted from Conover & Schultz, 1995).

a)



b)

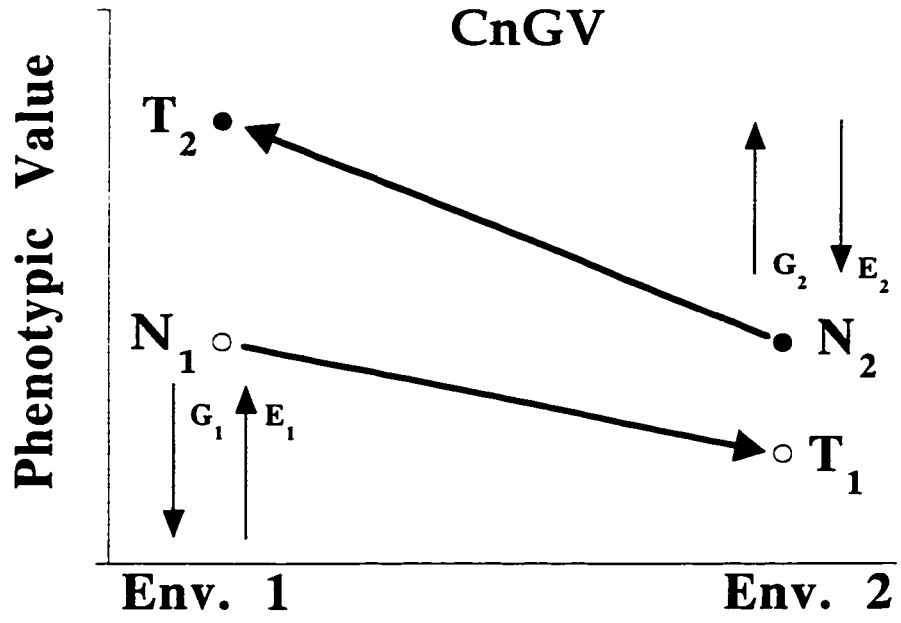


Figure 8. Mean (±SE) water temperature at the two sites used in the reciprocal transplant experiment. "Experimental Period" refers to 90 days during which the experiment was conducted. Linear contrasts indicated that water temperatures were significantly greater at the southern site between June and October (all p 0.00001) when most snail growth occurs. See Results for statistical analyses.

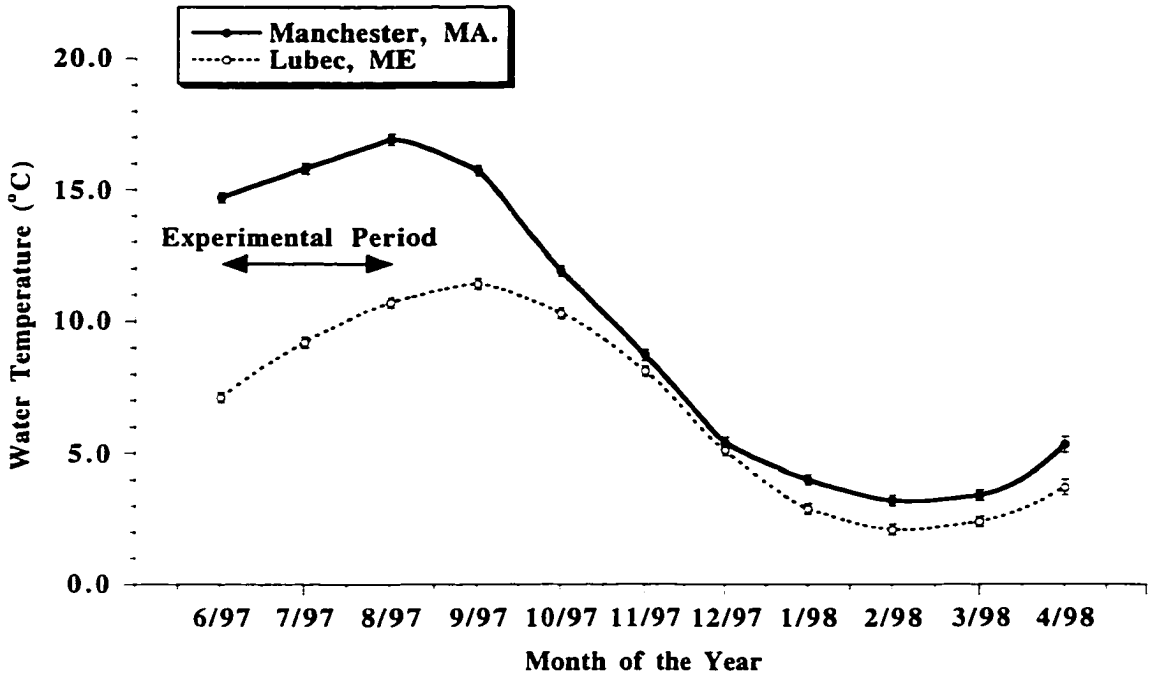
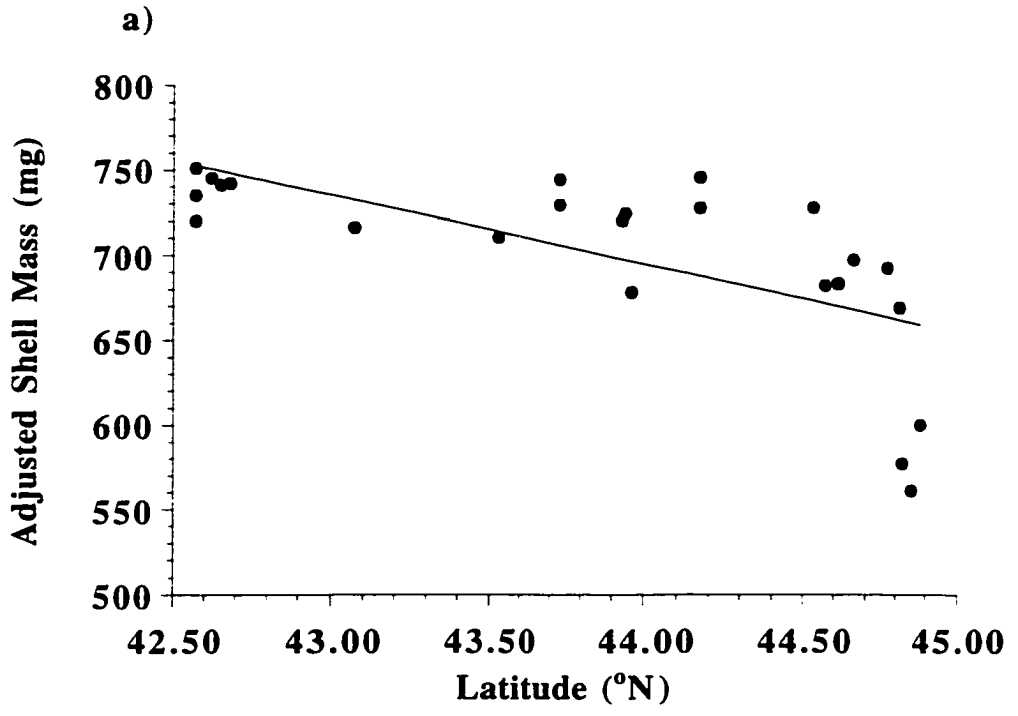


Figure 9. (a) Adjusted shell mass (Y) and (b) adjusted shell thickness (Y) from ANCOVA as a function of latitude (X) for 25 *L. obtusata* populations in the Gulf of Maine. Both shell mass ($Y = -40.486X + 2,475.9$; $R^2 = 0.46$) and thickness ($Y = -0.14X + 7.57$; $R^2 = 0.52$) decrease with increasing latitude. Each point represents the adjusted mean (±SE) for a sample of 50 snails from each population. Error bars are smaller than data symbols.



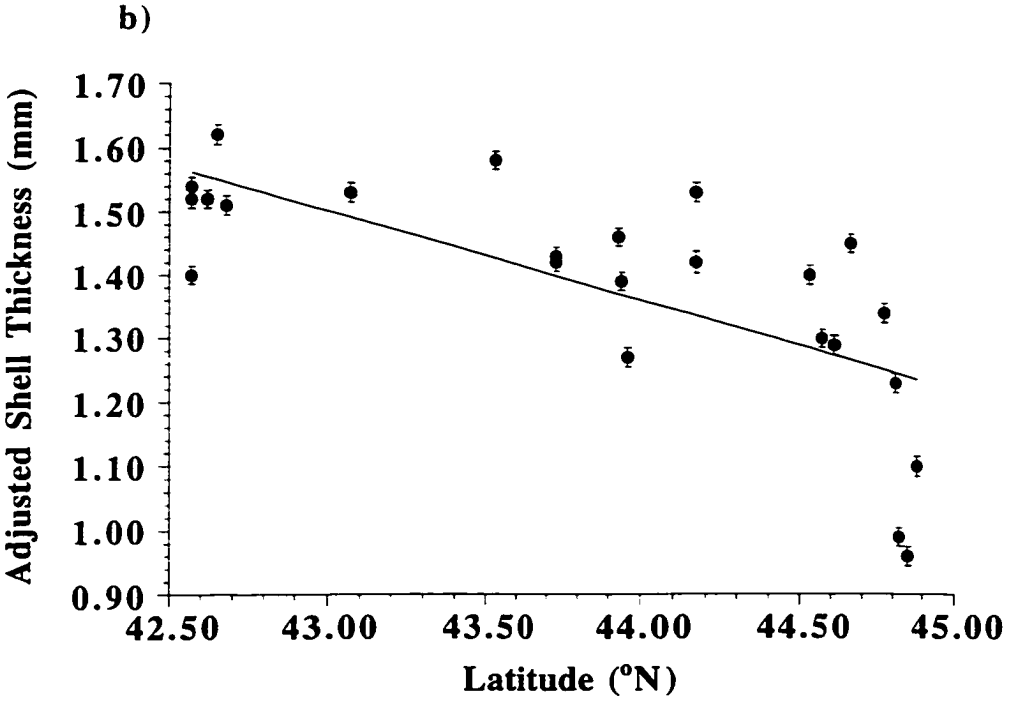


Figure 10. Adjusted breaking force (±SE) of *L. obtusata* from seven southern and six northern populations in the Gulf of Maine. Snails from southern populations required significantly more force ($p < 0.00001$) to break than snails from northern populations.

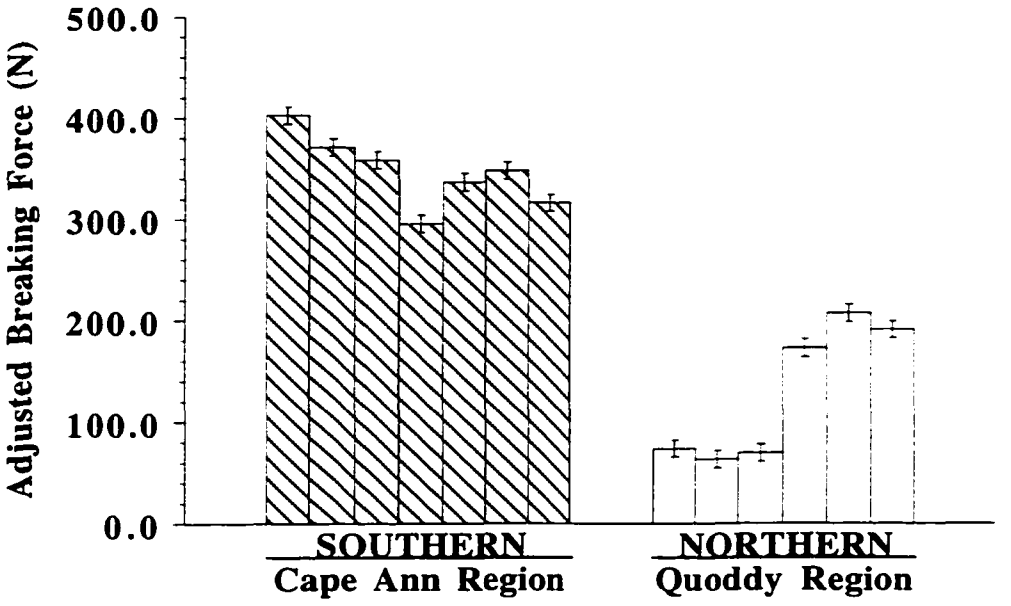


Figure 11. Adjusted body mass (Y) from ANCOVA as a function of latitude (X) for 25 *L. obtusata* populations in the Gulf of Maine. Body mass increases significantly with increasing latitude ($Y = 11.483X - 240.53$; $R^2 = 0.36$). Each point represents the mean (±SE) for a sample of 50 snails from each population.

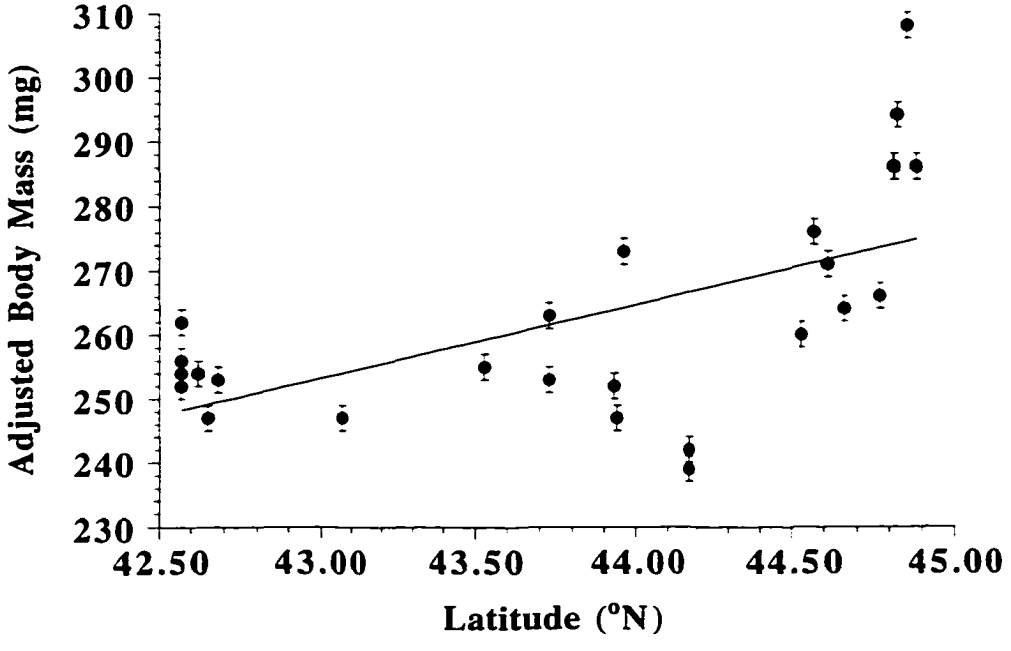
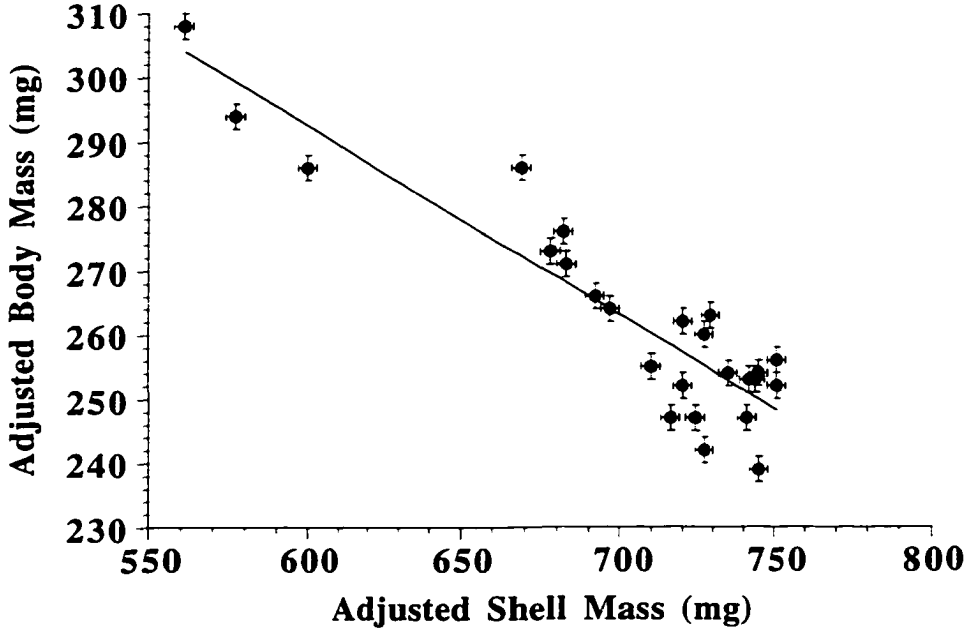


Figure 12. Adjusted body mass (Y) from ANCOVA as a function of (a) adjusted shell mass (X) and (b) adjusted shell thickness (X) from ANCOVA for 25 *L. obtusata* populations in the Gulf of Maine. Body mass decreases with increasing shell mass ($Y = -0.294X + 469.08$; $R^2 = 0.83$) and thickness ($Y = -89.368X + 386.51$; $R^2 = 0.83$). Each point represents the mean (±SE) for a sample of 50 snails from each population.

a)



b)

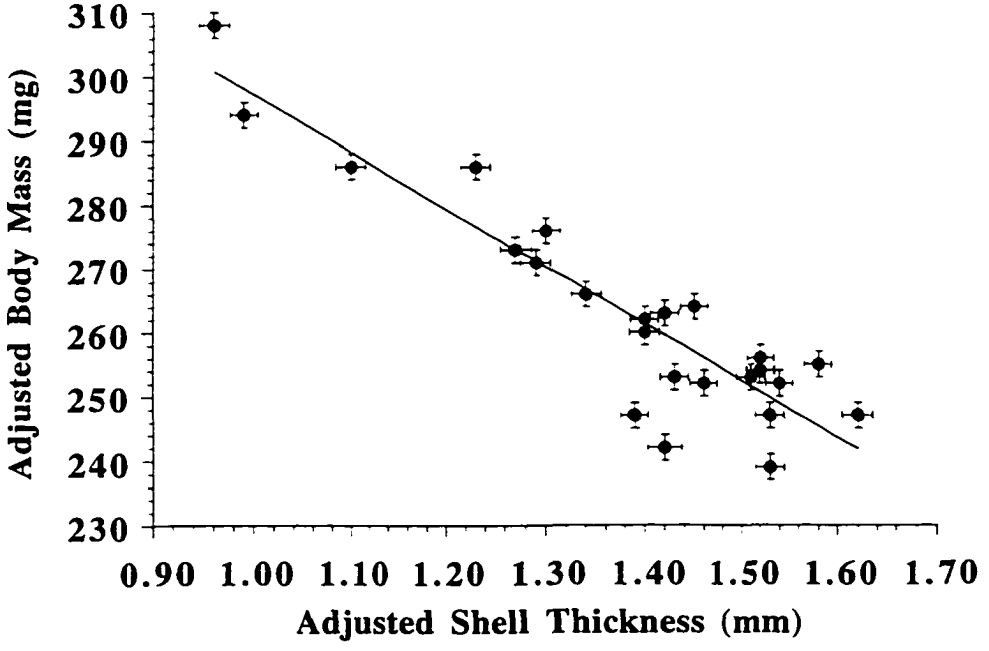
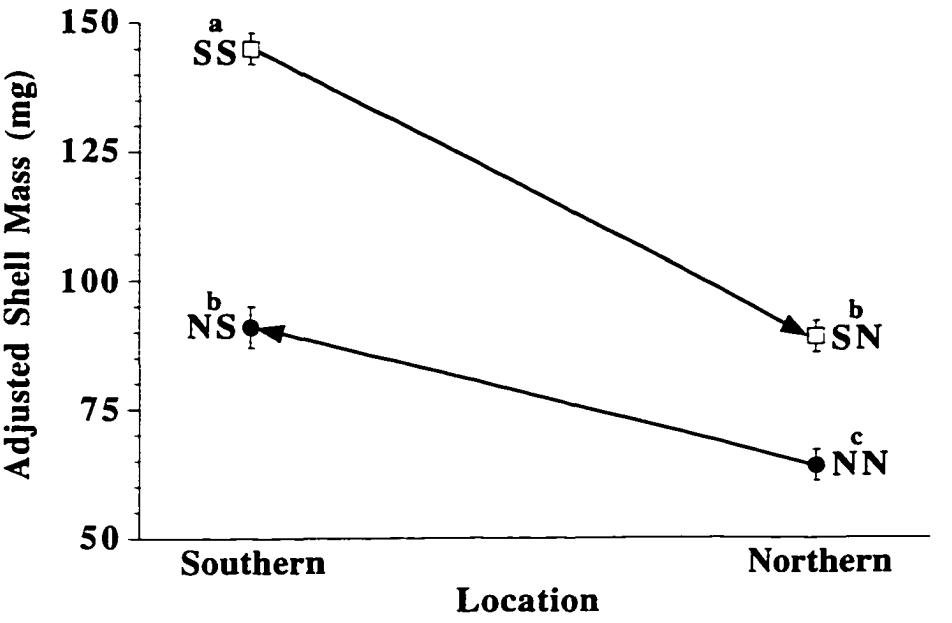


Figure 13. (a) Adjusted shell mass (±SE) and (b) adjusted shell mass growth (±SE) from ANCOVA for *L. obtusata* reciprocally transplanted between a northern and southern site in the Gulf of Maine for 90 days. NN = North to North; NS = North to South; SS = South to South; SN = South to North. Groups not sharing a common letter are significantly different (all $p < 0.001$). Error bars were sometimes smaller than data symbols. See Table 3 for statistical analyses.

a)



b)

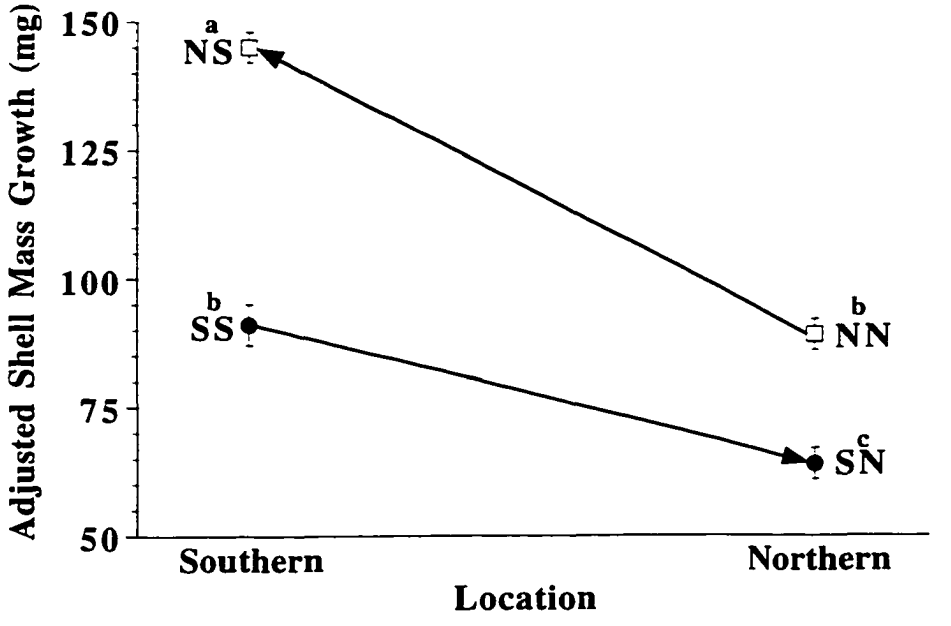
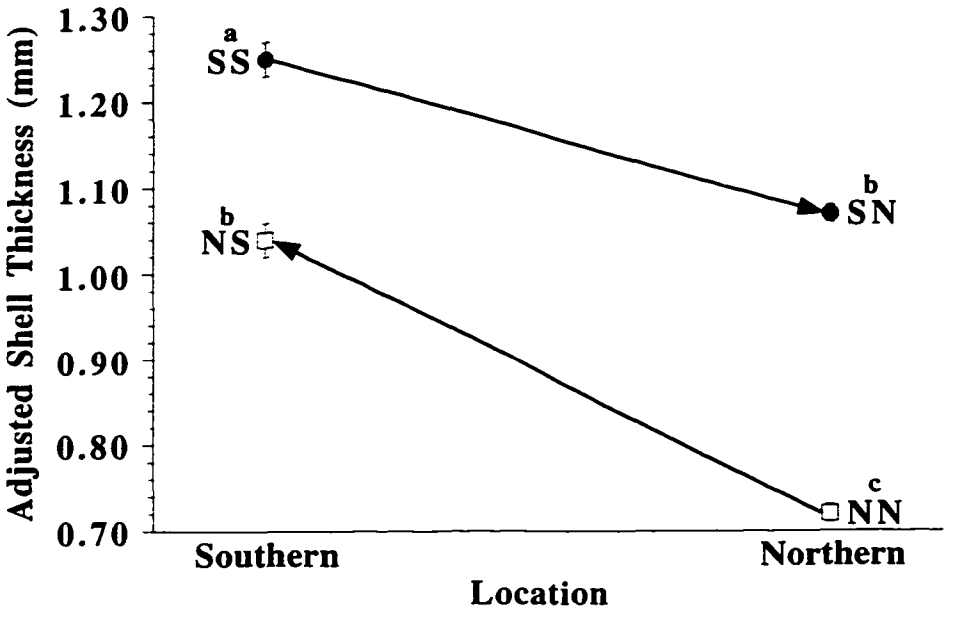


Figure 14: (a) Adjusted shell thickness (±SE) and (b) adjusted shell thickness growth (±SE) from ANCOVA for *L. obtusata* reciprocally transplanted between a northern and southern site in the Gulf of Maine for 90 days. Symbols as in 13. Error bars were sometimes smaller than data symbols. See Table 3 for statistical analyses.

a)



b)

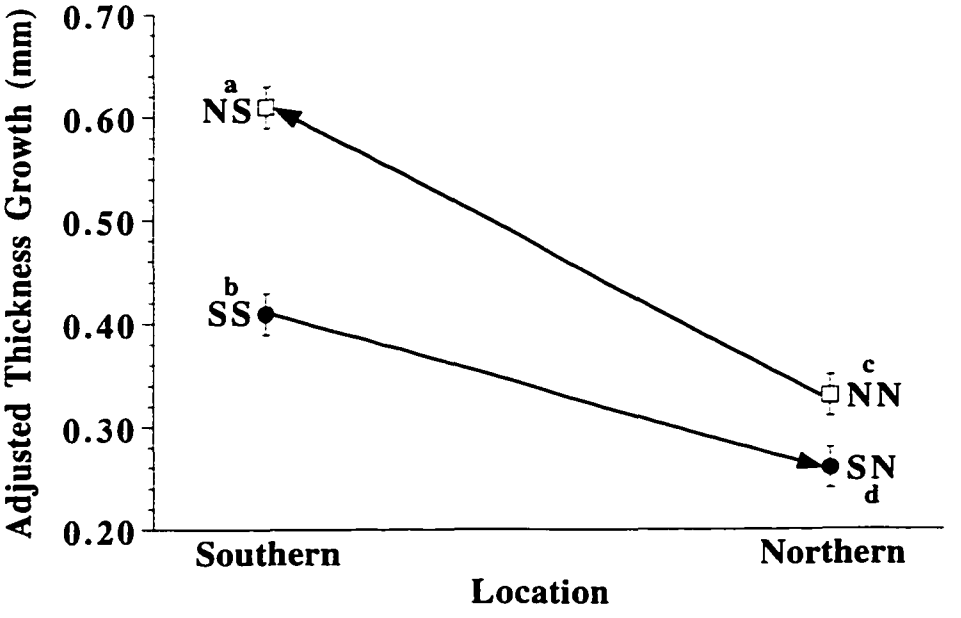
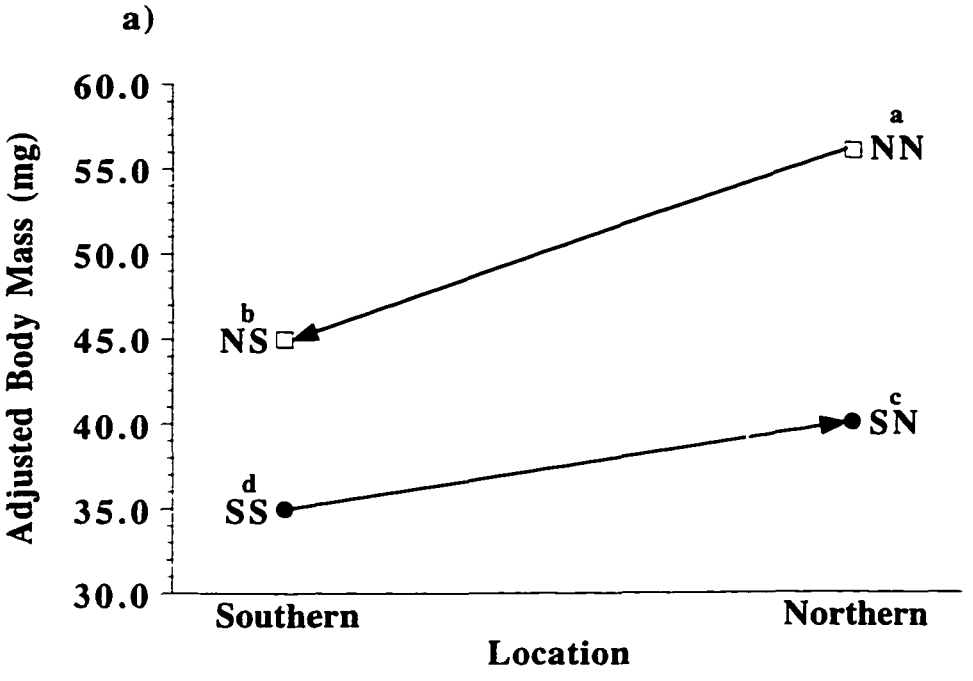


Figure 15. (a) Adjusted body mass (±SE) and (b) adjusted body mass growth (±SE) from ANCOVA for *L. obtusata* reciprocally transplanted between a northern and southern site in the Gulf of Maine for 90 days. Symbols as in Figure 13. Error bars were sometimes smaller than data symbols. See Table 5 for statistical analyses.



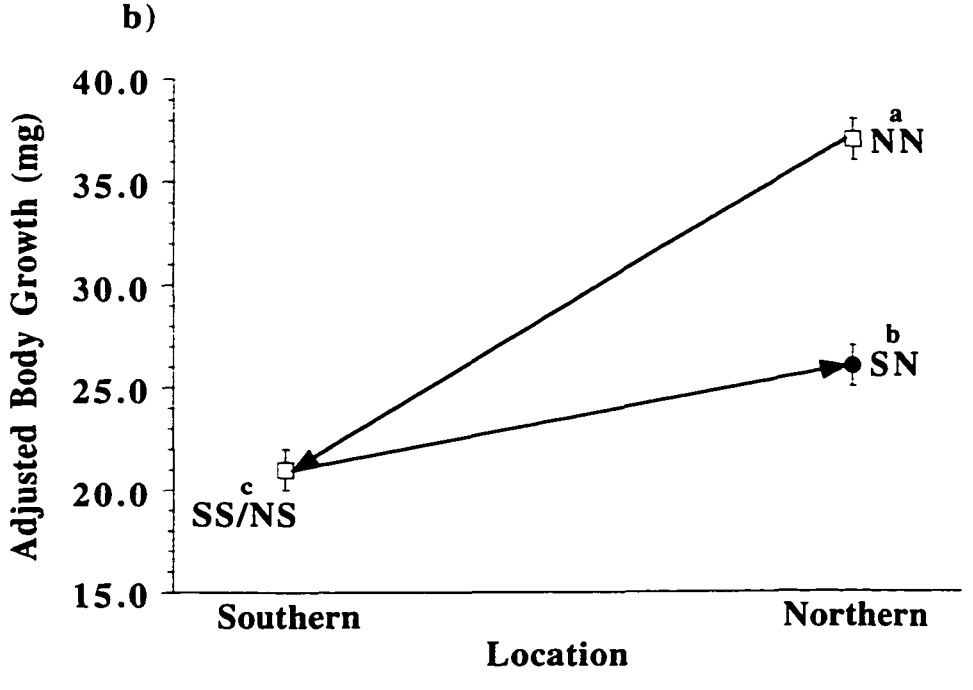
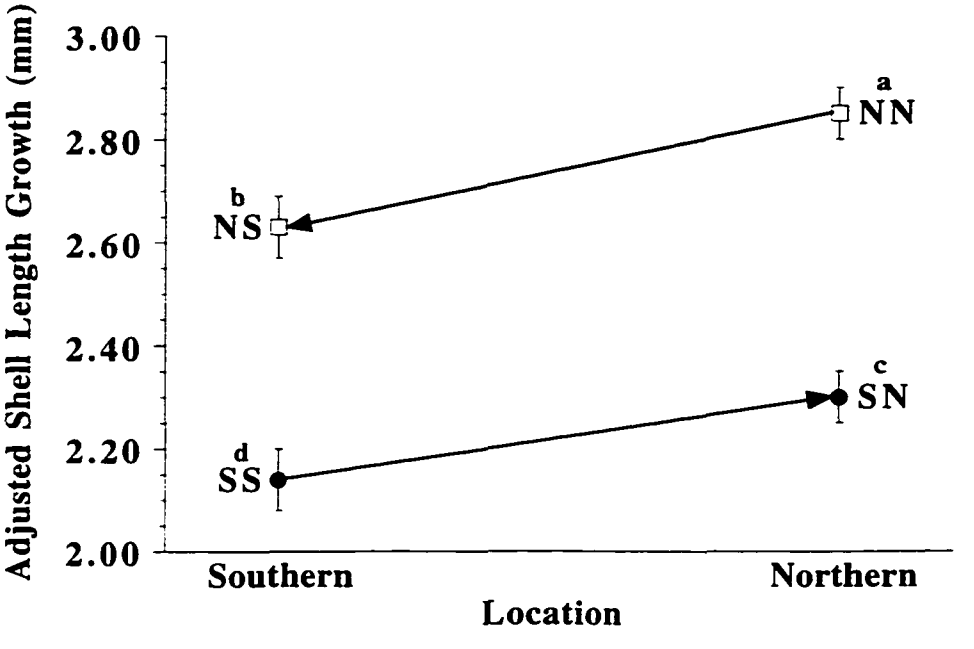


Figure 16. Adjusted shell length growth (±SE) from ANCOVA for *L. obtusata* reciprocally transplanted between a northern and southern site in the Gulf of Maine for 90 days. Symbols as in Figure 13. See Table 4 for statistical analyses.



CHAPTER 3:

THE EFFECTS OF PREDATOR EFFLUENT AND WATER TEMPERATURE ON PLASTICITY AND MORPHOLOGICAL TRADE-OFFS IN AN INTERTIDAL SNAIL

ABSTRACT

Paleontological and biogeographical evidence suggest that predation by crushing predators has strongly influenced the evolution of gastropod shell form. Although natural selection by crushing predators is often invoked to explain evolutionary and biogeographic patterns in shell morphology, recent experiments have shown that gastropods can alter shell form during ontogeny in response to predator effluent. Both predator effluent and water temperature are known to induce plastic changes in marine gastropod shell form, but there have been no attempts to address the relative importance of each cue. Snails (*Littorina obtusata*) collected from a northern (Lubec, Maine) and southern (Manchester, Massachusetts) were reciprocally transplanted and subjected to two effluent treatments. By raising snails in the presence and absence of one of their principal predators *(Carcinus maenas*) in two distinct geographic locations (water temperature effect), this study sought to identify the relative importance of each factor as inducers of plasticity in shell thickness. Regardless of source population or transplant location, snails raised in the presence of *Carcinus* produced significantly thicker shells than conspecifics raised in the absence of *Carcinus*. In addition, the data indicate that predator effluent has effects on shell form that are comparable to those of geographic differences in water temperature, which averaged 5.9°C colder at the northern site. Although natural selection can drive coevolutionary relationships between predator and prey, these results support the alternative view that historical transitions in gastropod shell form in response to crab predators need not reflect evidence of rapid evolution via natural selection.

Evidence of trade-offs accompanying predator-induced increases in shell thickness was also found. Snails raised with *Carcinus* grew more slowly in terms of shell size and also exhibited reductions in tissue mass and tissue growth. These trade-offs likely reflect geometric constraints imposed by shell form via reduced growth in terms of shell size and reduced available space inside the shell due to increased thickening. The existence of such trade-offs may explain why inducible defenses have evolved in *Littorina obtusata* and other intertidal gastropod species.

INTRODUCTION

An important goal of evolutionary ecology is to understand how organisms adapt to changing environments (Levins, 1968; Stearns, 1989, 1992; Schlichting & Pigliucci, 1998). This knowledge is essential to properly interpret biogeographic or historical phenotypic patterns (e.g., morphological changes in the fossil record, Williamson, 1981) or predict individual or population level responses to future environmental change (e.g., biological invasions, global climate change, NRC 1995). Adaptation to different environments is often reflected in patterns of phenotypic variation over a range of spatial and temporal scales (Vermeij, 1978, Conover & Schultz, 1995). Until recently, both narrow and broad scale patterns of phenotypic variation within species have been interpreted primarily as adaptive products of natural selection rather than ecophenotypic phenomena (Kitching et al., 1966; Endler, 1977, 1986; Futuyma, 1988). However, it is increasingly clear that intraspecific phenotypic variation can also reflect phenotypic plasticity; the within-generation response of an organism's genotype to its environment (Via & Lande, 1985; Stearns, 1989; Real, 1994; Schlichting & Pigliucci, 1998). Hence, a thorough understanding of intraspecific phenotypic variation requires determination of (1) the relative roles of genetic vs. ecophenotypic phenomena and (2) the relative contributions of important environmental cues in producing this variation.

Adaptive phenotypic plasticity should be favored when (1) environmental cues are temporally or spatially variable, (2) modification of a trait confers some direct benefit

to the organism, such as improved survival, growth, or reproduction, and (3) the cost of producing a fixed phenotype is greater (Stearns, 1989). Among the best documented examples of adaptive phenotypic plasticity are predator-induced defensive responses in prey. Examples include spine formation in bryozoans (Yoshioka, 1982; Harvell, 1984, 1991), crest enlargement in cladocerans (Grant & Bayly, 1981; Krueger & Dodson, 1981; Dodson, 1989; Tollrian, 1995), shell thickening in mussels (Leonard et al., in press), shell thickening in marine gastropods (Appleton & Palmer, 1988; Palmer, 1990; Trussell, 1996), and shell size and shape change in freshwater snails and marine barnacles (Crowl & Covich, 1990; Lively, 1986). Despite the recent surge in interest in phenotypic plasticity (Schlichting & Pigliucci, 1998), to date, most studies have examined its adaptive role only on very local scales (Harvell, 1984; Etter, 1988; Stearns, 1989; Smith & Palmer, 1994; Trussell, 1996, 1997, but see Trussell, 1999a). However, phenotypic plasticity may also contribute to larger scale patterns of biogeographic variation and macro-evolutionary phenomena such as speciation (West-Eberhard, 1989).

Predator-Induced Plasticity in Crab Molluscan Systems

Paleontological and biogeographical evidence suggest that predation by crushing predators has strongly influenced the evolution of gastropod shell form (Vermeij, 1976, 1978, 1981, 1987; Palmer, 1979; West & Cohen, 1996). For example, post-Paleozoic fossil assemblages show higher frequencies of shell repair (Vermeij, et al., 1981) and more robust, better defended shell morphologies than Paleozoic assemblages (Vermeij,

1978, 1987; Signor & Brett, 1984), and these morphological shifts coincided with the diversification of shell crushing predators in the Mesozoic (Vermeij, 1977). Particularly compelling are the recent transitions to better-defended shells in two intertidal species (*Nucella lapillus* and *Littorina obtusata*) that coincided with the invasion of a crab predator (*Carcinus maenas*) into the Gulf of Maine (Vermeij, 1982, Seeley, 1986).

Biogeographic evidence reveals more robust gastropod shell form in regions where shell crushing predators are more taxonomically diverse and powerful and where there has been a longer time for co-evolution between predator and prey (Vermeij, 1978, 1987; Vermeij & Veil, 1978). Thus, tropical gastropod shells are more robust than temperate snails (Vermeij, 1978; Vermeij & Currey, 1980); Indo-West Pacific snails are better defended than Caribbean congeners (Vermeij, 1976); and freshwater snails from ancient African rift valley lakes are stronger than snails from nearby, but younger lakes (West et al., 1991). These patterns are thought to be adaptive as numerous experiments demonstrate the greater efficacy of thick vs. thin shelled morphs in resisting crab predation (Bertness & Cunningham, 1981; Reimchen, 1982; Palmer, 1985a; Seeley, 1986; West & Cohen, 1996).

Although natural selection by crushing predators is often invoked to explain evolutionary and biogeographic patterns in shell morphology, recent experiments have shown that gastropods can alter shell form during ontogeny in response to predator effluent (Appleton & Palmer, 1988; Crowl & Covich, 1990; Palmer, 1990; Trussell,

1996). For example, both local and widely separated populations of *Littorina obtusata* can increase shell thickness in response to effluent associated with feeding *Carcinus maenas* (Trussell, 1996, 1999a). The taxonomic and geographic diversity of this response indicate that it is a general phenomenon and has invited reinterpretation of views that recent or fossil transitions in shell form are evidence of rapid selection (Vermeij, 1982; Seeley, 1986) or speciation (Williamson, 1981; Palmer, 1985b). Clearly, there is a close co evolutionary relationship between predators and their gastropod prey (Vermeij, 1987), but it appears that the relationship may be driven by plastic rather than strictly genetic, responses to predators.

Phenotypic plasticity is a developmental phenomenon; consequently, the speed and magnitude of an induced response can depend on the organism's growth rate. Because growth rate is often a function of temperature (Cossins & Bowler, 1987; Atkinson, 1994), this important background variable may greatly influence the production of inducible defensive structures. For many invertebrates having wide altitudinal or latitudinal ranges, growth rates and maximum body size are inversely correlated with temperature (Dehnel, 1955, 1956; Levins, 1969; Ament, 1979; Berven et al., 1979; Conover & Present, 1990). The consequences of this inverse relationship to expression of predator-induced defensive traits, however, remain unknown. They may be particularly important to marine molluscs because many shallow water species (1) are subject to latitudinal temperature gradients (Levinton, 1995), (2) experience differential

growth rates over these ranges (Dehnel, 1955, 1956; Ament, 1979; Palmer, 1994; Trussell, 1999b), and (3) exhibit inducible defensive traits in response to predators (see references above).

Phenotypic Plasticity in Shell Form in Response to Water Temperature

The effects of water temperature on induced defenses may be particularly profound for marine gastropods given its strong influence on the deposition of calcium carbonate ($CaCO_3$) based shells (Lowenstam, 1954a, b; Dodd, 1963, 1964; Kennedy et al., 1969; Graus, 1974; Vermeij, 1978, 1993). Because $CaCO_3$ saturation decreases and solubility increases with decreasing water temperature, both the deposition and maintenance of shells are expected to be more difficult in colder vs. warmer waters (Malone & Dodd, 1967; Graus, 1974; Clarke, 1983; Vermeij, 1978, 1993). This prediction is supported by increased calcification indices (the ratio of shell mass to its internal volume) in tropical versus temperate molluscs (Nicol, 1967; Graus, 1974) and by experimental evidence of increased calcification rates in *Mytilus edulis* at higher temperatures (Malone & Dodd, 1967). Moreover, both Lowenstam (1954a, b) and Dodd (1963) found that the calcite:aragonite ratio in *Mytilus edulis* increased with latitudinal decreases in water temperature. This latitudinal trend in calcite:aragonite ratio likely reflects the higher solubility of aragonite (versus calcite) in colder waters (Pytkowicz, 1969). Thus, for gastropods distributed along a latitudinal temperature gradient, shells in

colder waters are likely to be thinner, weaker, and more vulnerable to crushing predators than those in warmer waters (Trussell, MS a, b).

Littorina obtusata and *Carcinus maenas* in the Gulf of Maine: A Model System

The historical and geographic relationship between *Littorina obtusata* and one of its principal predators (*Carcinus maenas*) provides an outstanding system to test the influence of water temperature on inducible defenses. Before 1900, *Carcinus* did not occur north of Cape Cod, Massachusetts. After 1900, *Carcinus* began to move northward reaching the Bay of Fundy in the mid 1950's (Figure 17). Although *Carcinus* is currently present in the northern Gulf of Maine, its populations are small or ephemeral (G.C. Trussell & L.D. Smith, pers. obs.). In contrast, *Carcinus* is well-established on protected shores in the southern Gulf of Maine. The historical and present day differences in the contact between *L. obtusata* and *Carcinus* may be responsible for the pronounced decrease in shell thickness, shell strength and shell mass of *L. obtusata* at higher latitudes (Trussell, MS a, b). Although these patterns may reflect historical variation in the intensity of selection imposed by *Carcinus*, previous research also indicates that snails from northern and southern populations exhibit plasticity in shell form in response to *Carcinus* effluent (Trussell, MS a) and water temperature (Trussell, MS b).

Identifying the relative importance of *Carcinus* effluent and water temperature to the observed latitudinal gradients in shell form has been difficult because (1) both the duration of historical contact and present-day distributions of *Carcinus* and (2) latitudinal

gradients in water temperature could explain these patterns either separately or synergistically. In the northern Gulf of Maine, both reduced *Carcinus* abundance and colder water temperatures should yield thinner shells, while increased *Carcinus* abundance and warmer water temperatures in the south should promote the development of thicker shells. Here, I present the results of an experiment in which *L. obtusata* were reciprocally transplanted between a northern Maine and Massachusetts population and subjected to two effluent treatments (Crab and No-Crab). This experimental design allowed me to simultaneously test the effects of *Carcinus* effluent and water temperature on plasticity in shell form.

MATERIALS AND METHODS

Non-Destructive Estimates of Body Mass and Shell Mass

To non-destructively measure growth in body and shell mass of snails to be used in the reciprocal transplant experiment (described below), it was necessary to generate destructive regressions between measurements of actual shell mass (Y) on measurements of shell mass while submerged in seawater (X; hereafter, submerged mass) for each population. Following the methods outlined in Palmer (1982), these regressions revealed that submerged mass is a reliable predictor of actual shell mass (see Trussell, MSb for regression equations). By inserting measurements of initial submerged mass of snails collected for the reciprocal transplant experiment into the respective regression equations for each population, I estimated actual initial shell mass from initial submerged mass. To

calculate initial body mass, I subtracted the estimate of actual initial shell mass from the total intial mass of snails when weighed in air (Palmer, 1982).

Reciprocal Transplant Between a Northern and Southern Site, With and Without *Carcinus Effluent*

In late April 1998, juvenile (6 mm shell length) *Littorina obtusata* were collected from a northern site in Lubec, Maine and a southern site in Manchester, Massachusetts (Figure 1). After collection, each snail was tagged with permanent markers and their initial shell length and lip thickness measured with digital calipers (±0.01 mm; see Trussell, 1996). After measurements, 6 snails were placed in each of 48 replicate cylindrical containers (5 cm ht. x 10 cm dia.) (i.e., 24 containers with northern snails; 24 with southern snails). Each container had plastic mesh panels (3.75 mm x 2.90 mm) on the top and bottom to permit water flow. Each container housing these snails (hereafter, response snails) was secured underneath a similarly constructed container housing either (1) a single mature male *Carcinus* ('Crab' treatment) and 30 conspecific snails (hereafter, stimulus snails) for food, or (2) no crab ('No-Crab' treatment) and 30 conspecific stimulus snails. This design allows crab effluent to drip directly onto the response snails. Each pair of stimulus-response containers were then secured with cable ties inside a larger cylindrical chamber (11 cm ht. x 28 cm dia.) having plastic mesh panels on the sides, top, and bottom. The experimental design yields eight treatment

combinations (2 transplant locations x 2 source populations x 2 effluent treatments) and six replicate chambers per treatment combination.

Twenty four chambers were deployed at the northern site and 24 at the southern site (Figure 1). Chambers were anchored to bricks with cable ties at each site in the mid-intertidal zone. Response snails in each container were provided with 30 g (wet mass) of the alga *Ascophyllum nodosum* for food. Snails grow well under these conditions for at least 90 days (Trussell, MSb). A generous amount of *Ascophyllum* was also placed with the large chambers to alleviate potential desiccation stress to crabs during low tides. Chambers were monitored every 21 days to replace stimulus snails (in both Crab and No-Crab treatments) and the food supply for response snails. Measurement of final shell thickness and shell length was made after 90 days of growth in the field. Non-destructive estimates of body and shell mass were also calculated as described above except that new regressions of final actual shell mass (Y) as a function of final submerged mass were determined for each experimental group. I generated these new regressions because experimental treatments can change the relationship between submerged mass and actual shell mass. New regressions were generated with 35 snails randomly sampled from each experimental group by crushing the shell with a C-clamp and separating soft tissue and shell material. Both shell material and tissue were dried in an oven at 60°C for 48 hours and then weighed on a Mettler PG503 (±0.001 g). These new regressions were then applied to each experimental group to estimate actual shell mass and body mass a

described above. Like the initial regressions, the new regressions indicate that submerged mass is a reliable indicator of actual shell mass (all R^2 0.992).

Water temperature was recorded with submersible dataloggers (TidBit, Onset Computer Corporation, Pocasset, Massachusetts) deployed at each site. Loggers were programmed to record temperature once every hour. Monthly mean water temperature calculated from daily values recorded at high tide was used in statistical analyses.

Statistical Analyses

Data from the reciprocal transplant experiment were analyzed with a three-factor, nested analysis of covariance (ANCOVA) that treated Transplant Location, Source Population, and Effluent Treatment as fixed effects. Replicate chambers were nested within each Transplant Location x Source Population x Effluent Treatment combination. Because shell thickness is positively correlated with shell size, I used final shell length as the covariate. Analysis of shell growth in terms of shell length employed the same ANCOVA model with final-initial shell length as the response variable and initial shell length as the covariate. To examine the relationship between shell thickness and shell length growth, the adjusted least squares means generated by the ANCOVA on shell thickness were regressed against the adjusted least squares means generated by the ANCOVA for shell length growth.

To address the potential trade-offs associated with induced increases in shell thickness, similar ANCOVAs were conducted on the ratio of final body mass to shell

mass as the response variable. I chose this approach because I wanted to examine changes in the relative proportions of body and shell mass. Shell length was used as a covariate to adjust for the effects of shell size on this ratio. Body growth relative to shell growth was examined with an ANCOVA that used final body mass/shell mass as the response variable and initial body mass as the covariate to adjust for initial differences in body size among source populations . To examine the relationship between changes in body mass and body growth as a function of changes in shell thickness, the least squares adjusted means generated by the ANCOVA models for body mass and body growth were regressed against shell thickness.

Water temperature data were analyzed with a two way ANOVA that treated month and site as fixed effects. All statistical analyses were conducted using JMP software (Version 3.2.1, SAS Institute Inc., Cary, North Carolina) on a PowerMac 7500/100 and data were $\log_{(10)}$ transformed data when necessary. Because nested replicate tubs were declared a random effect in models, JMP used the Satterthwaite approximation to calculate mean squares, F-ratios, and their respective degrees of freedom. Based on two sets of earlier experiments (Trussell, MSa, b) I am confident that significant differences between northern and southern populations reflect broad scale geographic influences and not differences unique to the two study populations (*sensu* Hurlbert, 1984). Previous work (Trussell, MSa) revealing geographic differences in shell form and strength and shell responses to crab effluent in multiple populations from each

location clearly showed that functional and phenotypic differences *between* northern and southern populations greatly exceed population differences *within* locations.

RESULTS

Geographic Differences in Water Temperature

Despite significant seasonal variation (ANOVA: $F_{(7, 771)} = 923.84$; $p < 0.0001$), there were also significant differences in water temperatures between the two transplant locations (ANOVA: $F_{(7, 771)} = 1{,}003.77$; $p < 0.0001$; Figure 18). *Post hoc* linear contrasts that compared each site for a given month indicated that water temperatures were significantly different among sites for every month except December (all p 0.0001). Water temperatures during the course of the experiment averaged 5.9°C colder at the northern site.

Phenotypic Plasticity in Shell Thickness

ANCOVA revealed that effluent treatment, geographic location and source population all had significant effects on size-adjusted shell thickness (Tables 7-8; Figure 19). Snails from both source populations, regardless of the geographic location in which they were raised, produced significantly thicker shells when raised in the presence of *Carcinus* than conspecifics raised in the absence of *Carcinus*. The average effect of crab effluent across both source populations and transplant locations produced a 15.6% increase in shell thickness. Analysis of the two-way interactions, Treatment x Location and Treatment x Source Population, indicated that overall the response to *Carcinus*

effluent was greatest for snails of northern origin (+24.7% vs.+9.1%) and for snails raised at the northern study site (24.4% vs. 7.5%). However, these trends likely reflect the dramatic response exhibited by northern snails raised at the northern site with and without crabs. For these snails, the presence of crabs induced a 46.3% increase in shell thickness, while the response for other experimental groups was between 6.9-10.6%.

Location effects, regardless of effluent treatment and source population, indicated that snails raised at the southern location produced significantly thicker shells (+14.6%) than snails raised at the northern location (Tables 7-8; Figure 19). Similarly, source population effects indicate that snails originating from the southern population produced significantly thicker shells (+26.4%) than snails originating from the northern population. The significant two-way Location x Population interaction indicated that increases in shell thickness due to location effects were significantly greater for northern snails transplanted to the southern site (+20.5%) than the decrease in shell thickness for southern snails transplanted to the northern site (-9.1%).

Analysis of size-adjusted shell growth in terms of shell length indicated that only effluent treatment, transplant location, and source population had significant effects (Table 9; Figure 20). Regardless of source population and transplant location, snails raised in the presence of *Carcinus* grew less (-23.6%) than snails raised in the absence of *Carcinus*. A significant source population effect indicated that northern snails consistently grew more (+35.1%) than southern snails across both effluent treatments and

transplant locations. Finally, location effects revealed that overall snails grew more (+8.4) at the southern site than at the northern site. Regressions between adjusted least squares means from ANCOVA for final shell thickness (Y) and shell length growth (X) revealed a negative relationship; snails that grew the most were also the thinnest (Figure 21).

Trade-Offs in Body Mass and Body Growth Relative to Shell Mass and Shell Mass Growth

Effluent treatment, transplant location, and source population also had profound effects on allocation to size-adjusted body mass relative to shell mass (Tables 8, 10a; Figure 22a). Snails from both populations, regardless of the location in which they were raised, had less body mass (31.6%) when raised in the presence of *Carcinus* than snails raised in the absence of *Carcinus*. Analysis of two-way interactions revealed patterns similar to those found for shell thickness. Overall, effluent effects were greater at the northern location (-43.8% vs. -20.0%) and for snails originating from the northern population (-42.9% vs.-20.7%). Again these significant interactions likely reflect the large response found for northern snails raised at the northern site snails (-75.9%) versus those for southern snails at the southern (-24.5%) and northern site (-18.0%) and northern snails at the southern site (-16.1%).

Location effects indicated that snails, regardless of effluent treatment and source population, raised at the southern site exhibited significantly greater reductions in body

mass (40.2%) than those raised at the northern site (Tables 8, 10a; Figure 22a). Like the pattern found for shell thickness, the effect of transplant location was significantly greater for northern snails. The reduction in the relative body mass of northern snails transplanted to the southern site (-51.7%) was greater than the increase in relative body mass of southern snails transplanted to the northern site (+30.0%).

The patterns in final size-adjusted body mass relative to shell mass appear to be the direct result of differential size-adjusted body growth due to effluent treatment, transplant location, and source population (Tables 8, 10b; Figure 22b). Regardless of source population, snails raised with *Carcinus* (-62.9%) and those raised at the southern site (-71.7%) grew significantly less than snails raised without *Carcinus* and snails raised at the northern site. Effluent treatment had significantly greater effects at the northern location (-82.6% vs. -34.7%) and for snails originating from the northern population (-70.0% vs. -45.9%). Again, the decrease in tissue growth for northern snails transplanted to the southern site (-78.7%) was significantly greater than the increase in tissue growth for southern snails transplanted to the northern site (+60.7%).

Regressions between adjusted least squares means from ANCOVA for size-adjusted body mass/shell mass (Y) versus size-adjusted shell thickness (X) and size-adjusted body mass/shell mass growth (Y) versus size-adjusted shell thickness (X) indicated negative relationships. Snails having the thickest shells also had the least body mass (Figure 23a) and the least body growth (Figure 23b).

DISCUSSION

Natural selection is thought to drive co-evolution between shell crushing predators and their prey as well as produce historical transitions (Vermeij, 1982; Seeley, 1986) and biogeographic variation in gastropod shell form (Vermeij, 1977, 1978, 1987). However, it is clear that ecophenotypic responses to predator effluent (Appleton & Palmer, 1988; Palmer, 1990; Trussell, 1996, MSa) and water temperature (Graus, 1974; Vermeij, 1978, 1993; Trussell, MSb) also contribute to the production of more robust shell morphologies. Although we recognize the importance of these cues as inducers of phenotypic plasticity, there has been no attempt to address the relative importance of each factor. This deficiency makes explanations of biogeographic patterns in shell form (e.g., temperate vs. tropical) particularly difficult because the effects of predator effluent and increased water temperature can influence shell form in the same manner. Hence, in terms of plasticity, the increased calcification of tropical versus temperate mollusc assemblages may reflect more favorable conditions for calcium carbonate deposition and/or induced responses to effluent associated with the greater abundance and taxonomic diversity of shell crushing predators.

The relatively recent invasion of *Carcinus maenas* into the northern Gulf of Maine and present day latitudinal variation in water temperatures (Figure 18) provide an ideal system to study how water temperature and predator effluent may shape biogeographic patterns in shell form. Previous work (Trussell, 1999b) found a latitudinal

gradient in *Littorina obtusata* shell thickness with northern snails having thinner shells than southern conspecifics. This pattern in shell thickness was consistent with *a priori* expectations; both reduced *Carcinus* abundance (and thus, effluent concentration) and colder water temperatures should promote the development of thinner shells. Trussell (MSb) suggested that while snails from the northern and southern Gulf of Maine are capable of *Carcinus*-induced increases in shell thickness (Trussell, MSa), plastic responses in shell form in response to geographic differences in water temperature were also important. I made this argument because *Carcinus*-induced increases in shell thickness in laboratory experiments (~10% increase) were smaller than those induced by reciprocally transplanting *L. obtusata* between a northern and southern population when water temperatures at the northern location averaged 6.8°C colder. Northern snails transplanted to a southern site produced shells that were 44% thicker than northern snails raised at their native site while southern snails transplanted to the northern site produced shells that were 17% thinner than southern snails raised at their native site.

By raising snails with and without *Carcinus* (effluent effect) in two distinct geographic locations (water temperature effect), this study sought to identify the relative importance of each factor as inducers of plasticity in shell thickness. Regardless of both the population from which snails originated and the geographic location in which they were raised, snails raised in the presence of *Carcinus* produced significantly thicker shells than those raised in the absence of *Carcinus* (Figure 19). In particular, the large

response to effluent exhibited by northern snails raised at their native site illustrates the potential of plasticity to produce dramatic phenotypic change; after only 90 days, snails raised with *Carcinus* produced shells that were 46.3% thicker than those of conspecifics raised without *Carcinus*. This response was considerably greater than that exhibited by southern snails raised at both locations and northern snails raised at the southern location (responses between 6.9-10.6%). These results support previous arguments (Trussell, 1996, MSa) that predator-induced plasticity contributes to historical and biogeographic changes in shell form. Hence, the recent shifts (<100 years) in *L. obtusata* shell form from thin, high-spired morphs to thick, low-spired morphs following *Carcinus*' invasion into the northern Gulf of Maine (Seeley, 1986) may not be an unequivocal example of rapid evolution via natural selection in response to increased *Carcinus* predation.

Are Geographic Effects Indicative of Differences in Water Temperature?

The effects of water temperature on calcium carbonate kinetics dictate that both shell deposition and maintenance should be more difficult in colder waters. In general, the reciprocal transplant revealed variation in shell thickness that was consistent with the anticipated effects of water temperature. Regardless of effluent treatment and source population, snails raised at the northern site produced thinner shells than snails raised at the southern site. Interestingly, the overall effect of geographic location resulted in increases in shell thickness (14.6% greater at the southern site) that were comparable to the overall effects of *Carcinus* effluent (15.6% greater versus controls; Table 8).

Although these patterns are intuitively appealing, caution must be exercised when interpreting location effects as unambiguous evidence of the influence of water temperature. This limitation arises because both transplanted and native snails may be responding to background *Carcinus* effluent. The effect of background effluent is likely more pronounced at the southern site where *Carcinus* density is greater (G.C. Trussell & L.D. Smith, pers. obs.). Comparison of responses to effluent by northern snails at both locations illustrate this point. At the northern site, northern snails raised with *Carcinus* produced shells that were 46.3% thicker than their controls, while at the southern site northern snails produced shells that were 8.3% thicker. Hence, the relative magnitude of differences in shell thickness between effluent treatments decreased dramatically among locations. This relative decrease is likely due to the fact that northern snails raised at the southern site without *Carcinus* produced shells that were 43.3% thicker than northern snails raised at the northern site without *Carcinus*. In addition, northern snails raised at the southern site with *Carcinus* produced shells that were only 6.1% thicker than northern snails raised with *Carcinus* at the northern site. If warmer water temperatures were responsible for the trend of increased thickness at the southern site, then one would expect the shells of northern snails raised with Carcinus to be considerably thicker than those of controls raised at the southern site and those of northern snails raised with crabs at the northern site.

The relatively small difference among experimental effluent treatments at the southern site may therefore reflect the response of controls to background effluent. In contrast, at the northern site it is unlikely that northern snails raised without *Carcinus* are sensing any effluent; hence, the relative magnitude of the response to effluent is greater. This argument suggests that the relative difference in shell thickness among experimental effluent treatments should also be greater for southern snails raised at the northern site. This was not the case because the relative difference among effluent treatments for southern snails at each location was remarkably similar. The consistency in the response of southern snails to effluent treatment at both locations may reflect the evolution of a limited range of flexibility in shell thickness due to their more consistent and prolonged contact with *Carcinus* in the last 100 years. Evidence of reduced flexibility in snails originating from the southern population in response to the overall effects of effluent and transplant location (Table 8) support this assertion.

Future laboratory experiments that eliminate the potentially confounding effects of background effluent should allow the effects of water temperature and predator effluent on shell form to be teased apart more precisely. However, assuming that location effects are due entirely to differences in water temperature, one arrives at the conclusion that the overall effects of predator effluent on shell form are comparable to the effects geographic differences in water temperature (15.6% vs. 14.6%, respectively), which averaged 5.9°C colder at the northern site during the experiment. If true, then arguments

in favor of *Carcinus*-induced plasticity as an explanation of historical and biogeographic variation in *L. obtusata* shell form are even more compelling.

Trade-offs Associated With Induced Increases in Shell Thickness

'True costs' (*sensu* DeWitt et al., 1998) or trade-offs associated with predator-induced defenses are presumed to exist; otherwise, organisms would produce fixed rather than conditional defensive phenotypes (Lively, 1986 b; Stearns, 1989). Understanding the adaptive value of phenotypic plasticity and the constraints on its evolution requires an assessment of the accompanying costs or trade-offs (Stearns, 1989, 1992; DeWitt et al., 1998; Schlichting & Pigliucci, 1988). The nature of these costs may fall into two categories (see Dewitt et al., 1998): (1) 'True' or 'pure' costs and (2) limits to the benefit of plasticity. Both are likely subject to natural selection and instrumental in determining the benefits and evolutionary potential of inducible defenses (Schlichting & Pigliucci, 1998). The distinction between the two is critical, however, because the implications of true costs to the evolution of plasticity over canalized strategies are likely different than those due to limits to plasticity.

Dewitt et al., (1998) define costs of plasticity, such as those required to maintain plasticity machinery or to assess the environment, as "fitness deficits associated with plastic genotypes relative to fixed genotypes producing the same mean phenotype in a focal environment". In contrast, trade-offs associated with inducible-defenses, may reflect limits imposed by plasticity in that allocation of energy to the production of a

defensive trait may come at the expense of other functions. However, trade-offs associated with producing a defensive trait cannot necessarily be viewed as costs of plasticity because a fixed genotype may have to pay the same costs. For example, in the case of shell thickness, the direct costs associated with producing a thicker shell should be the same for a fixed and plastic genotype. Hence, only those production costs for plastic genotypes in excess of those for fixed genotypes should be considered a true cost of plasticity (DeWitt et al., 1998). If one accepts this premise, then the trade-offs complementing the production of thicker shells directly reflects the costs derived from producing more shell material rather than plasticity in shell thickness *per se*. Production costs are still important to determine because they are necessary to determine the net benefit of plasticity; that is, the benefit of not producing a defensive trait when it is unnecessary (DeWitt et al., 1998).

Although trade-offs and costs in the form of reduced fecundity (Lively, 1986 c), growth (Harvell, 1986; DeWitt in press; Trussell, MSa, b) and delayed maturity (Crowl & Covich, 1990) are known to accompany the production of better defended morphologies, this issue requires more attention (DeWitt et al., 1998; Schlichting & Pigliucci, 1998). Three trade-offs associated with predator-induced increases in shell thickness were evident in this study. First, the production of thicker shells by snails raised in the presence of *Carcinus* were accompanied by reductions in shell growth in terms of shell length (Figures 19, 20). Second, snails raised in the presence of *Carcinus* had reduced

body mass and body growth (Figures 22a, b). Regression analysis of both body mass (Figure 23a) and body growth (Figure 23b) as a function of shell thickness revealed significant inverse relationships; the thickest snails had the least body mass and body growth.

Although these trends may manifest themselves due to increased energetic costs incurred by increased shell deposition, this cost is expected to be small relative to other metabolic costs; especially in areas where surface seawater is saturated with calcium carbonate (Palmer, 1981, 1992). Palmer (1981) concluded that non-energetic, 'geometric' costs (the 'skeleton-limitation' hypothesis), best explained reduced body mass and body growth in thick-shelled snails. Because body growth cannot proceed ahead of the protective shell, body mass and growth are limited by the linear rate of shell growth (defined as change in shell length). There is a maximum rate at which calcification can occur (Palmer, 1992). Hence, the more shell material that is devoted to shell thickening the shell, the less that is available for linear growth of the shell (i.e., growth in terms of size). Kemp & Bertness (1984) documented the inverse relationship between shell thickness and linear growth in *Littorina littorea*. Rapidly growing shells were thinner and more globose and, thus, able to accommodate more body growth than slowly growing, thicker shells. This relationship was also apparent in this study (Figure 20) suggesting that constraints on body mass and body growth were imposed by reduced

growth in terms of shell size as well as by increased shell thickening, which limits the internal space of the shell available for body growth.

Growth can influence a number of other life history traits (Stearns, 1989; Stearns & Koella (1986) such as fecundity (Spight & Emlen, 1976) and the size and age of maturity (Crowl & Covich, 1990). In intertidal snails, fecundity is often a function of size (Spight & Emlen, 1976; Palmer, 1983) and faster growing snails are therefore expected to have the potential to produce more offspring at a given age (Etter, 1996). Hence, increased survivorship conferred by plastic increases in shell thickness in response to predators may be at the expense of future reproduction. The existence of these life history trade-offs may explain, in part, why inducible defenses have evolved in *L. obtusata* and other intertidal gastropods (Appleton & Palmer, 1988; Palmer, 1990).

There has been a recent enthusiasm for examples of rapid evolution in natural populations (Reznick, 1997; Svensson, 1997; Losos et al., 1997; Thompson, 1998) and the implications of these studies to the structure of ecological communities and classical Neo-Darwinian theory. Although we can appreciate the often stunning pace of evolution by means of natural selection, a more pluralistic view of evolution must adequately incorporate the dramatic potential of phenotypic plasticity. In particular, interactions between predator and prey are thought to represent powerful arenas of natural selection. And yet many of the best examples of phenotypic plasticity involve inducible defenses against predators across numerous taxa and ecosystems. The ubiquity and often dramatic

magnitude of this phenomenon suggests that evolving reaction norms may play an equally important role in structuring ecological communities and (co-) evolutionary processes.

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Table 7. Nested ANCOVA on Shell Thickness (Y) vs. Shell Length (X) for *Littorina obtusata* reciprocally transplanted between a Northern (Lubec, Maine) and Southern (Manchester, Massachusetts) site and subjected to two effluent treatments ('Crab' and 'No-Crab').

SOURCE	df	F	p
Treatment (Treat):	1, 49	172.79	< 0.0001
Location (Loc):	1, 41	162.58	< 0.0001
Source Population (S. Pop):	1, 57	406.52	< 0.0001
Treat X Loc:	1, 40	34.74	< 0.0001
Treat X S. Pop:	1, 40	23.50	< 0.0001
Loc X S. Pop:	1, 49	9.79	< 0.01
Loc X S. Pop X Treat:	1, 40	22.71	< 0.0001
Repl.{S. Pop, Loc, Treat}:	40, 236	1.98	< 0.001
Slope:	1, 235	0.58	NS

Table 8. Percent changes in shell thickness, body mass/shell mass, and body mass/shell mass growth due to effects used in the ANCOVA models presented in Tables 7, 10 a, b. Note that all main effects and their respective interactions were statistically significant. Treat = Effluent Treatment; Loc=Transplant Location; Pop=Source Population; C=Crab; NC=No-Crab; S=South; N=North.

Effect	Comparison	% Difference		
		Shell Thickness	Body Mass	Body Growth
Treat:	C vs. NC	15.6%	31.6%	62.9%
	Effluent Effect:	(C > NC)	(NC > C)	(NC > C)
Loc:	North vs. South	14.6%	40.2%	71.7%
	Location Effect:	(S > N)	(N > S)	(N > S)
Pop:	North vs. South	26.4%	72.0%	70.6%
	Source Effect:	(S > N)	(N > S)	(N > S)
Treat x Loc:	North/C vs. North/NC	24.4%	43.8%	82.6%
	South/C vs. South/NC	7.5%	20.0%	34.7%
	Effluent Effect:	(N > S)	(N > S)	(N > S)
Treat x Pop:	North/C vs. North/NC	24.7%	42.9%	74.0%
	South/C vs. South/NC	9.1%	20.7%	45.9%
	Effluent Effect:	(N > S)	(N > S)	(N > S)
Loc x Pop:	North/North vs. North/South	20.5%	51.7%	78.7%
	South/South vs. South/North	9.1%	30.0%	60.7%
	Transplant Effect:	(N > S)	(N > S)	(N > S)

TABLE 8 (Continued)

Effect	Comparison	% Difference		
		Shell Thickness	Body Mass	Body Growth
Loc x Pop x Treat:	SS/C vs. SS/NC	6.9%	24.5%	58.9%
	NS/C vs. NS/NC	8.3%	16.1%	20.8%
	SN/C vs. SN/NC	10.6%	18.0%	38.5%
	NN/C vs. NN/NC	46.3%	75.9%	116.8%
	Effluent Effect:	(C > NC)	(NC > C)	(NC > C)

Table 9. Nested ANCOVA on Shell Length Growth (Y) vs. Initial Shell Length (X) for *Littorina obtusata* reciprocally transplanted between a Northern (Lubec, Maine) and Southern (Manchester, Massachusetts) site and subjected to two effluent treatments ('Crab' and 'No-Crab'). Rep=Replicate.

SOURCE	df	F	p
Treatment (Treat):	1, 40	27.55	< 0.0001
Location (Loc):	1, 40	4.15	< 0.05
Population: (Pop):	1, 40	54.39	< 0.0001
Treat X Loc:	1, 40	0.20	NS
Treat X Pop:	1, 40	0.02	NS
Loc X Pop:	1, 40	1.86	NS
Loc X Pop X Treat:	1, 40	0.06	NS
Rep{Pop,Loc,Treat}:	40, 236	3.75	< 0.0001
Slope:	1, 235	0.27	NS

Table 10. Nested ANCOVA for *Littorina obtusata* reciprocally transplanted between a Northern (Lubec, Maine) and Southern (Manchester, Massachusetts) site and subjected to two effluent treatments ('Crab' and 'No-Crab'). (a) $\log_{(10)}$ (Body Mass/Shell Mass) (Y) vs. $\text{Log}_{(10)}$ (Final Shell Length) (X), and (b) Body/Shell Growth (Y) vs. Initial Body Mass (X). Rep=Replicate.

(a)

SOURCE	df	F	p
Treatment (Treat):	1, 47	170.75	< 0.0001
Location (Loc):	1, 41	278.65	< 0.0001
Population (Pop):	1, 52	629.51	< 0.0001
Treat X Loc:	1, 40	20.46	< 0.0001
Treat X Pop:	1, 40	17.00	< 0.001
Loc X Pop:	1, 40	14.51	< 0.001
Loc X Pop X Treat:	1, 40	33.01	< 0.0001
Rep{Pop, Loc, Treat}:	40, 236	2.56	< 0.0001
Slope:	1, 235	0.34	NS

(b)

SOURCE	df	F	p
Treatment (Treat):	1, 40	139.25	< 0.0001
Location (Loc):	1, 40	168.62	< 0.0001
Population (Pop):	1, 40	60.19	< 0.0001
Treat X Loc:	1, 40	43.88	< 0.0001
Treat X Pop:	1, 40	23.67	< 0.0001
Loc X Pop:	1, 49	19.89	< 0.0001
Loc X Pop X Treat:	1, 40	35.01	< 0.0001
Rep{Pop,Loc,Treat}:	40, 236	2.63	< 0.0001
Slope:	1, 235	0.15	NS

Figure 17: Map of the Gulf of Maine showing the northward progress of *Carcinus maenas*' biogeographic range expansion from 1900 to present day (based on Scattergood (1952) and Vermeij, 1978). Also shown are the locations of the two study areas in the southern (Manchester, Massachusetts) and northern (Lubec, Maine) Gulf of Maine.

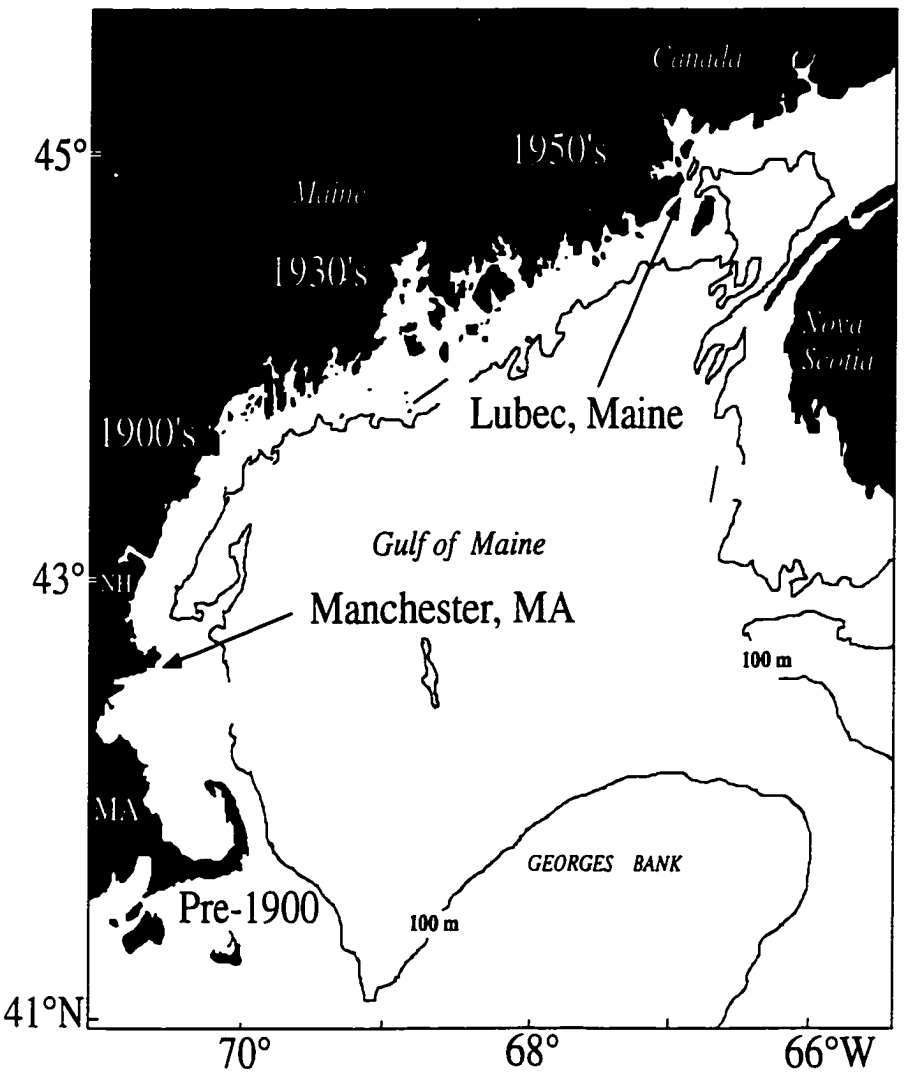

Figure 18. Mean (±SE) monthly water temperature at the two sites used in the reciprocal transplant experiment. "Experimental Period" refers to the time during which the experiment was conducted. Water temperatures averaged 5.9°C colder at the northern site during the experiment. See Results for statistical analyses.

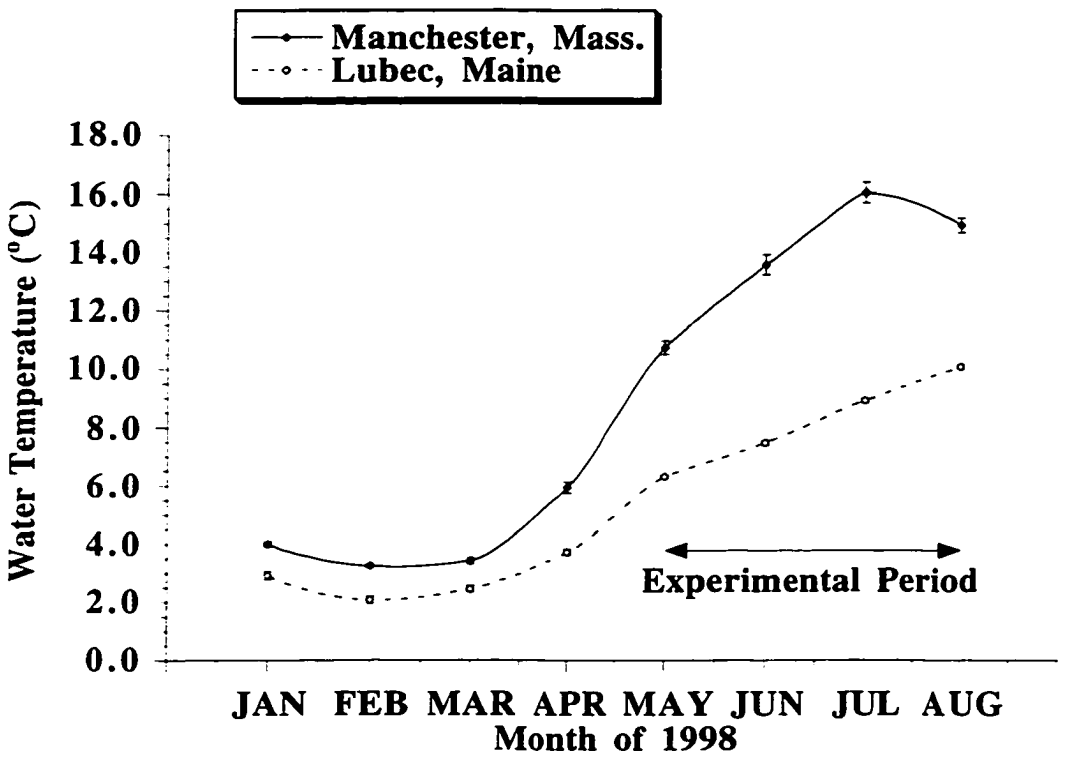

Figure 19. Adjusted mean shell thickness (±SE) from ANCOVA for *Littorina obtusata* reciprocally transplanted between a northern and southern site in the Gulf of Maine and subjected to two effluent treatments ('Crab" and 'No-Crab'). NN = North to North; NS = North to South; SS = South to South; SN = South to North; C = Crab; NC = No-Crab. See Table 7 for details of ANCOVA and Table 8 for a summary of percent changes in shell thickness.

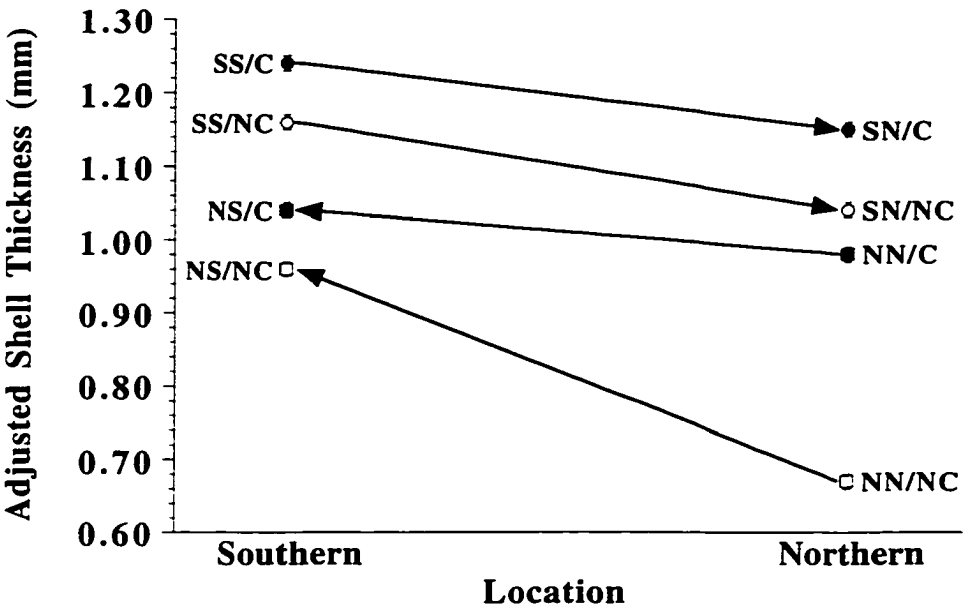

Figure 20. Adjusted mean shell length growth (±SE) from ANCOVA for *Littorina obtusata* reciprocally transplanted between a northern and southern site in the Gulf of Maine and subjected to two effluent treatments ('Crab" and 'No-Crab'). NN = North to North; NS = North to South; SS = South to South; SN = South to North; C = Crab; NC = No-Crab. See Table 9 for details of ANCOVA.

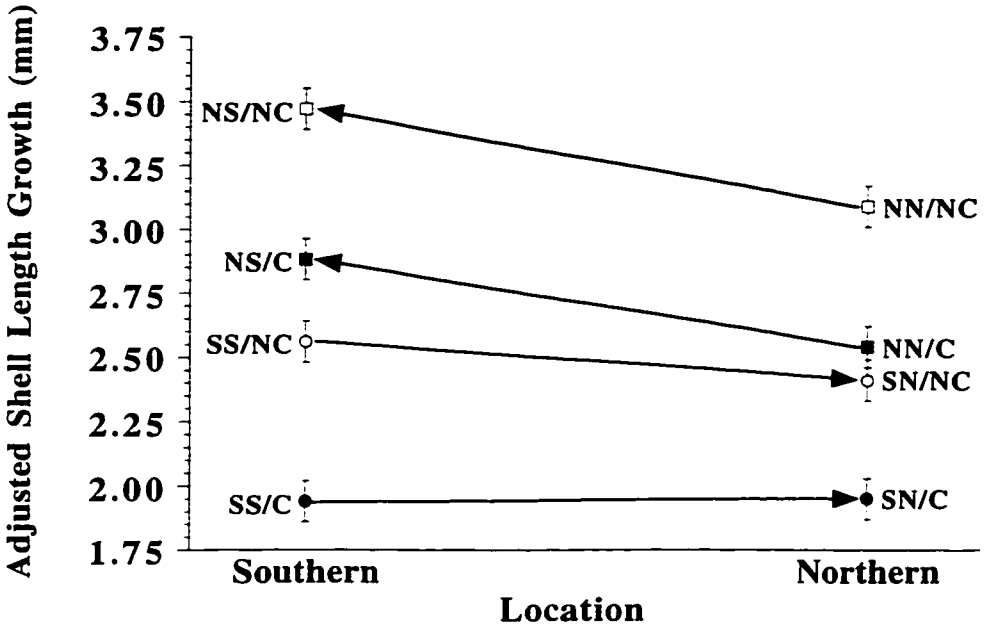

Figure 21. Adjusted mean (±SE) shell thickness (Y) as a function of adjusted mean (±SE) shell length growth (X) from ANCOVA for *Littorina obtusata* reciprocally transplanted between a northern and southern site in the Gulf of Maine and subjected to two effluent treatments ('Crab" and 'No-Crab'). There is a significant inverse relationship between shell thickness and shell length growth ($Y = -0.23X + 1.63$, $R^2 = 0.50$; $F_{(1, 6)} = 5.93$, $p = 0.0508$). Group codes as in Figure 19.

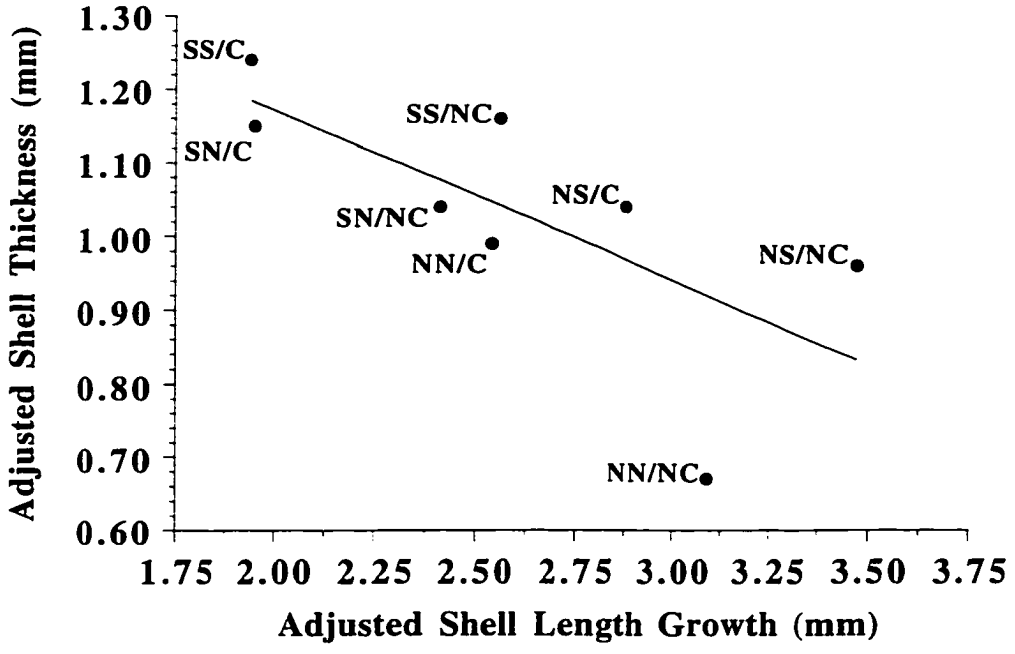

Figure 22. (a) Adjusted mean (±SE)body mass/shell mass and (b) adjusted mean (±SE) body/shell mass growth from ANCOVA for *Littorina obtusata* reciprocally transplanted between a northern and southern site in the Gulf of Maine and subjected to two effluent treatments ('Crab" and 'No Crab'). Group codes as in Figure 19. See Tables 10a, b for details of ANCOVA and Table 8 for a summary of percent changes in body/shell mass and body/shell mass growth.

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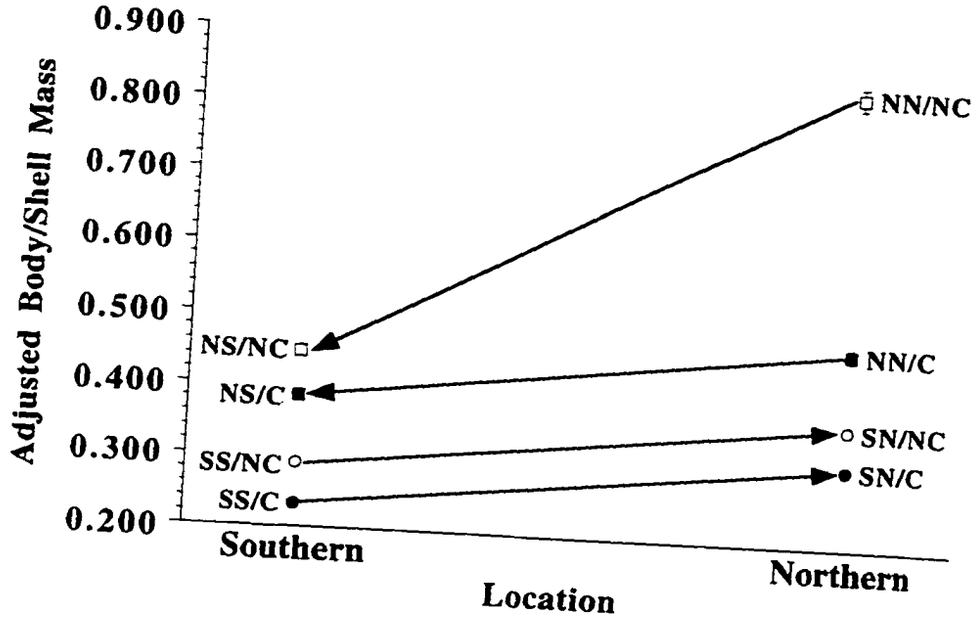
a)



b)

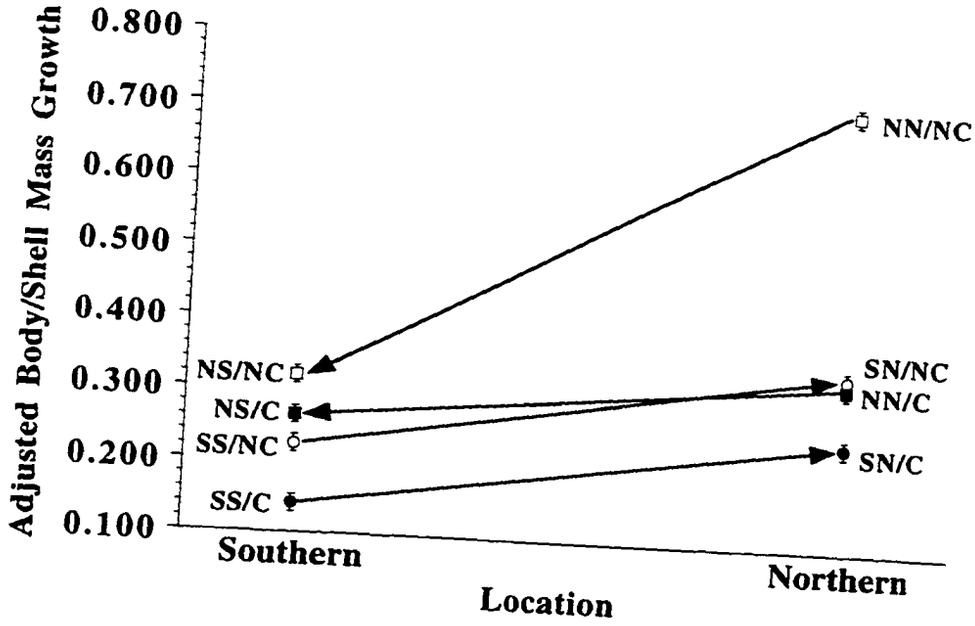
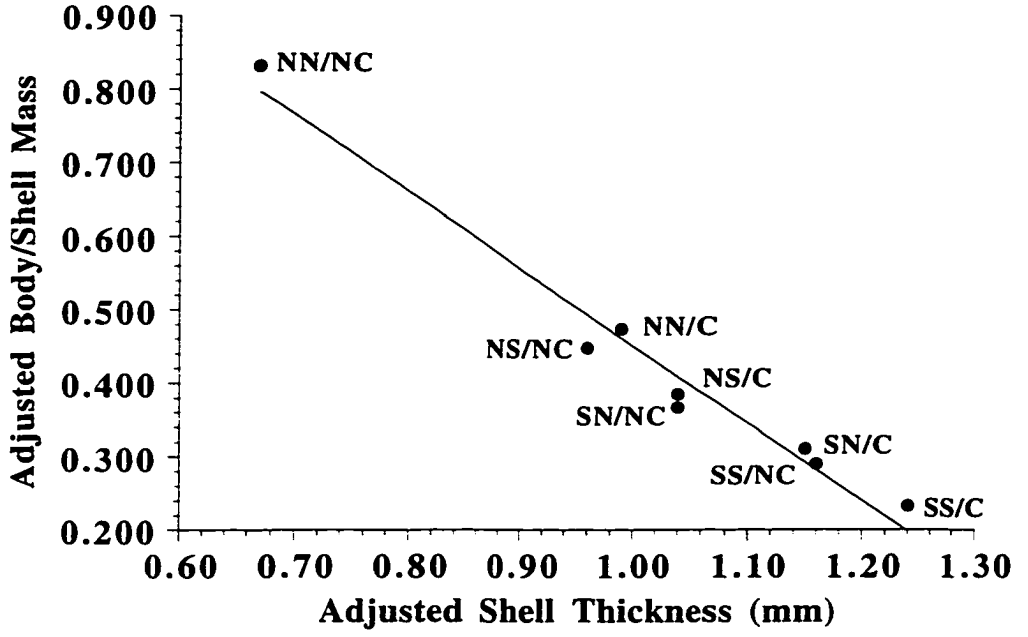
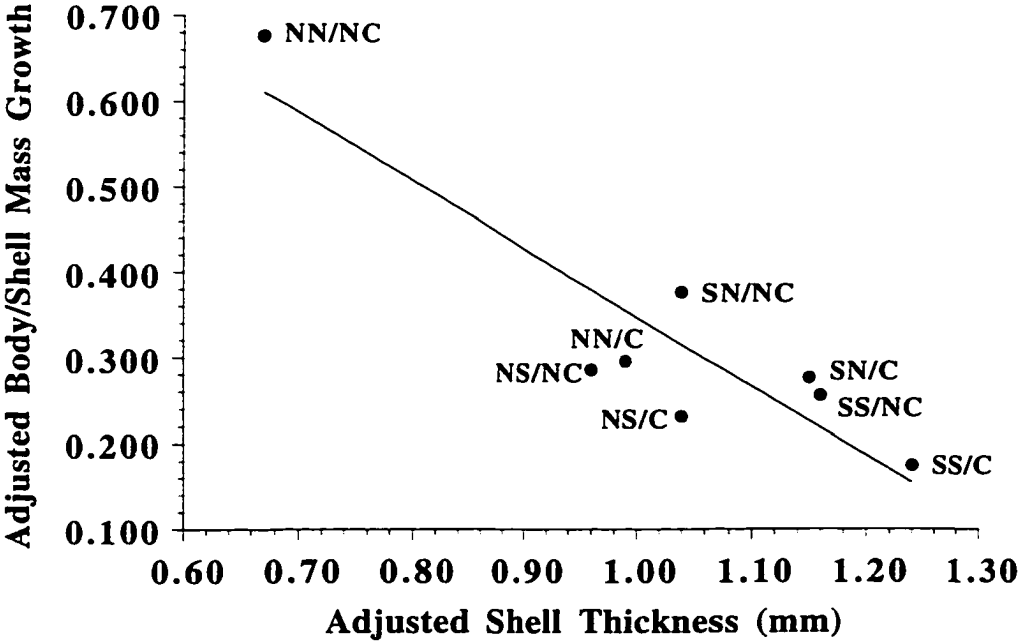


Figure 23. (a) Adjusted mean (±SE) body mass/shell mass (Y) as a function of adjusted mean (±SE) shell thickness (X) and (b) adjusted mean (±SE) body/shell mass growth (Y) as a function of adjusted mean (±SE) shell thickness (X) from ANCOVA for *Littorina obtusata* reciprocally transplanted between a northern and southern site in the Gulf of Maine and subjected to two effluent treatments ('Crab" and 'No-Crab'). Group codes as in Figure 19. Both body/shell mass ($Y = -1.05X + 1.50$, $R^2 = 0.97$; $F_{(1, 6)} = 192.56$, $p < 0.0001$) and body/shell mass growth ($Y = -0.95X + 1.30$, $R^2 = 0.93$; $F_{(1, 6)} = 82.41$, $p = 0.0001$) are inversely related to shell thickness.

a)



b)



CHAPTER 4:

EVIDENCE OF CO- AND COUNTERGRADIENT VARIATION IN THE GROWTH OF INTERTIDAL SNAILS IN RESPONSE TO INCREASED FLOW AND WAVE ENERGIES

ABSTRACT

Growth is an important component of an organism's life history. For snails inhabiting intertidal shores, growth is known to vary across habitats exposed to different wave energies. While habitat-specific variation in gastropod growth is known to have both genetic and plastic bases, little is known about how the influence of these factors covary across different environments. Here I present the results of two experiments designed to test the effects of flow and wave energy on the growth of two intertidal gastropods, *Littorina obtusata* and *Nucella lapillus*. A laboratory flume experiment tested the effects of flow velocity on the growth of a wave-exposed and protected population of *L. obtusata* while reciprocal transplant experiments between a wave-exposed and protected shore using both juvenile and adult whelks tested the effect of different wave energies on the growth of *N. lapillus*. For all measures of growth, *L. obtusata* exhibited a countergradient variation in growth. Wave-exposed snails raised in low flow exhibited increased growth relative to wave-exposed snails raised in high flow, while protected shore snails raised in high flow exhibited reduced growth relative to protected snails raised in low flow. The growth of wave-exposed snails in low flow exceeded that of all other experimental groups. Growth of juvenile and adult *N. Lapillus* produced contrasting results. Juvenile whelks exhibited a cogradient pattern in growth with protected shore whelks raised on the protected shore growing more than both protected and wave-exposed whelks raised on the wave-exposed shore. In contrast, differences in the growth of adult whelks raised on their native shores were not as dramatic as those for juvenile whelks raised on their native shores. In addition, wave-exposed adult whelks grew more than protected whelks transplanted to the wave-exposed shore and showed a trend for more rapid growth than protected whelks raised on the protected shore. These results suggest that countergradient variation in the growth of *L. obtusata* may reflect selection for genotypes conducive to more rapid growth on wave-exposed shores to offset the limitations on foraging time imposed by increased hydrodynamic stress. This argument may also apply to the countergradient pattern found for adult *Nucella*, but the cogradient pattern found for juvenile whelks suggest that other factors, such as increased predation on protected shores, may also influence habitat-specific variation in growth. The ontogenetic shift in patterns of growth for *Nucella* across habitats having different wave energies likely reflects ontogenetic shifts in the importance of predation as a selective agent on growth.

While most, if not all, examples of countergradient variation in growth of gastropods have involved temperature effects on latitudinally separated populations, this study provides evidence that countergradient variation in growth can occur on localized scales in response to other environmental cues. Moreover, factors other than

environmental characteristics such as ontogeny, likely contribute to the shaping of patterns of variation in growth across intertidal habitats.

INTRODUCTION

Understanding the genetic and environmental basis of phenotypic variation has been a major focus of evolutionary ecology. This approach has been instrumental in improving our understanding of adaptation and phenotypic evolution, but we are still limited in our knowledge of how genetic and environmental influences act to produce patterns of phenotypic variation across environmental gradients. Recently, Conover and Schultz (1995) suggested that attention to the nature of the covariance relationship between genetic and environmental influences, and the consequent effects on phenotypic expression, may provide a more lucid understanding of how patterns of phenotypic variation emerge.

When both genetic and environmental influences affect phenotypic expression, two patterns are likely to emerge: cogradient or countergradient variation. With cogradient variation, selection and plasticity act in the same direction on phenotypic values. Hence, the covariance relationship between genetic and environmental influences is positive. In this scenario (also termed synergistic selection; Falconer, 1989), phenotypic differentiation is promoted in native phenotypes across environmental gradients (N_1 vs. N_2 in Figure 24a) and phenotypes of transplanted organisms converge towards a similar phenotypic value (T_1 vs. T_2 in Figure 24a). In contrast, countergradient variation occurs when selection and plasticity act on phenotypic values in opposition; the covariance relationship between genetic and environmental influences is thus negative.

In this scenario (also termed antagonistic selection; Falconer, 1989), little or no phenotypic differentiation occurs in native phenotypes across environments (N_1 vs. N_2 in Figure 24b), while phenotypic values of transplanted organisms diverge from one another (T_1 vs. T_2 in Figure 24b).

Most examples of countergradient variation have involved growth of organisms having wide altitudinal (Levins, 1969; Berven 1982 a, b; Berven et al., 1979) or latitudinal distributions (Dehnel, 1955; Ament, 1979; Conover & Present, 1990; Conover & Schultz, 1995) with dramatic temperature gradients. That temperature differences affect growth is not surprising (Cossins & Bowler, 1987); in general, reduced temperatures are expected to suppress growth (Clarke, 1987; Atkinson, 1994). However, without the aid of reciprocal transplant or common garden experiments, the importance of genetic and environmental influences and their underlying covariance relationship to variation in growth remains unclear.

For example, in his study of body size in *Drosophila melanogaster*, Levins (1969) found that natural phenotypic patterns in body size conflicted with genetic differences among populations. While there was little change in body size among coastal and montane (1000 m) populations, coastal flies produced larger body sizes than montane flies under similar conditions in the laboratory. This pattern emerged because plastic and genetic responses to temperature differences acted in opposite directions on body size. At coastal locations, the environmental effects of increased temperature on development

tend to reduce body size while selection for increased resistance to desiccation stress favors larger body sizes. Conversely, in montane locations, plastic development in response to reduced temperatures yields larger body sizes while selection due to desiccation stress is expected to be negligible. Clearly, an appreciation of the covariance relationship between genetic and environmental influences in this and other studies (see Conover and Schultz, 1995) allows a more complete understanding of why patterns of phenotypic differentiation and/or similarity emerge across different environments.

Levinton (1983) proposed the latitudinal compensation hypothesis to explain comparable growth rates between high and low latitude organisms. Within a species, individuals from high latitude populations have evolved the ability to grow more rapidly than low latitude individuals at reduced temperatures, while low latitude individuals grow more rapidly than high latitude individuals at increased temperatures. While data support this model (Levinton, 1983; Levinton & Monahan, 1983; Lonsdale & Levinton, 1985), they cannot explain the more rapid growth of high latitude vs. low latitude individuals at temperatures typical of low latitude environments (Conover & Present, 1990; Conover & Schultz, 1995).

Intertidal snails exhibit remarkable morphological variation on both micro- and macro-geographic scales (Kitching et al., 1966; Vermeij, 1978; Johanesson, 1986; Trussell et al., 1993). Considerable evidence indicates that these patterns can reflect both selection (Kitching et al., 1966; Struthsaker, 1968; Trussell, 1997) and plasticity

(Appleton & Palmer, 1988; Etter, 1988a; Trussell, 1996, 1997, MS a, b). Although not recognized as such, evidence of countergradient variation in the growth of latitudinally separated populations also exists (Dehnel, 1955; Ament, 1979; but see Parsons, 1997; Trussell, MS a, b). As with other taxa (see Conover & Schultz, 1995), this phenomenon appears to be tied to latitudinal differences in temperature. However, the presence of countergradient variation on more localized scales in response to different environmental characteristics has not been addressed.

This study examines variation in the growth of two intertidal gastropod species raised under different flow conditions in the laboratory and field. Both co- and countergradient variation in growth can occur across high and low flow environments, suggesting that countergradient variation in growth may evolve on micro-geographic spatial scales and in response to environmental factors other than temperature, more work is needed to understand under what conditions countergradient variation emerges.

MATERIALS AND METHODS

Laboratory Flume Experiment with Littorina obtusata

To examine how source population and flow velocity influence variation in the growth of *Littorina obtusata*, snails from a wave-exposed (East Point, Nahant, MA) and a protected (Lobster Cove, Manchester, MA) shore were raised under high- and low-velocity flow in experimental flumes. In early June 1998, snails were collected from each shore and tagged (see Trussell, 1997). Twenty snails from each shore were placed

in four replicate high-velocity (HV) and four replicate low-velocity (LV) flumes constructed from acrylic tubes connected with PVC pipe to a reservoir containing seawater (see Figure 2 in Trussell, 1997). Tube length was 0.81 m with an internal diameter of 58.5 mm. Water flow through tubes was gravity-driven, and high- and low-velocities were generated by different heights of the water reservoir: high-velocity reservoirs 1.45 m and low-velocity reservoirs 0.35 m above the flume. The frequency of wave events was determined by an electronic timer that opened and closed motorized solenoid ball valves. To avoid overheating the valves, snails were exposed to a wave every 2 minutes.

Feeding snails during the course of the experiment required periods of reduced water velocities in the high-velocity flumes because high flow prevented placement of food within the flumes. Hence, the experiment had feeding periods and wave periods that were alternated every 5 6 days. During feeding periods, 200 g wet mass of the alga *Ascophyllum nodosum* were placed in each flume. Periods of high tide and low tide occurred throughout the experiment by changing the fitting on the end of each flume. During high tide an inverted PVC trap prevented tubes from draining; this trap was removed during low tide, and flumes were able to drain after each wave event. For logistical reasons, snails were exposed to only one low tide (6-8 hours in duration) per day.

Flow velocities for each treatment were calculated as described in Trussell (1997). The mean flow velocities for each treatment were 1.70 m/s in high velocity flumes and 0.60 m/s in low velocity flumes. During feeding periods, flow velocities in each treatment were 0.66 m/s in high velocity flumes and 0.60 m/s in low velocity flumes.

Reciprocal Transplant Experiment in the Field with Nucella lapillus

Two separate reciprocal transplant experiments also were conducted in the field with *Nucella lapillus* to examine the effect of different wave energies on growth. The first experiment used juvenile whelks that were collected from an exposed (No Name Point, Nahant, MA) and protected (Mackerel Cove, Beverly, MA) shore in May 1985. Each snail was then tagged and measured for initial shell length, which was the maximum distance between the shell apex and the tip of the siphonal canal. Collection, measurement, and placement of adult whelks utilizing the same sites were delayed until June to limit unnatural gene flow among wave-exposed and protected populations (Etter, 1996). Size categories for juveniles and adults were based on previous knowledge of the size at which whelks begin to mature (Etter, 1989).

After marking, approximately two-thirds of the wave-exposed snails were returned (= controls) to a separate wave-exposed shore (Bennet Head, Nahant, MA) and the remaining third transplanted (= transplants) to a separate protected shore at Mackerel Cove. Protected whelks were treated in the same manner. Instead of using the typical reciprocal transplant protocol, in which half of each group is placed on each shore,

slightly more were used at the wave-exposed shore to offset the higher mortality rates suffered by whelks at this site (Etter, 1989). The exception to this protocol was for wave-exposed juveniles, which were equally split between the wave-exposed and protected shore. I used this protocol because the mortality rates of wave exposed juveniles were expected to be higher due to their thin, pigmented shells that likely increase their vulnerability to increase desiccation stress (Etter, 1988b) and more intense predation (Kitching et al., 1966; Menge, 1983) on protected shores. A quantity of whelk biomass similar to the biomass released was removed from each site to prevent increasing biomass. All tagged whelks were recollected in October 1985 and their final shell length measured.

Statistical Analyses

All analyses were conducted using JMP software (Version 3.2.1, SAS Institute, Cary, North Carolina) on a PowerMac 7500/100. Data were $\log_{(10)}$ transformed when necessary to homogenize variances. Data from the flume experiment were analyzed with a three-factor, nested analysis of covariance (ANCOVA). Both flow treatment and source population were treated as fixed effects and replicate flumes were treated as a random effect nested within flow. Because I was interested in whether variation due to flow treatment and source population was significant relative to that among replicate flumes, the mean squares for these effects were tested against the mean square for nested replicates. Analyses of growth in terms of shell length, shell mass, and body mass were

conducted by using the final value for each trait as the response variable and the initial value of each trait as the covariate. Slopes in all cases were homogeneous (p 0.20) and therefore pooled before final analysis. Growth of whelks from the reciprocal transplant experiment in the field was analyzed with a two factor ANCOVA that treated source population and transplant location as fixed effects. In this analysis, final shell length was treated as the response variable and initial shell length as the covariate. Slopes in both analyses were homogeneous (all $p > 0.05$).

RESULTS

Laboratory Flume Experiment

For *Littorina obtusata*, all measures of growth reflected countergradient variation in growth. For growth in terms of shell length, ANCOVA revealed significant effects of both flow treatment and source population (Table 11; Figure 25). Snails from both populations raised in low velocity flumes grew significantly more than snails raised in high-velocity flumes. In addition, snails collected from the wave-exposed population grew significantly more in both flow treatments than conspecifics collected from the protected shore. The lack of a significant Flow * Population interaction indicated that snails from both populations responded similarly to flow treatment.

Growth in shell mass (Table 12; Figure 26) and total mass (Table 13; Figure 27) mirrored those found for shell length. Snails from both populations grew more in low-velocity flow, and wave-exposed snails grew significantly more than protected snails in

both flow treatments. Differences in growth among flow treatments were again similar for both populations.

Body mass growth differed from the patterns for shell length and shell mass. In this case, only flow treatment significantly affected growth; snails from both populations grew more when raised in low-velocity flow (Table 14; Figure 28). However, I was unable to detect significant variation in growth associated with source population and the lack of a Flow * Population interaction indicated that snails from both sites responded similarly to flow treatments.

Reciprocal Transplant Experiment

Growth of *Nucella lapillus* in the field exhibited somewhat different results. For juvenile whelks, ANCOVA indicated significant effects of source population and transplant location; overall, whelks from both source populations grew more when raised on the protected shore (Table 15a; Figure 29). There was also a significant source population effect, but the presence of a significant Population * Location interaction indicated that population effects on growth were not consistent across habitats; although protected snails raised at the native site (PP) grew more than wave-exposed transplants (EP), there were no differences in growth at the wave-exposed site (PE vs. EE). Juvenile growth was consistent with cogradient variation because there were (1) pronounced differences in growth among whelks raised in their native habitats (PP vs. EE), and (2) the growth of transplants shifted towards that exhibited by native phenotypes (EP & PE).

Adult whelks exhibited growth patterns that contrasted with those of juveniles. Adult whelks, regardless of source population, showed greater growth at the protected site, but the pattern of growth appears to be more consistent with countergradient variation (Table 15b; Figure 30). The difference in growth among whelks in their native habitats (PP vs. EE) was relatively small, and there was a trend for wave-exposed whelks to grow more than protected whelks in each environment. This difference was only apparent at the wave-exposed site, but there was a trend for more rapid growth of wave-exposed whelks compared to protected whelks at the protected site (EP vs. PP).

DISCUSSION

Intraspecific variation in growth can have profound consequences to other life history traits such as the age and size of maturity (Stearns & Koella, 1986; Stearns, 1992). Although understanding the role of genetic and environmental influences on variation in growth is important, knowing how genetic and environmental influences covary across different environments may help improve our understanding of how intraspecific differences in life histories evolve. For gastropods living on rocky intertidal shores, spatial variation in wave energies is thought to be particularly important in driving intraspecific variation in both morphological (Kitching et al., 1966; Etter, 1988a; Trussell, 1996, 1997 a, b; Trussell et al., 1993) and life history traits such as growth (Hughes, 1972; Janson, 1982; Brown & Quinn, 1988; Etter, 1996), fecundity and size at maturity (Etter, 1989). In addition, morphological and life history variation across

gradients in wave energies appear to have both genetic and plastic bases (Janson, 1982; Brown & Quinn, 1988; Etter, 1988a, 1996; Trussell, 1996, 1997a). However, the extent to which selection and plasticity act in concert to produce these patterns is poorly understood. The consequences of environmentally induced variation in growth for other life history traits may be determined, in part, by whether plasticity in growth is reinforced (cogradient) or opposed (countergradient) by the prevailing selection regime.

The influence of hydrodynamic forces imparted by breaking waves on several gastropod traits such as size, morphology and adhesive ability (Denny et al., 1985; Etter, 1988a) are likely mediated by differences in the risk of dislodgment on wave-exposed versus protected shores. This risk is expected to be greater on wave-exposed than protected shores because water accelerations and velocities are greater (Denny, 1985, 1988; Denny et al., 1985; Denny & Gaines, 1988). Reducing this risk is therefore paramount on wave-exposed shores because dislodgment can reduce foraging time (Denny et al., 1985; Judge, 1988) or sweep snails into atypical habitats (e.g., the subtidal zone) having more diverse predator assemblages (Sebens, 1981; Etter, 1988a). Because snails on wave-exposed shores must restrict their foraging activity to periods when it is mechanically safe to do so, the amount of time they are able to forage is likely less than that for conspecifics inhabiting protected shores. This constraint is probably further compounded by the fact that snail tenacity is reduced when snails are crawling

versus remaining stationary (Miller, 1974)-- a behavioral response during periods of increased wave energies.

For *Littorina obtusata* my results indicate that the pattern of growth across different flow environments reflect countergradient variation (Figure 1b). For all measures of growth, snails from the wave-exposed shore grew better than protected shore snails in both experimental flow environments (Figures 25-28). This pattern emerged because wave-exposed snails raised in low flow grew more than wave-exposed snails raised in high flow and protected snails raised in low flow. In contrast, protected snails raised in high flow grew less than protected snails raised in low flow and wave-exposed snails raised in high flow. We believe that countergradient variation in the growth of *Littorina obtusata* may reflect a genetic capacity for increased growth in wave-exposed snails that has evolved to offset the environmental constraints imposed by increased hydrodynamic stress on feeding time. By raising wave-exposed snails in an environment more favorable to growth (i.e., reduced flow velocities and accelerations) this genetic potential can be fully realized; for all measures of growth, wave-exposed snails raised in low flow grew the most of the four experimental groups.

Although not identified as such, a reciprocal transplant experiment with three gastropod species between a wave exposed and protected shore in the California Pacific also yielded evidence of countergradient variation in growth (see Brown & Quinn, 1988). Again, differences in growth between conspecifics raised on their native shores were

considerably less than those revealed after transplanting. This pattern was most striking for *Nucella emarginata*, but qualitatively similar results were also found for *Collisella digitalis* and *Collisella scabra*. Like several other studies (Roberts & Hughes, 1980; Menge, 1978; Hughes & Drewett, 1985; Burrows & Hughes, 1989), Brown & Quinn (1988) argued that reduced growth on wave-exposed shores may reflect restrictions on feeding time imposed by breaking waves. The argument that reductions in foraging time translate into reduced growth assumes that food quality and availability and assimilation efficiencies are similar among wave-exposed and protected environments. Although more work in this area is needed, Etter (1996) found that experimental inhibition of feeding for *N. lapillus* resulted in reduced growth rates. Hence, these results support the hypothesis that reduced growth rates on wave-exposed shores may partly reflect the consequences of reduced feeding time imposed by increased hydrodynamic stress.

Although the results for *Littorina obtusata* are consistent with the arguments above, the contrasting results yielded by *Nucella lapillus* in the field suggest that factors other than wave energies are shaping variation in growth. For example, predation by crabs on intertidal snails is thought to be more pronounced on protected shores because turbulence limits crab foraging on wave-swept shores (Kitching et al., 1966; Crothers, 1968; Menge, 1978). The thicker shells typical of protected morphs are important in deterring crab predators such as *Carcinus maenas* (Kitching et al., 1966; Palmer, 1985), but increased shell size also may limit the effectiveness of crab predation (Reimchen,

1982; Kaiser et al., 1993). Hence, more rapid growth by snails on protected shores may reduce the time required to reach a size refuge from predation. This scenario may explain the more rapid growth of protected juvenile whelks raised on the protected shore (PP; Figure 29). Wave-exposed whelks transplanted to the protected shore (EP) also increased their growth rates relative to EE whelks, but this growth did not exceed that of PP whelks. The cogradient pattern I found may reflect selection by crab predators for more rapid growth in juvenile whelks on protected shores, and this pressure may be more intense than that imposed by increased wave energies on exposed shores.

In contrast to the pattern found for juveniles, adult whelks exhibited a countergradient pattern in growth (Figure 30). The difference in growth between PP and EE adults was substantially less than that for juvenile PP and EE whelks. Also contrasting with the results for juveniles was the greater growth of EE whelks versus PE whelks and the trend of more rapid growth of EP whelks versus PP whelks. These results suggest that selection on growth rate by predation may be less important during later stages of ontogeny. Hence, once whelks native to the protected shore reach a critical size that provides a refuge from predation, energy can be channeled into reproduction in lieu of future growth. Although there appears to be an ontogenetic shift in the importance of selection imposed by predators on the growth of protected shore whelks, selection imposed by wave energies may continue to operate through adulthood. Indeed, the

increased growth of EP whelks versus EE whelks suggests that an asymptotic size was not yet reached.

Given the greater mortality of *Nucella lapillus* on wave-exposed shores (Etter, 1989) and that fecundity is often a positive function of snail size (Spight & Emlen, 1976; Palmer, 1983), adult wave-exposed whelks may continue to grow as quickly as possible within the constraints imposed by hydrodynamic forces. While the risk of dislodgment due to drag and acceleration reaction forces is likely independent of size (Denny et al., 1985), eventually growth will be limited by acceleration reaction forces. However, the size at which acceleration reaction forces become important to dislodging *N. lapillus* remains unknown. Despite these eventual constraints, adult wave-exposed whelks appear to posses the capacity for continued growth, and this capacity is likely maintained by selection. Moreover, the ontogenetic shifts in the importance of different selection pressures across intertidal environments may explain the presence of cogradient growth in juvenile whelks and countergradient growth in adult whelks.

Although most examples of countergradient variation in marine gastropods have involved the effects of temperature on growth in latitudinally separated populations, this study demonstrates that this phenomenon can occur on local scales and in response to other environmental factors. However, the contrasting results of this study and others documenting both countergradient (Brown & Quinn, 1988) and cogradient (Janson, 1982) patterns in growth preclude generalities regarding how selection and plasticity may shape

variation in growth across different flow environments. Clearly, attention to factors other than those that characterize the intertidal environment, such as how the relative importance of selection pressures change through ontogeny, may improve our understanding of the processes shaping habitat-specific differences in growth of gastropods on intertidal shores.

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Table 11. Results of ANCOVA on Final Shell Length (Y) vs. Initial Shell Length (X) for *Littorina obtusata* from a wave-exposed (East Point, Nahant, MA) and protected shore (Lobster Cove, Manchester, MA) raised under high- and low-velocity flow. Slopes were homogeneous ($p > 0.60$) and thus pooled before final analysis.

SOURCE	df	MS	F	p	Interpretation
Flow (F):	1, 6	1.79	21.60	< 0.01	Low > High
Population (P):	1, 6	6.38	77.07	< 0.001	Exposed > Protected
F * P:	1, 6	0.19	2.32	NS	
Replicate{Flow}:	6, 290	0.50	2.72	< 0.05	
Slope:	1, 289	0.01	0.19	NS	

Table 12. Results of ANCOVA on Final Shell Mass (Y) vs. Initial Shell Mass (X) for *Littorina obtusata* from a wave-exposed (East Point, Nahant, MA) and protected shore (Lobster Cove, Manchester, MA) raised under high- and low-velocity flow. Slopes were homogeneous ($p > 0.60$) and thus pooled before final analysis.

SOURCE	df	MS ($\times 10^{-4}$)	F	p	Interpretation
Flow (F):	1, 6	6.27	13.74	< 0.02	Low > High
Population (P):	1, 6	9.72	21.30	< 0.01	Exposed > Protected
F * P:	1, 6	1.02	0.02	NS	
Replicate{Flow}:	6, 290	2.74	3.24	< 0.01	
Slope:	1, 289	3.86	0.27	NS	

Table 13. Results of ANCOVA on Final Total Mass (Y) vs. Initial Total Mass (X) for *Littorina obtusata* from a wave-exposed (East Point, Nahant, MA) and protected shore (Lobster Cove, Manchester, MA) raised under high- and low-velocity flow. Slopes were homogeneous ($p > 0.60$) and thus pooled before final analysis.

SOURCE	df	MS	F	p	Interpretation
Flow (F):	1, 6	1.34 x 10-3	27.68	< 0.005	Low > High
Population (P):	1, 6	8.23 x 10-4	16.95	< 0.02	Exposed > Protected
F * P:	1, 6	2.45 x 10-5	0.50	NS	
Replicate{Flow}:	6, 290	2.91 x 10-4	2.05	= 0.06	
Slope:	1, 289	5.81 x 10-6	0.24	NS	

Table 14. Results of ANCOVA on Final Body Mass (Y) vs. Initial Body Mass (X) for *Littorina obtusata* from a wave-exposed (East Point, Nahant, MA) and protected shore (Lobster Cove, Manchester, MA) raised under high- and low-velocity flow. Slopes were homogeneous ($p > 0.20$) and thus pooled before final analysis.

SOURCE	df	MS	F	p	Interpretation
Flow (F):	1, 6	4.20×10^{-4}	12.52	< 0.05	Low > High
Population (P):	1, 6	4.44×10^{-5}	1.32	NS	Exposed > Protected
F * P:	1, 6	1.78×10^{-5}	0.53	NS	
Replicate{Flow}:	6, 290	2.01×10^{-4}	3.41	< 0.01	
Slope:	1, 289	1.53×10^{-5}	1.56	NS	

Table 15. Results of ANCOVA on Final Shell Length (Y) vs. Initial Shell Length (X) for (a) juvenile and (b) adult *Nucella lapillus* reciprocally transplanted between a wave-exposed (Bennet Head, Nahant, MA) and a protected shore (Mackerel Cove, Beverly, MA). P = Protected, E = Wave exposed. Orthogonal linear contrasts (indicated in boldface) of protected and wave-exposed whelks raised on their native shore (PP & EE) with their respective transplant groups (PE & EP) revealed highly significant differences ($p < 0.00001$).

(a)

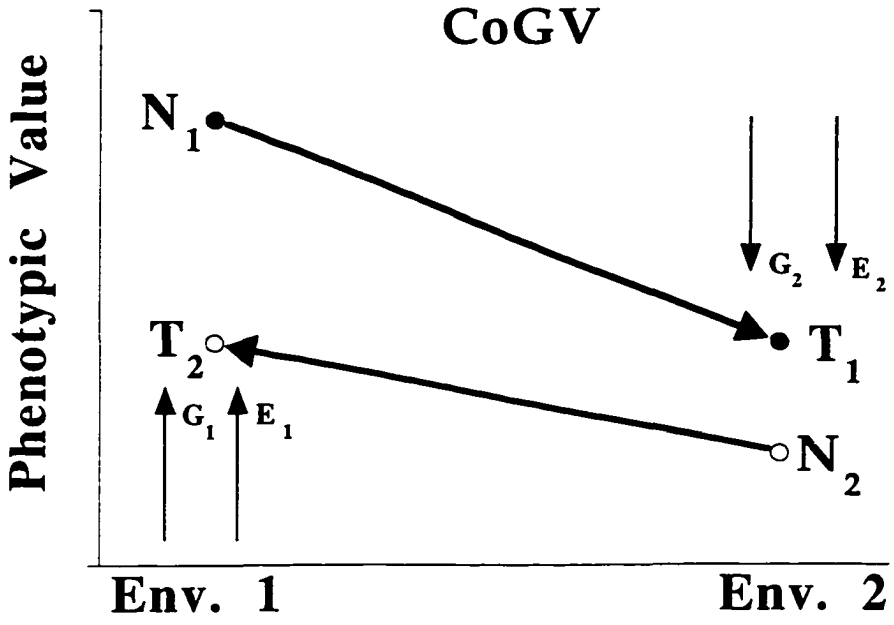
SOURCE	df	MS	F	p	Interpretation
Location (L):	1, 85	282.93	50.88	< 0.0001	P > E
Population (P):	1, 85	28.40	5.11	< 0.05	P > E
P*L:	1, 85	25.08	4.51	< 0.05	PP > PE; EP > EE
Slope:	1, 85	17.65	3.17	NS	

(b)

SOURCE	df	MS	F	p	Interpretation
Location (L):	1, 268	8.30	7.55	< 0.01	P > E
Population (P):	1, 268	1.71	1.56	NS	
P*L:	1, 268	2.43	2.21	NS	
Slope:	1, 268	2.35	2.14	NS	

Figure 24. (a) Phenotypic variation consistent with cogradient variation. Note the large difference in phenotypic values of phenotypes in their native environments (N_1 & N_2) and the shift of their respective transplants (T_1 & T_2) towards the phenotypic values of native phenotypes. (b) Phenotypic variation consistent with countergradient variation. Note the similarity in phenotypic values of phenotypes in their native environments (N_1 & N_2) and the divergences of their respective transplant phenotypes (T_1 & T_2). Arrows with G and E refer to the direction of genetic and environmental influences on phenotypes within their respective environments. See text for further explanation (Adapted from Conover & Schultz, 1995).

a)



b)

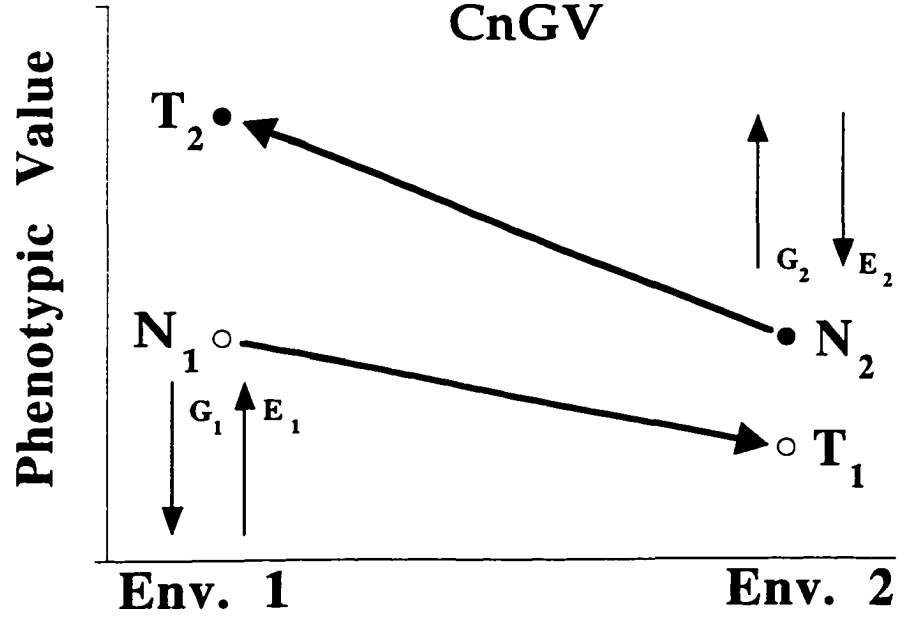


Figure 25. Least squares adjusted means (±SE) from ANCOVA of shell length growth for wave-exposed (E) and protected (P) *Littorina obtusata* raised under conditions of high (H) and low (L) velocity flow in experimental flumes. See Table 11 for results of ANCOVA.

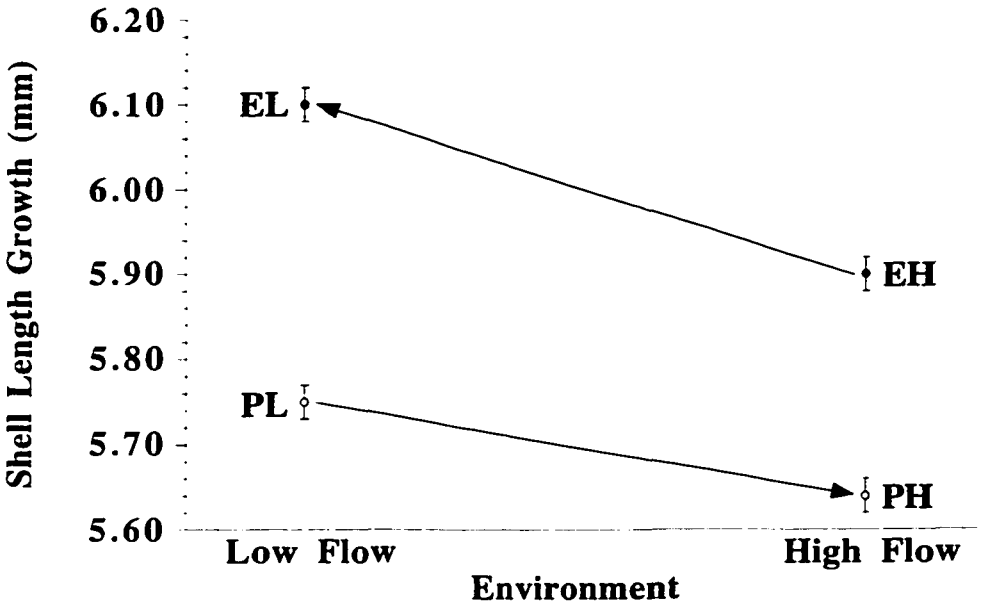


Figure 26. Least squares adjusted means (±SE) from ANCOVA of shell mass growth for wave-exposed (E) and protected (P) *Littorina obtusata* raised under conditions of high (H) and low (L) velocity flow in experimental flumes. See Table 12 for results of ANCOVA.

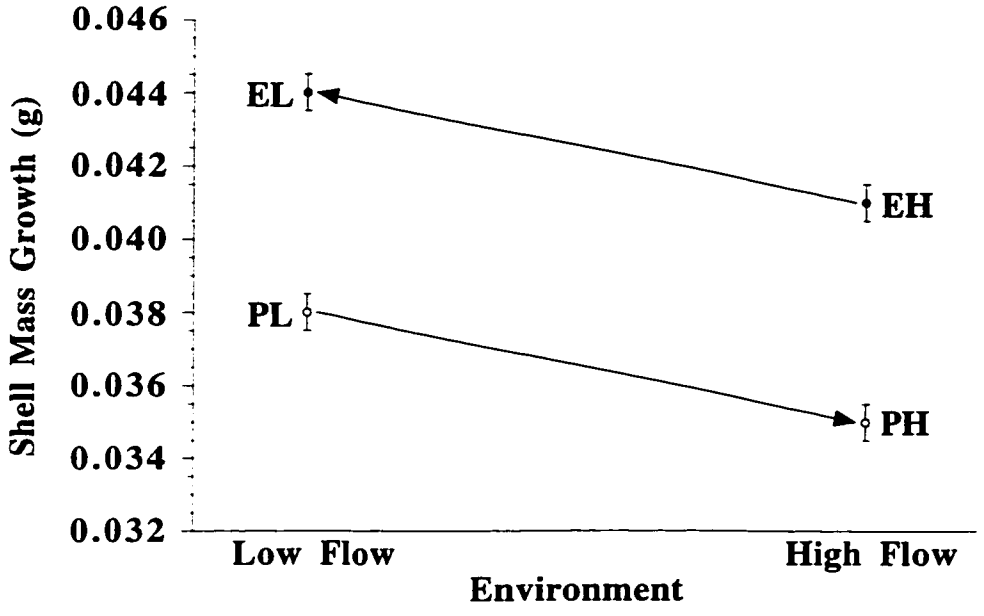


Figure 27. Least squares adjusted means (±SE) from ANCOVA of total mass growth for wave-exposed (E) and protected (P) *Littorina obtusata* raised under conditions of high (H) and low (L) velocity flow in experimental flumes. See Table 13 for results of ANCOVA.

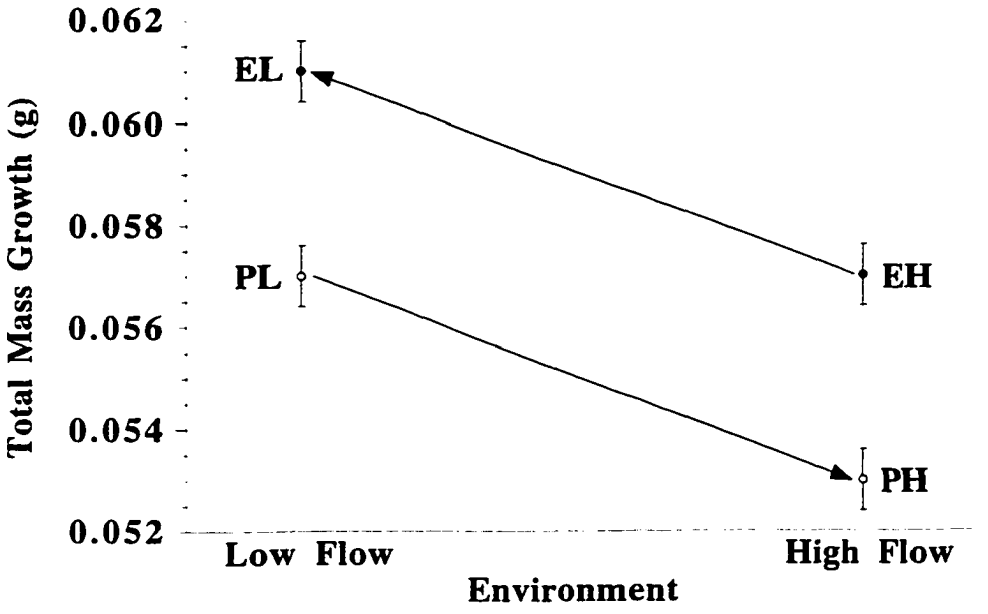


Figure 28. Least squares adjusted means (±SE) from ANCOVA of body mass growth for wave-exposed (E) and protected (P) *Littorina obtusata* raised under conditions of high (H) and low (L) velocity flow in experimental flumes. See Table 14 for results of ANCOVA.

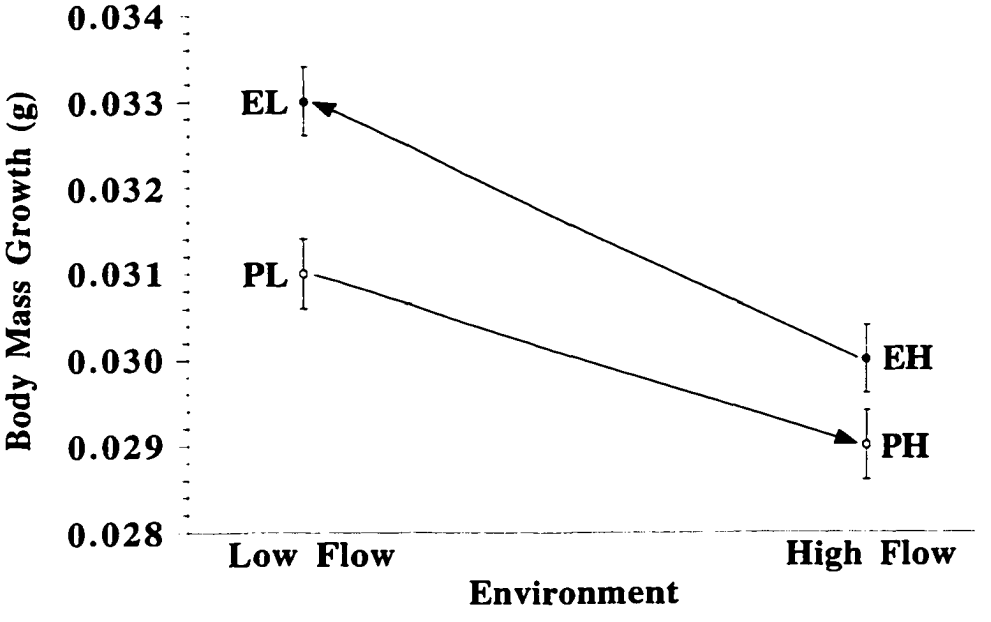


Figure 29. Least squares adjusted means (±SE) from ANCOVA of shell length growth for reciprocally transplanted wave-exposed (E) and protected (P) juvenile *Nucella lapillus*. EE = Wave exposed/Wave-exposed; EP = Wave-exposed/Protected; PP = Protected/Protected; PE = Protected/Wave-exposed. See Table 15a for results of ANCOVA.

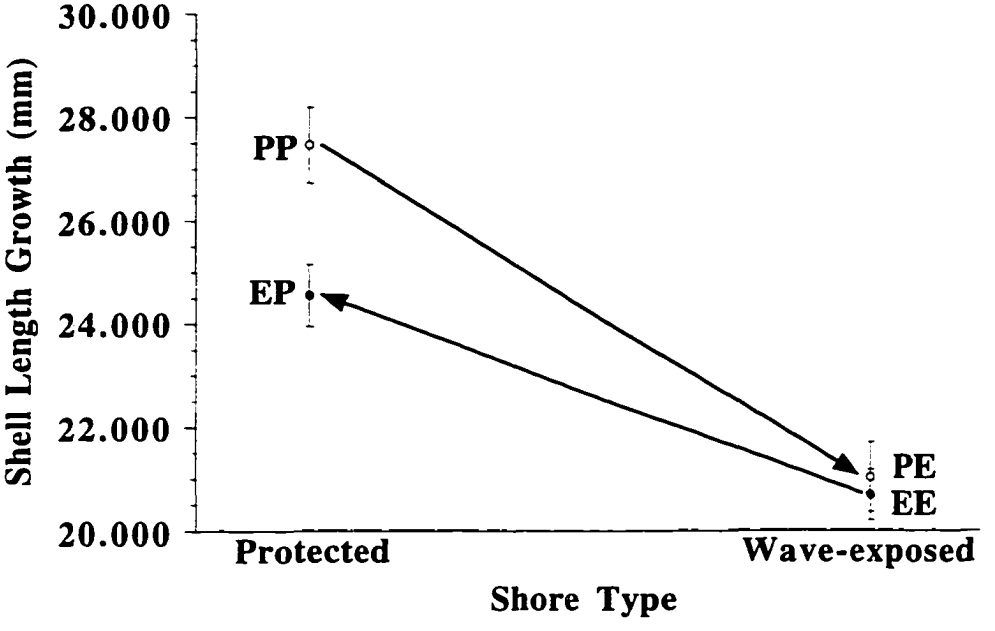
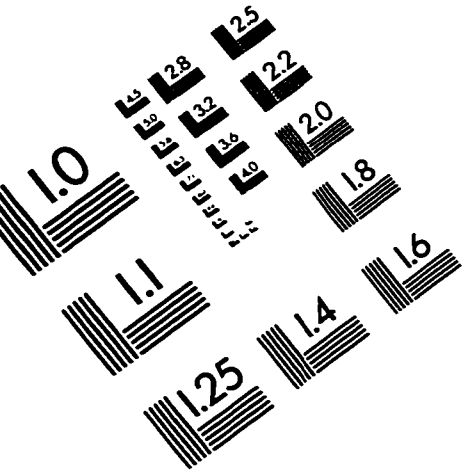
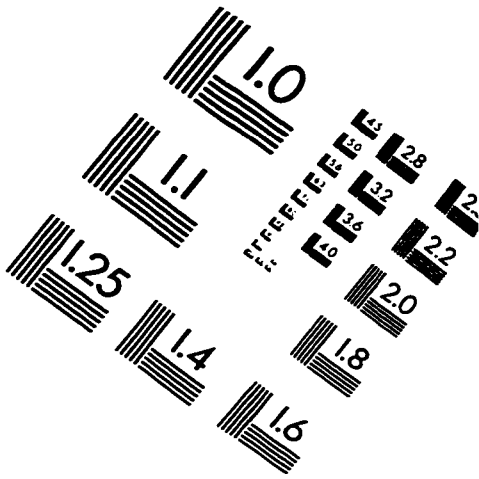
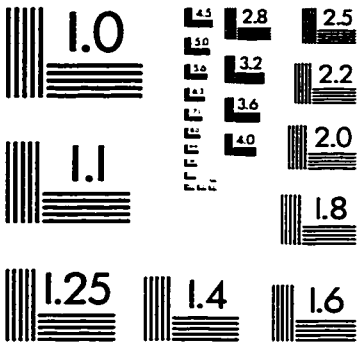


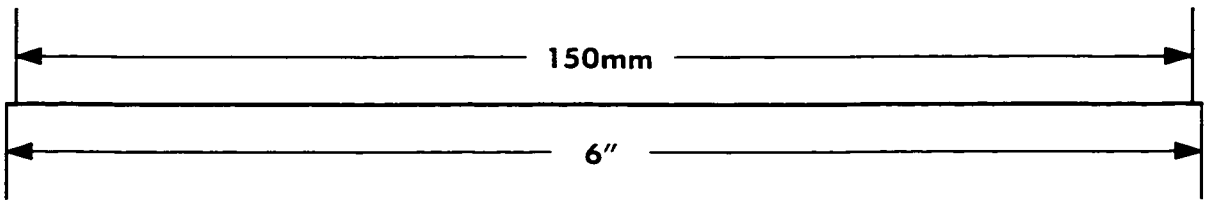
Figure 30. Least squares adjusted means (±SE) from ANCOVA of shell length growth for reciprocally transplanted wave-exposed (E) and protected (P) adult *Nucella lapillus*. Symbols as in Figure 29. See Table 15b for results of ANCOVA.

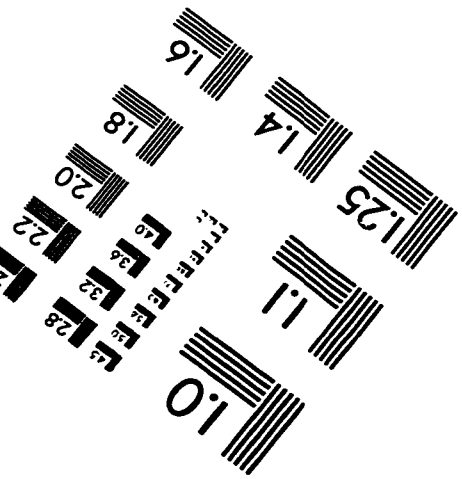
IMAGE EVALUATION TEST TARGET (QA-3)

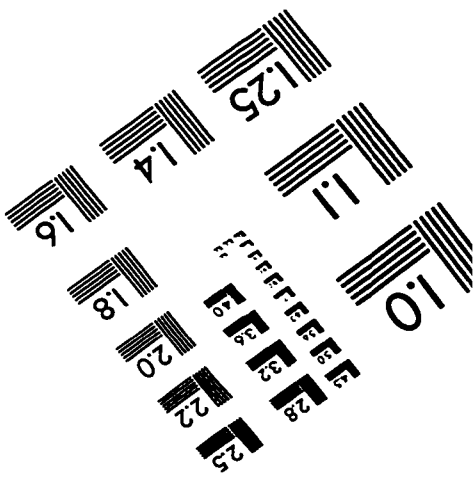












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