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# The maintenance, evolution, and impacts of inducible morphological defenses in Mytilus edulis: Responses to multiple and invasive predators

Aaren Scott Freeman
*University of New Hampshire, Durham*

# THE MAINTENANCE, EVOLUTION, AND IMPACTS OF INDUCIBLE MORPHOLOGICAL DEFENSES IN MYTILUS EDULIS: RESPONSES TO MULTIPLE AND INVASIVE PREDATORS.

BY

AAREN SCOTT FREEMAN
Baccalaureate Degree (BA) Swarthmore College, 1993
Master's Degree, Northeastern University, 1999

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This dissertation has been examined and approved.

Dissertation Director, James E. Byers, Associate Professor of Zoology

Mark D. Bertness, Robert P. Brown Professor of Biology, Brown University

Michael P. Lesser, Research Professor of Zoology

Michelle P. Scott, Professor of Zoology

Geoffrey C. Trussell, Assistant Professor of Biology, Northeastern University

March 9, 2007
Date

## DEDICATION

To my wife, Michelle, through all the high, lows, and long days, weeks, and months of graduate school, you have encouraged me to pursue this esoteric goal. Thank you!

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THE MAINTENANCE, EVOLUTION, AND IMPACTS OF INDUCIBLE MORPHOLOGICAL DEFENSES IN MYTILUS EDULIS: RESPONSES TO MULTIPLE AND INVASIVE PREDATORS.

by

Aaren Scott Freeman

University of New Hampshire, May, 2007

The burgeoning field of phenotypic plasticity and inducible defenses has documented a wide variety of predator-induced defenses. I this dissertation I have explored induced defenses in the marine mussel *Mytilus edulis* as they are affected by (a) shared evolutionary history with invasive crab predators, (b) specificity of responses to multiple predators (singly and combined) with different foraging strategies, and (c) spatial and temporal variation in the expression of predator specific induced defenses *in situ*.

*Mytilus* from southern New England expressed induced shell thickening when exposed to waterborne cues from the crab *Hemigrapsus*, but "naïve" northern mussel populations do not respond. Yet, both populations thicken their shells in response to a long-established crab, *Carcinus*. These results are consistent with the rapid evolution of an induced response to the recent invader *Hemigrapsus*.

*Mytilus* developed significantly heavier shells only in the presence of waterborne cues from *Carcinus,* thicker shells in response to *Carcinus,* the seastar *Asterias*, and the whelk *Nucella*, and heavier adductor muscles in response to cues from *Nucella* and *Asterias*. These induced defenses subsequently protected mussels from *Carcinus*, but only *Asterias* exposed mussel were defended from *Asterias*. However, mussels exposed to the combined cues from *Asterias* and *Carcinus* expressed neither inducible defense nor deterred foraging by the sea star or crab. Furthermore, *Mytilus* did not thicken shells in response to cues from the native crab *Cancer irroratus* or the combined cues from *Carcinus* and *Cancer;* yet mussels did increase adductor muscle in response to combined cues from *Asterias* and *Cancer.* Thus, multiple predator assemblages can disrupt predator specific induced defenses (resulting in risk enhancement for mussels), but these effects cannot be reliably predicted from the predator's functional grouping.

Finally, in field experiments, I found that mussels expressed predator specific responses to *Carcinus* in mid-intertidal cages (but not *Asterias*) and mussels in low intertidal cages increased adductor muscle only in response to *Asterias*, and only during a year with high tissue growth. Together these results suggest that inducible defenses can be influenced by shared evolutionary history with predators and the functional diversity of predator assemblages.

CHAPTER I

# DIVERGENT INDUCED RESPONSES TO AN INVASIVE PREDATOR IN MARINE MUSSEL POPULATIONS


## Abstract

Invasive species may precipitate evolutionary change in invaded communities. In southern New England (USA) the invasive Asian shore crab, *Hemigrapsus sanguineus*, preys on mussels (*Mytlius edulis*), but the crab has not yet invaded northern New England. We show that southern New England mussels express inducible shell thickening when exposed to waterborne cues from *Hemigrapsus*, while "naïve" northern mussel populations do not respond. Yet, both populations thicken their shells in response to a long-established crab, *Carcinus maenas*. Our findings are consistent with the rapid evolution of an inducible morphological response to *Hemigrapsus* within 15 years of its introduction.


## Introduction

Anthropogenic introductions increasingly bring organisms into contact that have no shared evolutionary history, resulting in novel interactions between non-

native and native competitors, prey and predators (Cox 2004). These novel species combinations create potentially strong selection pressure that can drive evolutionary change of heritable traits (Reznick and Endler 1982, Cox 2004, Strauss et al. 2006). While several studies have shown invaders can evolve rapidly in a novel, invaded environment (Cox 2004), examples of invader driven, rapid evolutionary change in native species are rarer (Cox 2004, Phillips and Shine 2004, Strauss et al. 2006). Rapid evolutionary change may particularly influence the ability of native prey to recognize and respond to novel invasive predators with inducible morphological defenses.

Inducible defenses are the expression of alternative forms (phenotypic plasticity) by organisms in response to cues from a predator or competitor. Some commonly noted inducible defenses include shape changes in barnacles, spines on bryozoans and cladocerans, thickened shells of mollusks, defensive chemicals in plants, and morphological and behavioral characters in anuran tadpoles (Tollrian and Harvell 1998, Trussell and Smith 2000). Although selection may act on inducible defenses (Trussell and Smith 2000), in terms of both the degree of plasticity (Trussell and Nicklin 2002) and the prey's capacity to recognize cues from predators (Kiesecker and Blaustein 1997, Schlichting and Pigliucci 1998), to date there have been no examples of an invasive species driving the rapid evolution and emergence of an inducible morphological response. To test for the evolution of predator recognition and expression of inducible morphological defenses in a marine mussel (*Mytilus edulis*), we

juxtaposed the induced defenses of two mussel populations having different historical contact with two invasive crab predators.

The Asian Shore Crab, *Hemigrapsus sanguineus*, was first reported in North America in New Jersey in 1988 and currently ranges from North Carolina to the mid-coast of Maine, U.S.A. (McDermott 1998; R. Seeley pers com). *M. edulis* is a large component of *H. sanguineus*' diet (Lohrer and Whitlatch 2002), but perhaps because this is a novel predator in the North Atlantic Ocean, nothing is known about inducible defenses by mussels to this crab. A longer term resident of New England, the green crab, *Carcinus maenas*, was introduced from Europe to the Mid-Atlantic United States in 1817 and currently ranges from New Jersey, U.S.A., to Prince Edward Island, Canada (Carlton and Cohen 2003). *C. maenas* has had significant impacts on native communities throughout its introduced range (Leonard et al. 1999, Trussell et al. 2002, Carlton and Cohen 2003) and is known to induce defenses in *M. edulis* from several populations (Leonard et al. 1999, Smith and Jennings 2000, Reimer and Harms-Ringdahl 2001). Small mussels are vulnerable to both crab species (Lohrer and Whitlatch 2002), show high relative growth amenable to detecting induced defenses, and represent a crucial, pre-reproductive stage under strong selection.

Given the invasion history of these two crabs, *M. edulis* in northern New England (specifically northeastern Maine) have never experienced predation by *H. sanguineus*. Because the genus *Hemigrapsus* is not native to the Atlantic, neither have they been exposed to any *Hemigrapsus* congeners. However, they have experienced predation by *C. maenas* for over 50 years. In contrast,

mussels in southern New England have experienced predation by *C. maenas* and *H. sanguineus* for 100+ and approximately 15 years, respectively. To determine if natural selection has altered the mussels' capacity to respond to these two crabs, we quantified the responses of mussels from these northern and southern populations to these two crab predators. If predator cues are species-specific and if selection has altered the capacity of mussels to recognize and respond to these invasive predators, we expected that mussels from southern New England would respond to cues from both crabs, while northern mussels would respond to cues from *C. maenas* but not *H. sanguineus*.

## Materials and Methods

**Mussel Collections and Initial Measurements**: To juxtapose the inducible responses of mussels from northern and southern New England to the two crab predators we began a laboratory induction experiment in May 2002. Mussels within the size range consumed by *Hemigrapsus sanguineus* and *Carcinus maenas* (i.e. 13-20 mm shell length) were collected from floating docks at least 15 km apart at 6 sites in northern Maine and 6 sites southern New England (Table 1, Figure 1), taken to Northeastern University's Marine Science Center, Nahant, MA (hereafter: Nahant) and allowed to acclimate for 2-3 weeks in tanks supplied with flowing, unfiltered seawater from the ocean. Mussels were always collected from the vertical or bottom sides of the docks, and at the end of the floating dock most exposed to waves or current. Collecting mussels from floating docks had the benefit of being a consistent habitat between sites with

few crabs (Freeman pers obs), suggesting low predation and background cues affected these mussels.

A Shell Thickness Index (STI) was used to compare mussel shell growth between treatments and populations (Reimer and Tedengren 1996, Frandsen and Dolmer 2002): STI = 1000 * dry shell wt/ $[L*(H^2 + W^2)^{0.5} * \pi/2]$, where L, H and W are length, height and width, respectively. As a measurement of shell weight/surface area, STI provides a valuable estimate of each living mussel's shell thickness at the beginning of the experiment and was highly correlated with measurements of actual shell thickness, but with less measurement error. A multiple regression of shell thicknesses measured at 4 locations (left and right valves, center and lip) on mussels not used in these experiments was well correlated with their STI ($P<0.0001$, $R^2 = 0.911$, $n = 48$). Similarly, the surface area of mussels estimated using the denominator in the STI equation was highly correlated with direct estimates of mussel shell volume using an immersed-displacement technique (surface area$^{1/2}$ vs. volume $^{1/3}$: $P < 0.0001$, $R^2 = 0.97$, $n = 165$). The dry shell weight of living mussels was obtained using a method described by Palmer (1982); specifically, the immersed mass of each live mussel was obtained while suspended in sea water below a balance. These immersed weights were then converted to dry shell weights using individual destructive regressions for each of the 12 sites (16 mussels/site). Regressions from each site of immersed, live mussel weight to dry shell weight were highly correlated ($R^2$ always > 0.99). Length, width, and height of mussel shells were measured using digital calipers ($\pm$ 0.01 mm) and used to calculate the initial STI. Mussels

were then individually marked with paint dots for re-identification and the paint sealed with cyanoacrylate glue.

**2002 Experiment** - To examine the effects of waterborne cues from the two crabs on both mussel populations, we employed a factorial design crossing mussel populations with various predator exposures. 60 - 3.5 liter buckets arranged in two sea-tables at Nahant (30 buckets/table) were each supplied with flowing, unfiltered seawater from an overhead manifold via vinyl tubing (1.5 to 2.0 liters/minute) and aerated from a common source. Seawater for these experiments originated in the shallow subtidal 20-40 m from shore before passing through a large settling tank and the rest of the seawater system. Because the seawater intake was away from shoreline and because in 2002 *H. sanguineus* was not abundant in the subtidal zone at Nahant, few background cues from *H. sanguineus* were likely present in the ambient water (particularly relative to the subsequent Woods Hole experiment). Water drained from each bucket through holes drilled ~ 2 cm below the bucket lip and ~ 4 cm above the surrounding water level, such that water never flowed back into buckets. 50 pre-measured mussels from each site were divided among 5 buckets. To expose these mussels to waterborne cues from crabs, without actual predation, crabs were housed in a single perforated container placed in each bucket. Control buckets had a similar, but empty, perforated container. Of the 30 buckets in each of the two sea-tables, 12 were assigned to contain *H. sanguineus*, 12 were controls, and 6 were assigned to contain *C. maenas*. Thus, *H. sanguineus* and controls were replicated twice from each of the 12 collection sites; *C. maenas*

exposed mussels were used as a positive control and were only replicated once/site. A similar biomass of crabs was used in respective cue treatments; to compensate for the small size of *H. sanguineus*, we used 4 *H. sanguineus*/container and 1 *C. maenas*/container. Every 4 weeks the buckets were cleaned to remove sediment and randomly rearranged within each sea-table. At this time, crabs were also removed from the buckets, fed crushed mussels, and returned to respective cue containers within 8 hours. Thus, by feeding crabs in a separate container mussels were exposed to minimal cues from crushed conspecifics. Cues from crushed conspecifics can also trigger induced shell thickening in *M. edulis* (Leonard et al. 1999), and magnify crab specific responses to improve the protection of some mollusks (Trussell and Nicklin 2002). After 84 days, mussels were removed from the experiment and frozen for later measurement. Measurements were conducted the same as initial measurements with the exception that dry shell weight was measured directly.

To compare the final STI of mussels raised with the various crab cue treatments we conducted a 3-factor, split-plot analysis of covariance with predator treatment (Control, *H. sanguineus*, and *C. maenas*) and population (North and South) as fixed effects, sites (6 northern and 6 southern) as random effects nested within population, and initial STI as a covariate. Initially, sea-table was used as a block, but was removed from the model because it and related higher order interactions were not significant ($P > 0.15$). Interactions of initial STI with all fixed effects (i.e. predator treatment, population, and predator treatment X population) were initially tested and removed from the model when they proved

non-significant ($P > 0.20$). *A priori* linear contrasts compared the 2 predator cue treatments to controls within each population ($\alpha = 0.05$). All analyses were conducted using JMP IN 5.1 (SAS Institute, Inc), which performs the Satterthwaite approximation for the denominator degrees of freedom.

**2003 *in situ* Experiment** -To determine if the previous results were robust or influenced by a laboratory setting more similar to northern collection sites (e.g., in regards to water temperature, higher concentration of background cues from *H. sanguineus*, etc.), we ran an additional induction experiment under field conditions more similar to southern sites (Woods Hole, MA). *H. sanguineus* was well established in the Woods Hole region prior to the experiment (McDermott 1998). In September 2003, mussels were collected from floating docks (as described above) at 5 sites in northern Maine and 5 sites in southern New England (Table 1), and held in seawater tanks (without flowing seawater) for 3-4 weeks at 9-10°C. Initial and final morphological measurements were made of mussels as in 2002. To estimate initial dry shell weights of mussels in 2003, separate destructive regressions of shell dry weight vs. immersed weight for northern and southern mussels were created by pooling the 2002 regressions for northern and southern mussels, respectively (both $R^2 > 0.999$). 30 pre-measured mussels from each site were divided among 3 cages with either 1 *C. maenas,* 4 *H. sanguineus* or no crabs in respective treatments. Each cage was constructed of stainless steel mesh (20cm x 20cm x 9cm, l x w x h: 0.5 cm mesh opening) with a large "arena" for crabs and a small (7 cm x 10 cm) stainless steel mesh compartment housing 10 pre-measured mussels from an individual site (as

described above). The 30 cages were then randomly suspended on ropes (2 cages/rope) under a floating dock in Eel Pond, Woods Hole, MA, with cages 0.5 m and ~2 m below the water surface. All ropes were spaced > 2 m apart and > 15 m from shore. Cages were deployed in October 2003; thereafter, every 2 weeks they were cleaned and any dead crabs replaced. Although the crabs were not fed, none died during the first two months of the experiment and very few died during the third month. Because crabs were not fed in this experiment, but minimally fed in the previous lab experiment, similar and robust responses in the two experiments indicate that responses of mussels to crabs are similar regardless of the crabs' past diet. Every 4 weeks cage positions were randomly rearranged. After 81 days all experimental mussels were removed and frozen, and final morphological measurements conducted within 2 months. Statistical analysis was conducted as for the 2002 laboratory experiment (with no blocking factor). Excessive mortality of mussels collected from Niantic, CT, resulted in the loss of 2 cages from that collection site. Thus, final analysis of the field experiment consisted of mussels from 5 northern sites and only 4 southern sites.

Some populations of *M. edulis* in northern Maine and Eastern Canada co-occur with a cryptic congener, *Mytilus trossulus*. When we excluded sites sympatric with *M. trossulus* (i.e. Lubec and Cutler, ME) from our 2002 analysis our results and conclusions were no different. In addition, all mussels for the 2003 experiment were collected from populations consisting of negligible *M. trossulus* (Rawson et al. 2001).

Figure 1. Collection and experimental sites. Sites of the induction experiments at Nahant in 2002 and Woods Hole in 2003 (asterisk). Also indicated are collection sites for mussels used in the Nahant laboratory experiment (open squares) and the Woods Hole field induction experiment (filled circles).

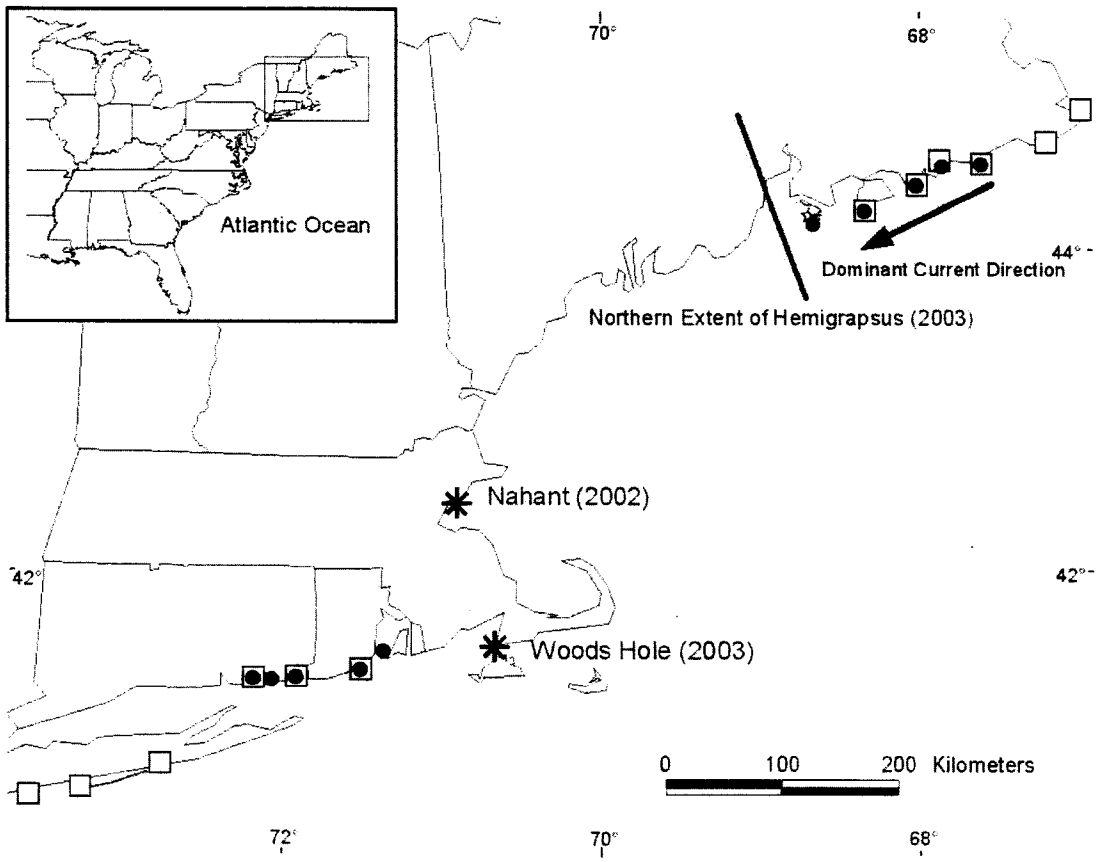


Table 1. Collection sites for mussels.

| Nahant (MA) 2002 laboratory | | | |
|---|---|---|---|
| Site | Latitude | Longitude | Source Population |
| Pt. Judith, RI (PJRI) | 41° 22.6 | 71° 31.0 | South |
| Stonnington, CT (SCT) | 41° 20.1 | 71° 54.5 | South |
| Niantic, CT (NCT) | 41° 19.4 | 72° 10.5 | South |
| Moriches, NY (MNY) | 40° 47.6 | 72° 44.8 | South |
| Captree, NY (CNY) | 40° 38.5 | 73° 15.3 | South |
| Pt. Lookout, NY (PLNY) | 40° 35.5 | 73° 35.1 | South |
| Lubec, ME (LME) | 44° 51.7 | 66° 59.2 | North |
| Cutler, ME (CME) | 44° 39.4 | 67° 12.2 | North |
| Jonesport, ME (JME) | 44° 31.7 | 67° 36.9 | North |
| Millbridge, ME (MME) | 44° 32.7 | 67° 52.7 | North |
| Prospect Harbor, ME (PHME) | 44° 23.9 | 68° 01.4 | North |
| Bernard, ME (BME) | 44° 14.4 | 68° 21.1 | North |
| | | | |
| Woods Hole (MA) 2003 *in situ* | | | |
| Jamestown, RI (JRI) | 41° 28.0 | 71°23.0 | South |
| Pt. Judith, RI (PJRI) | 41° 22.6 | 71° 31.0 | South |
| Avery Pt, CT (APCT) | 41° 19.0 | 72° 03.6 | South |
| Stonnington, CT (SCT) | 41° 20.1 | 71° 54.5 | South |
| Niantic, CT (NCT) | 41° 19.4 | 72° 10.5 | South |
| Stonnington, ME (SME) | 44° 09.0 | 68° 40.0 | North |
| Jonesport, ME (JME) | 44° 31.7 | 67° 36.9 | North |
| Bernard, ME (BME) | 44° 14.4 | 68° 21.1 | North |
| Prospect Harbor, ME (PHME) | 44° 23.9 | 68° 01.4 | North |
| Wyman, ME (WME) | 44° 30.5 | 67° 51.5 | North |

## Results

After being raised for 3 months at Nahant, mussels had grown and mussels from northern and southern New England had thickened their shells differently in response to water-borne cues from the two invasive crab predators (i.e. there was a significant population by predator treatment interaction)(Table 2; Figure 2). Mussels from southern sites thickened their shells in response to waterborne cues from *H. sanguineus* relative to controls (P=0.011), and mussels appeared to thicken their shells in response to *C. maenas,* though the trend was not significant (P=0.145; Table 2; Fig. 2). In contrast, although mussels from northern sites developed significantly thicker shells in response to cues from *C. maenas* (P=0.001), they did not respond to cues from *H. sanguineus* (P=0.573; Table 2; Fig. 2). In addition, there were clear population differences in the temperature sensitive process of shell accretion, with mussels from northern populations thickening their shells more than mussels from southern populations (Fig. 2). These findings suggest that northern and southern mussel populations are genetically distinct. This pattern of warm water-adapted mollusks secreting shell more slowly than northern conspecifics is consistent with counter-gradient variation, a pattern seen in the New England snail *Littorina obtusata* (Trussell 2000). Finally, in 2003, mussels raised in cages suspended from a floating dock in Woods Hole also responded to waterborne cues from the above crabs. These mussels responded to the cue crabs nearly identically to the previous laboratory

experiment, with only northern mussels not responding to *H. sanguineus* (Table 3; Figure 3).

Table 2. ANOVA of Nahant (2002) induction experiment. Split plot analysis of covariance of final Shell Thickness Index (STI) of mussels raised as controls or with cues from *Carcinus maenas* or *Hemigrapsus sanguineus* in a laboratory induction experiment at Nahant, MA (2002). Also, results of *a priori* linear contrasts comparing predator cue treatments to respective controls. (†: a = residual used as denominator, b = Site(Population) used to generate the denominator df using Satterthwaite's method, c = Site (Population) * Predator used to generate the denominator df using Satterthwaite's method). See Table A1 (Appendix) for unadjusted means for each site.

| Source | df | MS | F | P† |
|---|---|---|---|---|
| Response variable: Final STI | | | | |
| Site (Population) | 10, 22.9 | 0.0168 | 12.36 | <0.0001 c |
| Predator | 2, 21.5 | 0.0103 | 7.53 | 0.0033 c |
| Population | 1, 10.2 | 0.1067 | 7.45 | 0.0207 b |
| Predator * Population | 2, 20.6 | 0.0061 | 4.44 | 0.0249 c |
| Site (Population) * Predator | 20, 253 | 0.0014 | 1.11 | 0.3378 a |
| Initial STI | 1, 253 | 1.8452 | 1499.9 | <0.0001 a |
| Residual | 253 | 0.0012 | | |

Linear contrasts: *Carcinus* (North) vs. Control (North) p=0.0011; *Carcinus* (South) vs. Control (South) p=0.145 $^{(1-\beta=0.304,\ LSN=490)}$; *Hemigrapsus* (North) vs. Control (North) p=0.5729 $^{(1-\beta=0.085,\ LSN=3392)}$; *Hemigrapsus* (South) vs. Control (South) p=0.011

LSN=Least Significant Number, i.e. the minimum number of observations needed to achieve $\alpha = 0.05$ for the measured $\sigma$ and $\delta$.

Table 3. ANOVA of Woods Hole (2003) induction experiment. Analysis of covariance of final Shell Thickness Index (STI) of mussels raised as controls or with cues from *Carcinus maenas* or *Hemigrapsus sanguineus* in cages suspended from a floating dock in Woods Hole, MA (2003). (†: a = residual used as denominator, b = Site(Population) used to generate the denominator df using Satterthwaite's method, c = Site (Population) * Predator used to generate the denominator df using Satterthwaite's method). See Table A2 (Appendix) for unadjusted means.

| Source | df | MS | F | P† |
|---|---|---|---|---|
| Response variable: Final STI | | | | |
| Site (Population) | 7, 15.4 | 0.0134 | 3.81 | 0.0135 c |
| Predator | 2, 14.7 | 0.0440 | 12.62 | 0.0006 c |
| Population | 1, 10.0 | 0.1841 | 17.64 | 0.0018 b |
| Predator * Population | 2, 14.7 | 0.0158 | 4.54 | 0.0292 c |
| Site (Population) * Predator | 14, 207 | 0.0035 | 0.71 | 0.7647 a |
| Initial STI | 1, 207 | 1.0469 | 214.19 | < 0.0001 a |
| Initial STI * Population | 1, 207 | 0.0163 | 3.34 | 0.0692 a |
| Residual | 207 | 0.0050 | | |

Linear contrasts: *Carcinus* (North) vs. Control (North) P=0.0031; *Carcinus* (South) vs. Control (South) P=0.0049; *Hemigrapsus* (North) vs. Control (North) P=0.3996 $^{(1-\beta=0.128,\ LSN=1206)}$; *Hemigrapsus* (South) vs. Control (South) P=0.0006

Figure 2. Final STI of Nahant (2002) induction experiment . Adjusted final Shell Thickness Index (LSM) of mussels raised in a laboratory induction experiment at Nahant, Massachusetts, Gulf of Maine. Mussels from northern and southern populations were raised as controls or in the presence of cues from *Carcinus maenas* or *Hemigrapsus sanguineus*. Values are adjusted least square means (LSM) from an analysis of covariance with initial STI as a covariate. Error bars indicate 1 SE.

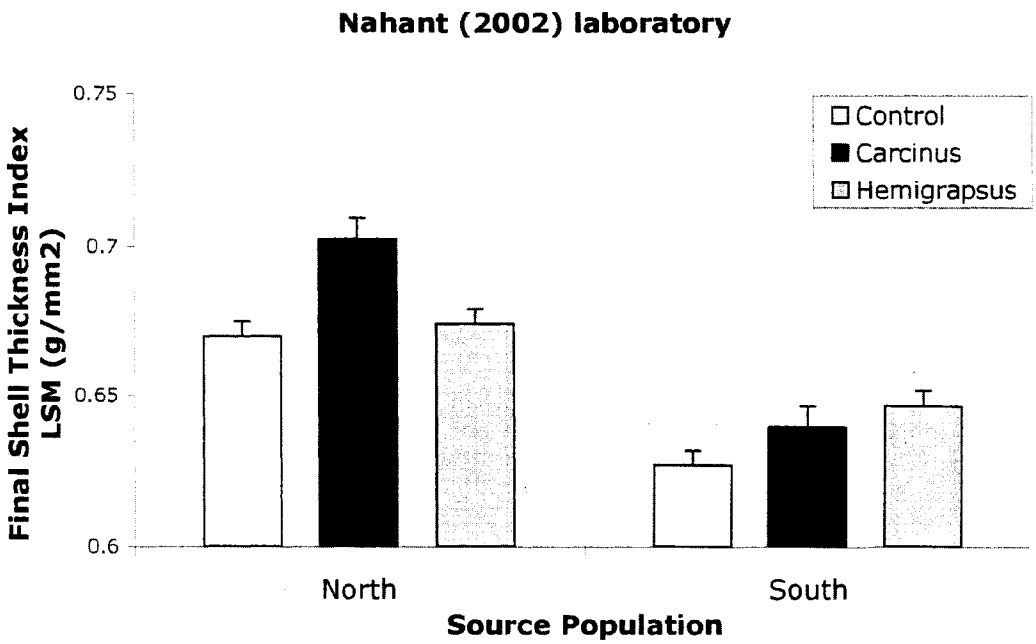
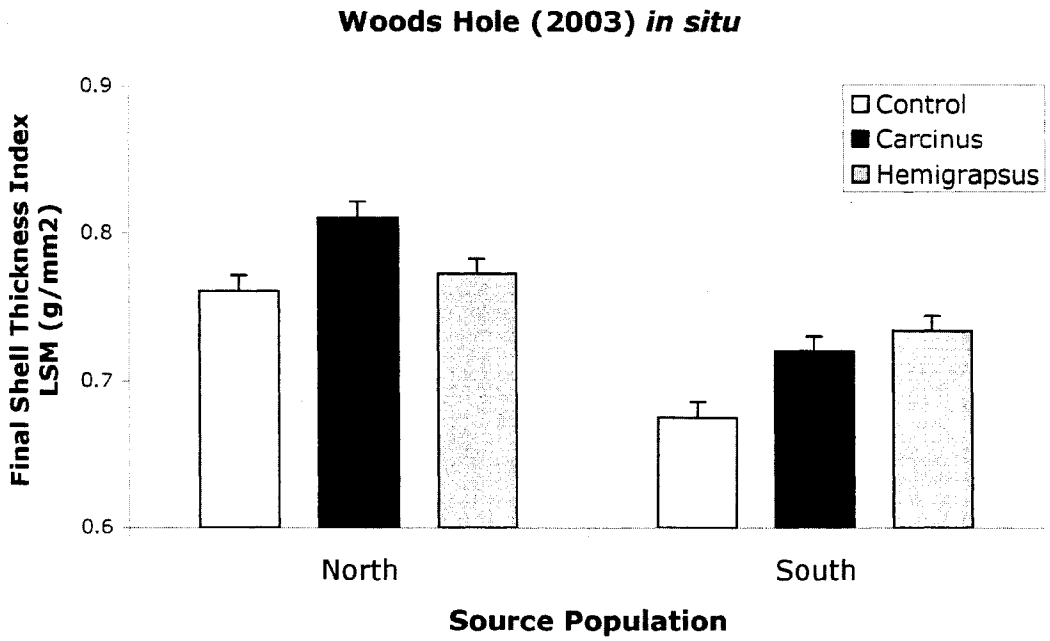


Figure 3. Final STI of Woods Hole (2003) induction experiment. Adjusted final Shell Thickness Index (LSM) of mussels raised *in situ* in cages suspended from a floating dock in Woods Hole, MA, in 2003. Details as in Figure 2.



## Discussion

Our results clearly indicate that mussels from populations in northern and southern New England respond differently to waterborne cues from *H. sanguineus*. Yet, mussels in both regions express similar induced shell thickening in response to *C. maenas,* a resident throughout this coast for more than 50 years. Although brief, we believe the historical contact with and predation by *H. sanguineus* accounts for the divergent mussel responses. The mussel's inducible response to *H. sanguineus* may reflect a novel mechanism of shell thickening, however it more likely reflects natural selection favoring the recognition of this novel predator through rapid evolution of cue specificity or thresholds (Payne et al. 2004), relying on mechanismsfor induced defenses to other crabs (Schlichting and Pigliucci 1998). Our experiments do not distinguish between these possibilities. Despite the mussel's planktonic larvae, the response to *H. sanguineus* manifested by southern *M. edulis* has not spread to northern mussels. This suggests strong local adaptation and/or mostly unidirectional gene flow due to dispersal barriers such as the predominantly southwestward currents in northern New England (Byers and Pringle 2006).

Although invasive predatory crabs can induce defenses in native mollusks (Leonard et al. 1999, Smith and Jennings 2000, Trussell and Smith 2000), these previous examples did not establish that predator recognition and an inducible morphological defense emerged due to selection from the invasive predator. Inducible morphological defenses are distinct from other prey defenses (i.e.

behavioral responses and fixed traits) because they are often irreversible and they may require a sizeable time lag to develop after predator cues are detected (Padilla and Adolph 1996, Sih 2004). The few examples of natural selection by invasive predators deal with the alteration of existing predator specific responses, fixed traits, and adaptive behavioral responses (Trussell and Nicklin 2002, Cox 2004, Phillips and Shine 2004, Strauss et al. 2006).

While recent historical contact with *H. sanguineus* appears to have selected for predator recognition in *M. edulis*, we cannot rule out non-heritable processes in individual mussels, such as learning by native prey (Maloney and Mclean 1995) or conditioned predator recognition. However, there are no examples of inducible morphological defenses resulting strictly from learning. In addition, *in situ* background cues necessary for learning (Brown and Chivers 2005) appeared to have a negligible effect in our system, although they likely differed between our experimental arrangements. At the time of the experiments, *H. sanguineus* was only recently established and thus much less abundant in Nahant compared to southern New England where the crab had been established for several years. If background cues were influential in our system, southern control mussels in the *in situ* experiment would have thickened their shells, diminishing the difference between control and *H. sanguineus* exposed mussels in our Woods Hole field experiment relative to the Nahant laboratory experiment. However, this difference was greater in the *in situ* field experiment than the Nahant lab experiment, suggesting that ambient background cues were

not sufficient to influence our experiments or learning in southern mussels prior to collection.

Alternatively, the differing mussel responses to the two crabs may be related to heritable population differences in *H. sanguineus* recognition unrelated to the introduction of *H. sanguineus*. However, because the genus *Hemigrapsus* is novel to the Atlantic Ocean there is little reason to believe that any Atlantic mussels recognized it prior to its invasion. Thus, even if the extremely limited gene flow of *M. edulis* between Europe and North America (Riginos et al. 2004) disproportionately influenced northern or southern New England mussels, this effect would not help to explain a population's predisposition to recognize *Hemigrapsus*. Moreover, even if *M. edulis* recognized *H. sanguineus* prior to its invasion, it is doubtful that the trait would be lost only in northern New England mussels, given the capacity of mussels to maintain cue recognition in the absence of reinforcing predation (Reimer and Harms-Ringdahl 2001). Alternatively, northern New England mollusks may generally experience lower predation than southern conspecifics (Bertness et al. 1981). Thus, although prior recognition of *H. sanguineus* (per se) seems unlikely, southern New England mussels may more readily express inducible defenses to many predator species by responding to a lower threshold of cues or with decreased specificity to predators (Brown and Chivers 2005). In fact, this potential gradient in cue thresholds and sensitivities may promote the rapid evolution of recognition of a novel, invasive predator in southern New England mussels.

Species interactions can differ on various geographic scales due to local selection and other processes (Dethier and Duggins 1988, Sanford et al. 2003). Similarly, there is considerable potential for the evolutionary history of invasive and native species interactions to vary spatially and temporally. Although we have only a nascent understanding of the role of inducible defenses in marine systems (Raimondi et al. 2000, Trussell et al. 2002), this phenomenon is likely highly influenced by the evolutionary history of the interacting species. The confluence of evolutionary and ecological interactions represents an essential field of inquiry to understand fully the impacts of invasive species.

CHAPTER II

# SPECIFICITY OF INDUCED DEFENSES IN *MYTILUS EDULIS* AND ASYMMETRICAL PREDATOR DETERRENCE.


## Abstract

Induced defenses of prey have become widely recognized in several marine taxa, yet their specificity to particular predators and impacts on subsequent predation are seldom investigated. In this study, *Mytilus edulis* showed highly specific induced defenses in response to predators with different attack strategies. The mussels developed significantly heavier shells only in the presence of waterborne cues from *Carcinus maenas,* a crushing crab predator; and significantly heavier adductor muscles only in the presence of waterborne cues from *Asterias vulgaris* (=*A. rubens*), a predatory sea star that pries open bivalves. However, mussels effectively thickened their shells in response to cues from predators by either increasing allocation to shell (in response to *C. maenas*), or reducing linear shell growth (in response to *A. vulgaris* and, to a lesser extent, the predatory whelk *Nucella lapillus*). These different mechanisms of shell thickening in response to all three predators defended the mussels from subsequent crab predation; increasing handling times of mussels by predatory *C.*

*maenas* by more than 48%. In contrast, only mussels with increased adductor muscle weight (an induced response to *A. vulgaris*) were subsequently defended from the sea star. These results suggest that although induced defenses in *M. edulis* are specific to predators with different attack strategies, diffuse selection by *C. maenas* may allow predator specific responses to be adaptive even when predator composition changes.

## Introduction

Inducible defenses are an adaptive response of prey to environments in which predation pressure varies spatially or temporally. Among marine taxa induced defenses in response to predator cues are widespread and observed in barnacles (Lively 1986b), bryozoans (Harvell 1984), gastropods (Appleton and Palmer 1988, Trussell 1996, Trussell and Smith 2000), and bivalves (Reimer and Tedengren 1996, Leonard et al. 1999, Smith and Jennings 2000, Whitlow et al. 2003). The effectiveness of induced defenses is potentially limited by the ability of prey to correctly identify predator cues and express appropriate defensive phenotypes (Moran 1992, DeWitt et al. 1998). Correctly identifying predator cues allows prey to express appropriate defensive phenotypes without the added costs of incongruous, ineffective, or mistakenly expressed defenses (Langerhans and DeWitt 2002). Although, there have been several tests of the specificity of induced defenses elicited by different predators in marine systems (Harvell 1990, Smith and Jennings 2000, Reimer and Harms-Ringdahl 2001, Iyengar and Harvell 2002, Cheung et al. 2004), fully understanding the benefits,

disadvantages, and possible selection pressure on induced defenses requires knowing their effectiveness against various predators with different foraging strategies.

Because the expression of many induced morphological defenses require time lags, mismatches can occur between a new predation environment and the prey's induced phenotype (Clark and Harvell 1992, Padilla and Adolph 1996, Van Buskirk 2002). In particular, when prey have evolved specific defenses to predators with differing attack strategies, inducible defenses to one predator may: 1) provide protection from a second predator with a different attack strategy (Van Buskirk 2001, Laforsch and Tollrian 2004), 2) leave prey more vulnerable to a second predator (Matsuda et al. 1993, Smith and Van Buskirk 1995, Turner et al. 1999, DeWitt et al. 2000, Relyea 2001), or 3) have no effect on predation by a different predator. The outcome of predation experiments in systems with multiple predators has only recently been explored, even in well-studied systems such as *Daphnia* spp. cyclomorphosis (Laforsch and Tollrian 2004), but may provide a more full understanding of the adaptive value of inducible defenses in multiple predator environments (i.e. the diffuse selection acting on inducible defenses)(Cipollini 2004, Strauss et al. 2005).

To address the question of specificity of induced defenses and the consequences of this specificity in subsequent predator-prey encounters, I focus on the common, intertidal marine bivalve *Mytilus edulis*. *M. edulis* is ideal for the study of specificity of induced defenses because it responds to several predators with very different attack strategies. Several independent studies have shown

that *M. edulis* develops thicker, more rounded shells and stronger adductor muscles in response to cues from *Asterias* spp. (a sea star that pries open mussels) (Reimer and Tedengren 1996, Reimer and Harms-Ringdahl 2001) and increases shell thickness in response to cues from the introduced crab *Carcinus maenas* (a crab that crushes the mussel's shell) (Leonard et al. 1999, Smith and Jennings 2000, Reimer and Harms-Ringdahl 2001, Frandsen and Dolmer 2002) and *Nucella lapillus* (a whelk that drills through mussel shells) (Smith and Jennings 2000). Without direct comparisons of induced morphologies it is difficult to determine if the mussel responds to all predators with varying degrees of a similar defensive strategy (Smith and Jennings 2000), or if different predators induce different traits (Reimer and Harms-Ringdahl 2001).

Finally, due to annual and inter-annual variation in abundance of various predators (Navarrete et al. 2000, Saier 2001, Witman et al. 2003) the induced morphological defenses of individual mussels may be subjected to disparate predator foraging strategies. Although it is clear that mussels expressing induced defenses to *Asterias* sp. take longer for the sea star to consume (Reimer and Tedengren 1996); and it is inferred from increased shell strength that mussels expressing induced defenses to *Carcinus maenas* take longer for the crab to consume (Leonard et al. 1999); it is not clear how these predator specific responses affect handling times when attacked by a predator with a different attack strategy. Until recently, there have been remarkably few estimates of effectiveness of induced defenses in mollusks through actual predation trials (but

see (Reimer and Tedengren 1996, Cheung et al. 2004) and no tests of their effectiveness against predators with different attack strategies.

Research presented in this study simultaneously documents the induced defenses of *Mytilus edulis* in response to waterborne cues from several individual predators: a whelk (*Nucella lapillus)*, a sea star *(Asterias vulgaris=A. rubens)*, and a crab (*Carcinus maenas*). In addition to having differing attack strategies, these predators differ in the sizes of mussels consumed; crabs are gape limited creating a size refuge for mussels (Ebling et al. 1964), whereas mussel do not have an absolute size refuge from whelk and sea star predation (Hunt and Scheibling 1998, Saier 2001). By contrasting the mussel's responses to these predators, I elucidate the ability of these bivalves to distinguish between predators and express defenses appropriate to predators with differing attack strategies. I further determine the effectiveness of these predator specific induced defenses against predators with disparate attack strategies, i.e. the crab, *C. maenas* and the sea star *A. vulgaris*.

## Materials and Methods

**Mussel Collection and Measurement** - To determine the specificity and effectiveness of induced defenses I evaluated the morphology of Mytilus edulis raised with waterborne cues from predators, then exposed similarly induced mussels to predation by Carcinus maenas and Asterias vulgaris. In late June 2002, I collected several hundred mussels from the low intertidal zone at Hilton Park, Great Bay Estuary, New Hampshire, USA (43° 7' N, 70° 50' W). From

these I randomly selected mussels (12-17 mm shell length, 10 replicate$^{-1}$) to be pre-measured for shell mass, length, width, and height. For these measurements length was the greatest anterior to posterior shell dimension, width was the axis perpendicular to the plane formed by left and right shells held firmly closed, and height was measured along the dorso-ventral axis, perpendicular to the hinge. To obtain the dry shell mass of live mussels I used the following technique described by (Palmer 1982): While a live mussel was suspended in seawater on a mesh net hung beneath a balance (Mettler-Toledo AG204, Greifensee, Switzerland) its immersed mass was measured. For a separate group of immerse-weighed mussels, all tissue was removed and their dry shell weights measured. A regression of these dry shell weights against immersed weights was then used to estimate the dry shell weight of the living, experimental mussels (Mussel dry shell weight = 1.5993*immersed weight + 0.0015, R square > 0.9999, n=29). Experimental mussel shells were also measured with digital calipers (length, width and height; $\pm$ 0.01 mm). For subsequent identification, I marked each mussel shell with paint pens (916 Brite-Mark, Roseland, N.J., USA) and sealed paint marks with cyanoacrylate glue.

**Experimental Apparatus** - In a sea table at the University of New Hampshire's Coastal Marine Laboratory (Newcastle, N.H.) I arranged 20, 3.5 liter buckets (16 cm tall x 18 cm diameter). All buckets were supplied with a continuous flow of unfiltered seawater via vinyl tubing (1.5 to 2 liters/minute). The 20 buckets were divided into three predator cue levels (Nucella lapillus, Asterias vulgaris, Carcinus maenas) and a no-predator control. I collected

predators from the rocky intertidal zone adjacent to the marine laboratory and placed predators individually in a single mesh-sided container in each replicate bucket. Seawater escaped each bucket through a dozen holes drilled 2 cm below the lip of each bucket. All holes were 5 cm above the water level of the rest of the sea table, such that water from the sea table did not flow back into the buckets and mix predator cues. I used one individual of A. vulgaris and one of C. maenas in their respective cue treatments. To provide similar cue levels to all treatments, I compensated for the small size of N. lapillus by using 6 whelks replicate$^{-1}$. In addition to the 10 pre-measured mussels in each growth chamber, I also raised 32 extra mussels (length 12-17 mm) for use in a predation experiment (described below).

The experiment began on July 7, 2002 and ran for 91 days. Every 4 weeks I randomly rearranged buckets in the sea table and fed crushed mussels to the predators. Predators were fed monthly in a separate container and returned to cue containers within 6 hours. One *Carcinus maenas* replicate was excluded from analysis because the crab escaped the mesh-sided container and consumed all the pre-measured mussels. At the end of the experiment, I froze all pre-measured mussels for later morphological measurements. Final morphological measurements were based on the 139 mussels surviving to the end of the experiment (of the original 200 pre-measured mussels).

***Morphological Statistics*** - I collected the following final morphological measurements on all pre-measured mussels: shell length, shell width, shell

height, shell dry weight, tissue dry weight, and posterior adductor muscle dry weight. In order to examine the growth and morphological changes of mussels during the experiments I compared the following means of each replicate container (dependent variables are listed first): 1) The residuals of a regression of the final shell weight against the initial shell weight of each mussel, 2) The changes in shell length, width, and height (final –initial) of each mussel, 3) The residuals of a regression of the final shell thickness index (STI) against initial STI. STI is an integrative estimate of shell thickness and correlates well with multiple measurements of actual shell thickness. STI = dry shell wt x $[L \times (H^2 + W^2)^{0.5} \times \pi/2]^{-1}$, where L, H and W are length, height and width, respectively (Reimer and Tedengren 1996, Frandsen and Dolmer 2002). A multiple regression of measurements of the shell thickness (left and right valves, center and lip) from 48 mussels against STI was well correlated ($P<0.0001$, $R^2 = 0.911$), 4) The residuals of a regression of final tissue weights against initial shell weights to compare relative tissue growth, and 5) The residuals of a regression of the posterior adductor muscle (dry weight) against the total tissue (dry weight) to determine the amount of tissue allocated to the posterior adductor muscle. In order to preserve experiment-wide Type-I error I compared the replicate mean residuals from the above data using a multivariate analysis of variance (MANOVA) and subsequent univariate analyses of variance (ANOVAs). If there was a significant treatment effect in univariate ANOVAs ($p<0.05$), I used a priori linear contrasts to compare the means of each predator cue treatment to the control treatment. Because graphs of the above residuals can be difficult to

interpret, I have used adjusted least square means from a nested analysis of covariance of the same relationships to produce Figure 4. I also compared the outcome of a nested ANCOVA of the above relationships and a nested ANOVA of the residuals from the above regressions to address concerns of potential biases of this residuals technique. These analyses produced similar results, confirmed the homogeneity of regression line slopes, and indicated the residuals technique was appropriate for this data set. Data used in these regressions were untransformed, because exploratory analysis of these data indicated that square-root or log transforming data did not improve the linear fit of bivariate plots. Finally, initial dimensions of mussels did not differ between treatments (all $p > 0.70$).

**Predation Experiment** - At the end of the 91-day experimental growth period, after removing the pre-measured mussels, I combined the extra 32 mussels from each replicate chamber into a common pool of mussels for each predator cue treatment. In a temperature-controlled room (9-10° C), I arranged a series of ten, 3.5-liter predation chambers (16 cm tall x 18 cm diameter) such that each replicate chamber could be viewed from above through a video camera housed on a tripod. Black plastic hung around the tripod and predation chambers also minimized visual disturbance of crabs. I placed one male *Carcinus maenas* (carapace width 48-59 mm) in each chamber and began predation trials. All crabs were healthy, with intact claws etc. After commencing recording with the video camera, a single pre-measured, mussel randomly selected from the various predator treatments was placed in each predation

chamber with a crab. Each predation trial lasted 12 hours with the first 1.5 hours recorded on video. The water in each predation chamber was replaced between trials and another handling time observation begun using randomly selected mussels and the same crabs. This process was repeated, allowing all 10 crabs multiple opportunities to consume mussels from each predator cue treatment. After all trials were complete, 7 crabs had consumed 1 to 5 mussels from each of the 4 cue treatments and 3 crabs had consumed 1 to 3 mussels from each of the 3 cue treatments. A total of 74 observations were made of crabs consuming mussels. Previous trials indicated that similar sized *C. maenas* can consume > 10 mussels in the size range used in this experiment in a 12 hour period, suggesting that the crabs were not satiated during the above trials. I later examined videos and estimated handling time from the moment the crab picked up the mussel until shell fragments were discarded and the crab continued searching. I then compared the handling times using an analysis of covariance with each crab as a blocking factor and mussel length as a covariate; followed by *a priori* linear contrasts to compare the handling times of each predator treatment to controls. I also attempted to obtain handling times for *Nucella lapillus*; however, the whelk did not feed in the laboratory and was not used in subsequent predation trials.

Similar attempts to quantify handling times for *Asterias vulgaris* were visually obscured because the sea stars' oral (i.e. lower) surface was not visible to the camera above; however, recording predation trials from below yielded reliable estimates of handling times. In 2004, after raising mussels in a second

cue experiment with *A. vulgaris, Carcinus maenas* or no-predator controls, and observing similar, predator specific induced defenses (Freeman *in prep*), I ran a second predation experiment with *A. vulgaris*. For this I placed a rack of 9 glass bowls (15 cm diameter x 7 cm tall) in a temperature controlled growth chamber (15° C; 1.4 m high x 0.6 m x 0.7 m, internal dimensions), each containing 5 cm depth of unfiltered sea water and a single *A. vulgaris* (5.3-7.5 cm, arm tip across oral disk). I obtained l x w x h measurements of randomly selected mussels raised in the cue experiment, placed a single mussel in each glass bowl and began recording time-lapse video from a camera placed 50 cm below the 9 glass bowls. These predation trials were run at least 8 hrs apart with no more than 2 trials in 24 hrs. These trials were repeated until each of the 9 sea stars had consumed 1 to 5 mussels from each of the 3 cue treatments. A total of 73 predation events were observed. By viewing the oral surface of sea stars preying on mussels, I was able to estimate handling time from the moment the sea star began opening the mussel to when the mussel valves opened and the sea star changed position and began digesting the mussel. These handling times were analyzed using an analysis of variance with *A. vulgaris* identity as a blocking factor and mussel shell height as a covariate; followed by *a priori* linear contrasts to compare the handling times of each predator treatment to controls.

## Results

The multivariate analysis of variance indicated that waterborne cues from the various predators significantly affected mussel morphology and growth (Table

4). A subsequent univariate analysis of variance indicated that final shell weight was affected by cue treatment (Figure 4a, Table 4). Mussels raised in the presence of cues from *Carcinus maenas* significantly increased their shell weights, while shell weights of *Asterias vulgaris* and *Nucella lapillus* exposed mussels were not affected. All changes in shell length, width, and height showed similar patterns, but the change in shell length and change in shell width showed a significant treatment effect (Figure 4b-d, Table 4). *A. vulgaris* exposed mussels had significantly reduced changes in shell length and width relative to control mussels, whereas *N. lapillus* exposed mussels had nearly significant reduced shell widths ($p=0.058$). The type of predator cue also had a significant effect on the shell thickness index (Figure 4e, Table 4). Linear contrasts indicated that mussels exposed to *C. maenas* or *A. vulgaris* had higher shell thickness indexes than control mussels, while *N. lapillus* exposed mussels showed similar trends. Total tissue weights of mussels showed no treatment effect (Table 4); however, the amount of soft tissue allocated towards adductor muscle was significantly greater in *A. vulgaris* exposed mussels (and *N. lapillus* to a lesser degree) than control mussels (Table 4, Figure 4g).

Predation experiments revealed asymmetrical benefits of specific inducible defenses. When exposed to lethal *Carcinus maenas*, the mussels' predator cue treatment had a significant effect on handling time (Table 5). Compared to handling times of control mussels that were not exposed to predator cues, *C. maenas* took approximately 48% longer to consume mussels previously exposed to cues from *C. maenas* ($p = 0.041$), 72% longer to consume

mussels previously exposed to cues from *Asterias vulgaris* ($p = 0.007$), and approximately 67% longer to consume mussels previously exposed to cues from *Nucella lapillus* ($p = 0.052$). Similarly, the mussels' cue treatment significantly affected handling times of *A. vulgaris* (Table 6). However, only mussels previously exposed to *A. vulgaris* cues had increased handling times relative to control mussels (i.e. 36%, $p = 0.019$).

Table 4. (M)ANOVA of mussel morphology – specificity. *Mytilus edulis* growth responses to cues from *C. maenas*, *A. vulgaris*, *N. lapillus* and controls. Univariate and multivariate tests were conducted on the means of each replicate. Degrees of freedom for univariate ANOVA treatment and error terms were 3 and 15, respectively. Where regressions were used as scaling variables the covariate appears in parentheses after the dependent variable.

| Full MANOVA | | | | |
|---|---|---|---|---|
| Source of variation | df | Wilks' λ | F | p |
| Treatment | 21,26.39 | 0.0078 | 5.5562 | <0.0001 |

Univariate ANOVAs

| Response variable | F | p | *A priori* linear contrasts |
|---|---|---|---|
| Final Shell Weight (Initial Shell Weight) | 3.656 | 0.0370 | *Carcinus* > Control p = 0.051; *Asterias* vs. Control p = 0.367; *Nucella* vs Control p = 0.454 |
| Change in Shell Length | 4.462 | 0.0198 | *Carcinus* vs. Control p = 0.343; *Asterias* < Control p = 0.030; *Nucella* vs Control p = 0.109 |
| Change in Shell Height | 3.129 | 0.0571 | |
| Change in Shell Width | 6.425 | 0.0052 | *Carcinus* vs. Control p = 0.189; *Asterias* < Control p = 0.018; *Nucella* vs Control p = 0.058 |
| Final STI (Initial STI) | 7.923 | 0.0021 | *Carcinus* > Control p=0.0003; *Asterias* > Control p=0.009; *Nucella* vs Control p=0.068 |
| Final Tissue Weight, mg (Initial Shell Weight) | 0.910 | 0.4597 | |
| Adductor Muscle Weight, mg (Total Tissue Weight) | 6.158 | 0.0061 | *Carcinus* vs. Control p = 0.838; *Asterias* > Control p = 0.002; *Nucella* vs Control p = 0.053 |

Table 5. *Carcinus maenas* handling times – specificity. (a) Analysis of handling times of *Carcinus maenas* consuming mussels raised with waterborne cues from *C. maenas*, *A. vulgaris*, or *N. lapillus* (ANCOVA). Final Shell Length*Treatment was not significant (p=0.12). (b) *C. maenas* handling times, SE, and *a priori* linear contrasts (vs. Control) when consuming mussels raised under 4 cue treatments. Handling time is the least squares mean (LSM) with final shell length as a covariate.

5a. *Carcinus* Handling Time

| Source | df | MS | F | p |
|---|---|---|---|---|
| Treatment | 3 | 115.871 | 3.1261 | 0.0323 |
| Crab ID | 9 | 278.758 | 7.5207 | <0.0001 |
| Final Shell Length | 1 | 156.228 | 4.2150 | 0.0444 |
| Error | 60 | 37.065 | | |

5b.

| Mussel Cue Treatment | Control | Carcinus | Asterias | Nucella |
|---|---|---|---|---|
| Handling time, min (LSM) | 7.9 | 11.7 | 13.5 | 13.2 |
| SE | 1.3 | 1.3 | 1.5 | 2.2 |
| Post-hoc linear contrasts (vs. Control) | | p = 0.041 | p = 0.007 | p = 0.052 |

Table 6. *Asterias vulgaris* handling times – specificity. Analysis of handling times of *Asterias vulgaris* consuming mussels raised with waterborne cues from *C. maenas*, *A. vulgaris* or no predator (ANCOVA). Final Shell Height*Treatment was not significant ($p > 0.20$). 6b. *A. vulgaris* handling times, SE, and *a priori* linear contrasts (vs. Control) when consuming mussels raised under 3 cue treatments. Handling time is the least squares mean (LSM) with final shell height as a covariate.

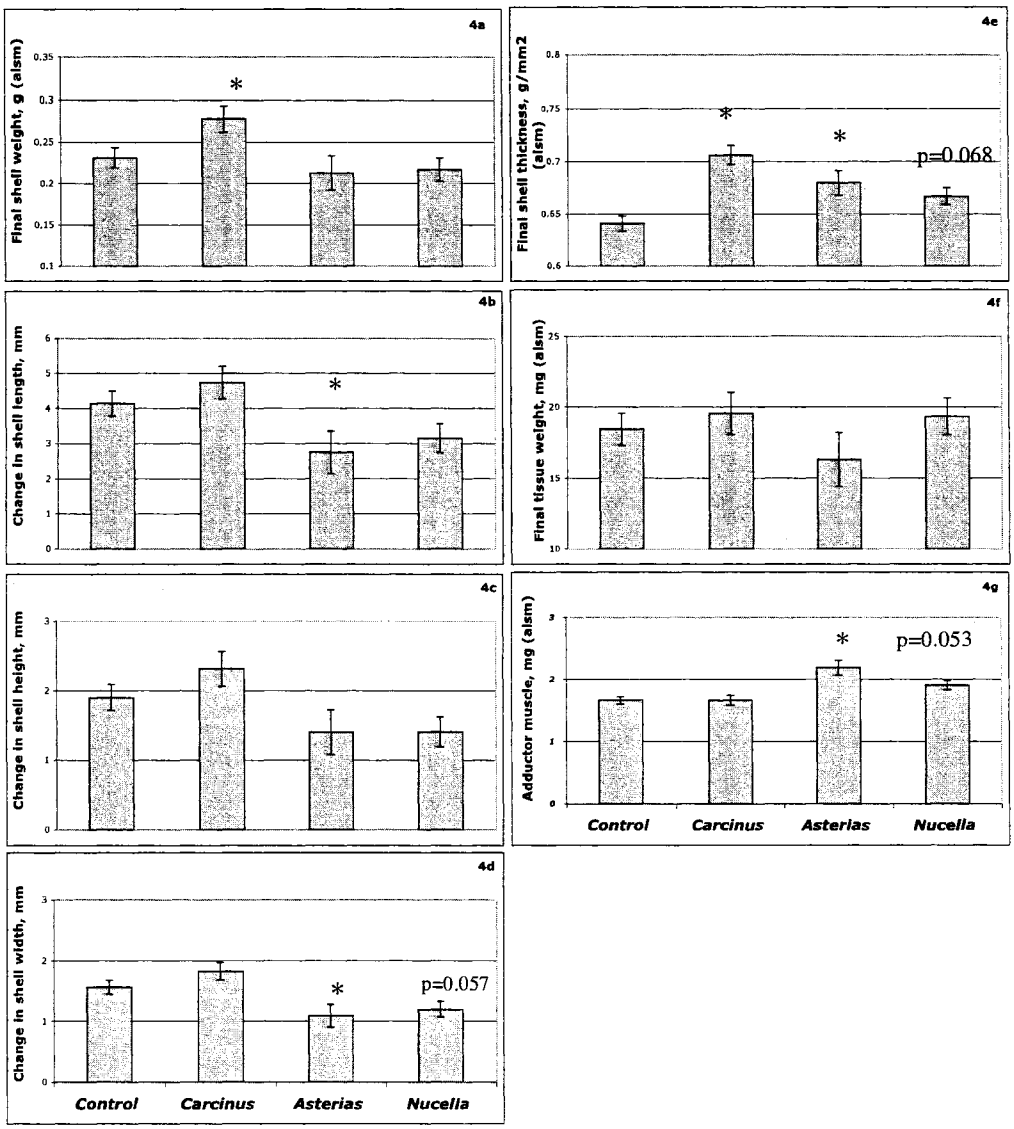
6a. *Asterias* Handling Time

| Source | df | MS | F | p |
|---|---|---|---|---|
| Treatment | 2 | 5406.3 | 5.4481 | 0.0066 |
| Asterias ID | 8 | 1378.6 | 1.3892 | 0.2190 |
| Final Shell Height | 1 | 11691.7 | 11.7822 | 0.0011 |
| Error | 62 | 992.32 | | |

6b.

| Mussel Cue Treatment | Control | Carcinus | Asterias |
|---|---|---|---|
| Handling time, min (LSM) | 59.5 | 49.8 | 81.2 |
| SE | 6.0 | 6.9 | 6.8 |
| Post-hoc linear contrasts (vs. Control) | | p = 0.290 | p = 0.019 |

Figure 4. Morphological measurements – Specificity. Final morphological measurements of mussels raised with various predator cues. Values for final shell dry weight (4a) and final tissue weight (4f) are least square means (+ SE) from a nested ANCOVA with initial shell weight as covariate. Values for final shell thickness index (4e) and final adductor muscle weight (4g) are the least square means (+ SE) from an ANCOVA with final shell length and final tissue weight, respectively, as covariates. Values for change in shell length, width and height (4b-d) are least square means from an ANOVA. Error bars are ± 1 SE. An "*" indicates significant difference from control treatment.



## Discussion

*Mytilus edulis* can distinguish among the three predators and express inducible defenses appropriate to each predator's foraging strategy. However, the resulting effectiveness of these defenses is asymmetrical; the mussels' response to *Carcinus maenas* does not deter *Asterias vulgaris*, yet responses to all 3 predators deter *C. maenas*. That these differing inducible defenses deter the crab, *C. maenas*, is due to distinct mechanisms of shell thickening. In the presence of cues from *C. maenas*, mussels develop thicker shells by allocating more to shell weight (see also Leonard et al. 1999). Accretion of shell $CaCO_3$ is not energetically costly relative to respiration costs; however, it is normally presumed to proceed at, or near, a maximum rate in other mollusks (Palmer 1992, but see Trussell 2002). In mussels, it appears that shell accretion is not maximized in the absence of predators, given that it increased in the presence of cues from *C. maenas*.

Unlike the response to *Carcinus maenas*, mussels thickened shells in response to cues from *A. vulgaris* (and to a lesser extent, *Nucella lapillus*) by decreasing linear shell growth but not altering shell accretion (i.e. adjusted final shell weights did not differ between predator cue treatments). Moreover, mussels developed relatively larger adductor muscles in response to waterborne cues from *A. vulgaris* (and to a lesser extent, *N. lapillus*) but not in response to *C. maenas* (Figure 4g, Table 4). Although this increase in adductor muscle is accompanied by a decrease in linear shell growth, this is probably not an

energetic trade-off, as the adjusted final shell weight remained unchanged in the presence of *A. vulgaris* (Figure 4a, Table 4). This apparent trade-off may represent an adaptive response (providing less surface area for the sea star to grasp) or a mechanical necessity (a thicker shell is less likely to break when resisting the stronger adductor muscle) (Kautsky et al. 1990, Reimer and Tedengren 1996).

In contrast to the significant and specific responses of mussels to *Asterias vulgaris* and *Carcinus maenas*, mussels did not show such strong responses to *Nucella lapillus*. In response to the whelk, mussels did not alter shell weight but showed reductions in linear growth (L, W, or H); effectively thickening their shells and deterring *C. maenas* predation. In addition, mussels increased relative adductor muscle weight in response to *N. lapillus*, suggesting that these mussels would be defended from sea star predation. Given that *N. lapillus* largely drills through mussel shells to access soft tissue the adaptive significance of mussels increasing adductor muscle size is perplexing. Occasionally, *N. lapillus* feeds on mussels through their gaping shell (Ebling et al. 1964), suggesting that shell closure is an adaptive response to the whelk. However, another whelk in the region (*Buccinum* spp.) often feeds on mussels by prying their valves open to access soft tissue, causing mussels to close tightly (Thompson 2002). If mussels express an induced defense to *Buccinum* spp., increasing their adductor muscle would likely deter the whelk. As such, mussels may have imperfect cue recognition, be unable to distinguish between *N. lapillus* and *Buccinum* spp., and show an over-generalized response to any whelk. Although no costs of induced

defenses were observed in this study, similar cases of mistaken identity in mollusk predators can entail costs of induced defense with no defensive benefit (Langerhans and DeWitt 2002).

Predator specific responses may also be influenced by size specific predation (e.g Black 1993). For instance, gastropods responding to chemical cues from gape limited decapod predators can increase their growth rates to attain a size refuge from these predators (Crowl and Covich 1990). While mussels can attain a size refuge from *Carcinus maenas* (Ebling et al. 1964) they generally do not have a size refuge from whelks and sea stars (Hunt and Scheibling 1998, Saier 2001). Thus, there is likely an adaptive advantage for mussels to maintain high growth rates and shell accretion in response to cues from *C. maenas*. In contrast, there is little or no adaptive advantage to rapid growth in responses to *Nucella lapillus* and *Asterias vulgaris*. Indeed, reduced linear shell growth may be an adaptive response to *Asterias* spp. as it provides a sea star with less surface area against which to pull the mussel valves open (Reimer and Tedengren 1996). Finally, mussels may be under pervasive selection to maximize feeding rates, growth rates, and reproductive output through high immediate growth. Any reduction in growth may result in "opportunity costs" of reduced future growth and reproduction (Harvell 1990).

Although these induced defenses are specific to a predator's attack strategy, they may also influence predator behaviors, and indirectly affect handling times. For instance, sea stars adjust their position and the pulse duration of shell pulling based on the size and shape of a mussel's shell (Norberg

and Tedengren 1995). As a consequence, mussels with large, rigid shells but relatively weak adductor muscles (e.g. *Carcinus maenas* exposed mussels) are consumed rapidly by a sea star, while mussels with small, rigid shells and strong adductor muscles may be consumed by a slower, "siege strategy" (Norberg and Tedengren 1995). Similarly, *C. maenas* feeding is influenced by shell thickness and shape, but may also be affected by the ability to resist perimeter assaults (prying, gape entry etc) (Moody and Steneck 1993). The effectiveness of a perimeter assault is likely influenced by valve closure ability and adductor muscle strength.

In many situations, predator species may be segregated by habitats; sea stars rarely forage in the high intertidal (Lubchenco and Menge 1978), whereas crabs predation is less intense in high flow sites (Leonard et al 1999). This leads to spatially predictable patterns in the expression of inducible defenses (Leonard et al. 1999, Frandsen and Dolmer 2002). Similarly, sea star and crab populations often fluctuate seasonally and annually, tracking mussel populations (e.g. Navarrete et al. 2000, Saier 2001, Witman et al. 2003), creating temporal variation in induced defenses that may influence their adaptive value. Because many inducible defenses develop slowly relative to community changes in predator assemblage, time lags in the expression of inducible defenses may represent fitness costs not normally considered in adaptive phenotypic plasticity (Padilla and Adolph 1996, DeWitt et al. 1998, Van Buskirk 2002).

Temporal variation in predator abundance and time lags in the expression of induce defenses can be detrimental to mollusks expressing induced defenses

to specific predators (DeWitt et al. 2000). This can be seen in the current study as mussels exposed to *Carcinus maenas* cues and subsequently preyed upon by *Asterias vulgaris* were not defended (and possibly may have been more vulnerable to the sea star). In contrast, mussels were effectively defended against *C. maenas* regardless of the previous predator cues (i.e. *C. maenas*, *A. vulgaris*, or *Nucella lapillus*). Due to this asymmetrical benefit of the induced defense, predation by *C. maenas* may reinforce the inducible defenses specific to *A. vulgaris* and *N. lapillus*, a pattern consistent with diffuse selection. Diffuse selection is often indicated when the adaptive value of traits influencing interactions with a predator species are altered through interactions with additional predator species (Strauss et al. 2005). Findings in this study are of interest, as the adaptive value of induced defenses are frequently interpreted as only being influenced by a single target predator (but see Cipollini 2004). Moreover, although diffuse selection is often invoked to describe how responses to similar predators can be reinforced (Stinchcombe and Rausher 2001, Van Buskirk 2001, Laforsch and Tollrian 2004), the present study is the first to suggest that specific responses to a predator can be reinforced by a predator with a different attack strategy.

Finally, because *M. edulis* expresses inducible defenses appropriate to the predator's foraging strategy, both when predators are feeding (Reimer and Harms-Ringdahl 2001) and when the predators are not feeding, the present study suggests that cues are emanating directly from the predators. Temporary reductions in mussel feeding behavior occur in response to crushed conspecifics,

but not to predator cues *per se* (Freeman and Meszaros in prep). Accordingly, if cue predators are feeding on crushed conspecifics, behaviorally mediated reduced growth could be confounded with predator specific induced defenses. Mussels in the present study were able to actually increase shell weight without slowing linear growth in response to *C. maenas*, perhaps because the predators were not fed (for comparisons see: Smith and Jennings 2000, Reimer and Harms-Rindgahl 2001). The ability of mussels to respond to non-feeding predators allows them to express these induced defenses even though predators may be in the area but feeding on alternative prey (barnacles etc.). Because mussels will also respond to crushed conspecifics (Leonard et al. 1999), responding to these additional cues may additively increase the defensive morphology expressed (Trussell and Nicklin 2002).

In conclusion, although several studies have independently examined the effects of individual predators on induced defenses of *Mytilus edulis*, a comparison of the impacts of different predators reveals that mussels can distinguish between non-feeding predators and respond with induced defenses appropriate to the predator's attack strategy. The induced response to several predators effectively increased handling time by the crab, *Carcinus maenas,* but not the sea star *Asterias vulgaris*. Thus, in addition to an apparent absence of costs of the inducible defense (i.e. no reduced tissue growth), costs associated with time lags in the expression of induced defenses are also minimized, as mussels responding to all three predators are defended from the invasive crab, *C. maenas*. The high specificity of the mussel's response to predators and the

apparent general effectiveness of the various defenses may facilitate the diffuse selection of these inducible defenses; however, it will also be important to examine the simultaneous effect of these predator cues.

CHAPTER III

# MULTIPLE PREDATOR RESPONSES IN BLUE MUSSELS: RISK ENHANCEMENT DUE TO POOR INTEGRATION OF INDUCIBLE MORPHOLOGICAL DEFENSES.


## Abstract

Prey are commonly exposed to multiple predators in natural environments, yet we know little about how prey integrate different predator-specific inducible morphological defenses. We experimentally compared the inducible defenses of the common marine mussel (*Mytilus edulis*) to waterborne cues from 2 predators with different attack strategies, the sea star, *Asterias vulgaris* (=*A. rubens*), and the crab, *Carcinus maenas*, both individually and together. The mussels expressed specific inducible defenses appropriate to each predator's attack strategy; they increased adductor muscle weight in response to cues from the sea star, a predator that pulls mussel shells open, and increased shell thickness in response to the crushing predatory crab. Both predator-specific responses successfully increased handling times by the respective predator. However, mussels exposed to the combined cues from both predators expressed neither inducible defense nor deterred foraging by the sea star or crab. These results

suggest that poor phenotypic integration of two predator-specific responses (induced adductor muscle and shell growth) underlie the diminished response to combined predators and resulting risk enhancement. The degree that prey can integrate potentially disparate defenses in a multiple predator environment may represent a seldom explored facet of the evolution of inducible defenses.

## Introduction

When predation threat varies spatially or temporally many prey organisms advantageously alter their defensive behaviors or morphologies based on cues from various predators. To date there have been hundreds of documented cases of phenotypic plasticity (behavioral and morphological) in response to the presence or absence of cues from single predator species (reviewed in Lima and Dill 1989, Tollrian and Harvell 1998). Inducible morphological defenses can take the form of increased spines on cladocerans and bryozoans (Tollrian and Harvell 1998), thickened shells of mollusks, (Appleton and Palmer 1988, Trussell 1996) and defensive chemicals in plants (Karban and Baldwin 1997). Most predator-prey systems involve multiple predators (Sih et al 1998) and a growing body of work has shown that behavioral responses to multiple predators interact to affect prey mortality through risk enhancement or reduction (Rahel and Stein 1988, Martin et al. 1989, Crowder et al. 1997, Losey and Denno 1998, McIntosh and Peckarsky 1999, Meyer and Byers 2005, Griffen and Byers 2006b). However, very few studies have examined inducible morphological defenses in situations involving simultaneous exposure to multiple predators (reviewed in Relyea 2003).

The increasing recognition of predator diversity on ecosystem function (Duffy 2002) points to our need to firmly understand influences of multiple predators on the ecology and evolution of inducible morphological traits (Relyea 2004b).

Several general predictions have emerged from studies of behavioral and morphological prey responses to multiple predators with different attack strategies. First, if two predator species have similar predation strategies, prey can effectively respond to both predators simultaneously and the combined impact of two predator species will induce prey phenotypes in the same direction (reviewed in Sih et al. 1998, Relyea 2003). These similar defenses may result in risk reduction for prey (Sih et al. 1998, Vance and Soluk 2005). A second situation occurs if predators have different predation strategies, and prey respond with defenses specific to the predator's attack strategy. Here, prey are often faced with conflicting defensive responses (reviewed in Sih et al. 1998, Turner et al. 1999) and can either respond to the most threatening predator species (Sih 1987, Rahel and Stein 1988, Lima 1992, McIntosh and Peckarsky 1999, Eklov and Werner 2000, reviewed in Relyea 2003, Teplitsky et al. 2004) or, if the threat from the two predators is similar, prey can compromise with an intermediate response (McIntosh and Peckarsky 1999, Turner et al. 2000). Sometimes, prey can effectively reduce predation from multiple predators with different attack strategies by exhibiting unique responses not expressed for either predator, individually (e.g. reduced movement instead of migrating to avoid habitat specific predators) (Crowder et al. 1997, Krupa and Sih 1998). However, it is also possible that these opposing defensive responses may not be effectively

integrated into a cohesive response, resulting in risk enhancement for the prey (Soluk 1993, Losey and Denno 1998).

The majority of the above examples are mediated by behavioral responses, with relatively few examples of morphological responses to simultaneous exposure to multiple predators (but see Relyea 2003, Teplitsky et al. 2004). Moreover, to date, there have been no examples of risk enhancement resulting from inducible morphological defenses to combined cues from multiple predators. The degree that prey can integrate potentially disparate defenses in a multiple predator environment may represent an important fitness component that has rarely been considered in the evolution of inducible defenses (DeWitt and Langerhans 2003).

Presumably, adaptive phenotypic plasticity allows organisms that can adjust their behaviors or morphologies with inducible defenses to maintain higher average fitness across various environments than organisms with constitutive defenses (Via and Lande 1985). Viewed in the context of adaptive phenotypic plasticity, the ability of prey to integrate distinct defensive phenotypes will have direct bearing on the overall net benefit of predator-sensitive inducible defenses, particularly when predators employ different attack strategies and elicit conflicting prey responses (Schlichting and Pigliucci 1998, Sih et al. 1998). Thus, while reduced vulnerability to a single predator species is a clear fitness benefit of induced defenses, if multiple predator environments are encountered frequently, prey responses to multiple predators should be considered in the overall fitness value of phenotypic plasticity. For instance, inducible defenses that are effective

against individual predators and integrate well in multiple predator environments may be more favored than predator specific responses that do not integrate well and result in risk enhancement in multiple predator environments.

The interacting influences of multiple predator species generate novel evolutionary forces on inducible traits, resulting in selection regimes that are often not predictable from pair-wise species interactions (DeWitt and Langerhans 2003); i.e., a form of diffuse selection by multiple predators (Strauss et al. 2005). However, this multi-species, diffuse selection paradigm has been largely neglected in the study of inducible defenses, despite clear ecological relevance (Agrawal 2001, Relyea 2004a). In this study, we compare defenses induced under various single and combined predator cues and their effectiveness in deterring subsequent predation to explore how multiple predator species interactively influence the value of inducible morphological defenses.

**Study System** - To examine the impact of multiple predators on induced defenses we used the common marine bivalve, *Mytilus edulis* and two important predators on this mussel, *Asterias vulgaris (=Asterias rubens* (Wares 2001)), a sea star, and *Carcinus maenas*, an introduced, but long-established, crab in the Northwest Atlantic. *Mytilus* is a common species in many near-shore marine communities and ideal for investigating the impacts of multiple predators on induced defenses because it responds with specificity to several predators employing different attack strategies. For example, mussels develop thicker, heavier shells in response to waterborne cues from *Carcinus* (a predator that breaks open mussel shells to access tissue) and allocate more towards adductor

muscle growth in the presence of cues from *Asterias* (a sea star that pries open mussel shells to access tissue; Reimer and Harms-Ringdahl 2001, Freeman 2007). High specificity of inducible morphological defenses have been observed in other systems (Relyea 2001, Iyengar and Harvell 2002, DeWitt and Langerhans 2003) and may indicate trade-offs associated with induced defenses in the presence of multiple predators with different attack strategies (Sih et al. 1998). We determine if mussels express appropriate morphological defenses in response to waterborne cues from this crab and seastar (in single and multiple predator situations) and subsequently quantify how effectively these inducible defenses deter the predators.

## Materials and Methods

**Mussel collection and measurement** - In June 2003, mussels were collected from a floating dock at the University of New Hampshire's Coastal Marine Laboratory (Newcastle, NH). Mussel shells were measured with digital calipers (length, width and height; $\pm$ 0.01 mm). Six mussels (14.4-19.3 mm shell length) were randomly assigned to each of 40 experimental replicate buckets. Using a technique described by Palmer (1982), the dry mass of each mussel shell was estimated by measuring the immersed mass of each live mussel using a below beam balance (Mettler-Toledo AG204, Greifensee, Switzerland) while the mussel was suspended in seawater on a mesh net. Because the mussel tissue is neutrally buoyant, this technique isolates the weight of the mussel shell. The dry shell weights of experimental mussels were then accurately estimated

from their immersed weights using a separately quantified relationship of immersed weight and dry shell weight. This regression relationship was generated through destructive sampling of immersed and dry shell weights of a group of mussels subsampled from the experimental source pool (mussel dry shell weight = 1.599*immersed weight + 0.002, $R^2 > 0.999$, n=28). Finally, the pre-measured, experimental mussels were marked with small color-coded dots using paint pens (916 Brite-Mark, Roseland, N.J., USA), and dots covered with cyanoacrylate glue to increase the mark's durability.

**Induction Experiment Set-up** - Forty replicate buckets (3.5 L) were arranged in a sea table at the University of New Hampshire's Coastal Marine Laboratory. Each bucket was independently supplied with flowing, unfiltered seawater (1.5-1.9 L minute$^{1}$) and aerated from overhead sources. Predators were collected from the intertidal and shallow subtidal zones at Fort Stark, NH. Each predator was placed into a small, perforated container, and two of these containers were randomly assigned to each bucket according to the 4 predator cue treatments: 2 *Carcinus*, 2 *Asterias*, 1 *Carcinus* & 1 *Asterias*, or a no predator control (2 empty containers). The perforated containers allowed cues from predators to permeate the bucket but prevented access of the predators to the mussels or interactions between predators. Having two predators in all predator addition treatments (a substitutive design) ensured that predator composition and predator density were not confounded (Relyea 2003). In previous work, the mussel's response to two predators of the same species is not different from their response to one individual (Freeman 2007). Every 4 weeks, the buckets were

cleaned and randomly rearranged on the sea table to diminish any effect of irregular air or water flow. At each 4-week cleaning, predators were removed to a separate container and fed crushed mussels. Within 8 hours, predators were returned to appropriate cue containers in experimental buckets. At the end of the experiment (i.e. after 104 days), all mussels were frozen for later morphological measurement. In one replicate from each of three treatments (i.e. *Asterias*, *Asterias/Carcinus*, and Control) all mussels died due to escaped predators or a lethal reduction in water and air flow. These replicates were not used in analyses.

**Morphological Statistics** - To determine whether mussels responded differentially to the various predator combinations, six different measures of growth were tracked: final shell weight (adjusted to initial shell weight), change in shell surface area, dry tissue weight (adjusted to initial dry shell weight), as well as final measurements of shell thickness at two locations (lip and center), and adductor muscle dry weight (adjusted to final shell surface area). Dry shell and tissue weights were obtained after samples were dried at 70°C for 36 hours. First, the measurements of shell length, width and height were used to calculate the initial and final shell surface area using the following equation: Surface Area $= L^2 \times (W^2 + H^2)^{-2} \times \pi/2$ (Reimer and Tedengren 1996, Frandsen and Dolmer 2002). This estimate of surface area correlated well with direct measures of the displaced shell volume upon immersion using a separate group of mussels (surface area$^{1/2}$ vs. volume $^{1/3}$: $P < 0.0001$, $R^2 = 0.97$, $n = 165$). Second, to standardize analyses of shell weight, the final shell weights (the dependent

variable) were regressed against the initial dry shell weights and residuals used for subsequent statistical analyses. Next, using a micrometer, shell thickness was measured on the right and left shells at the lip and center of each shell (6.4 mm from the ventral, posterior shell margin and where the axis perpendicular the mussel's sagittal plane meets the shell, respectively). The lip thicknesses of left and right valves were averaged together and used in analyses, as were the average center thicknesses of left and right valves. Next, to gain a relative measure of tissue growth during the experiment, final tissue dry weights (the dependent variable) were regressed against initial shell weights and residuals used for subsequent analyses. Finally, as a measure of the relative size of the adductor muscle, adductor muscle dry weights (the dependent variable) were regressed against final shell surface areas and residuals used for subsequent analyses. Adjusting adductor muscle weight to total tissue weight instead of final shell surface area did not change the observed pattern (Freeman unpublished data).

Statistical analysis of the above measurements is similar to that described by (Relyea 2003). To preserve experiment-wide significance values ($\alpha$=0.05) the replicate means of change in shell surface area, final shell thicknesses, and residuals from each of the regressions described above were analyzed using a one way multivariate analysis of variance (MANOVA). Upon finding a significant MANOVA (Table 7), univariate effects were then examined using individual analyses of variance (ANOVAs) of the replicate means. If a univariate ANOVA was significant, the replicate means of each predator exposed treatment were

then compared to controls using *a priori* linear contrasts ($\alpha$=0.05). All analyses were conducted using JMP IN version 5.1 (SAS Institute).

**Predation experiments** - To examine the effect of these various inducible traits on predation, predators were allowed to consume mussels previously raised with waterborne cues from the 4 relevant cue treatments (control, *Carcinus*, *Asterias*, and both predators). Mussels used in the predation trials had been raised with waterborne cues from the same 4 cue treatments for 118 days (July-September 2004), and expressed identical inducible defenses (Freeman in prep).

For the *Asterias* predation experiments, 9 glass bowls (15 cm diameter x 7 cm tall) were placed on a rack in a lit, temperature controlled growth chamber (15° C; 1.4 m high x 0.6 m x 0.7 m, internal dimensions). Each bowl contained 5 cm depth of unfiltered seawater and a single *Asterias* (5.3-7.5 cm, arm tip across oral disk). Individual mussels were then randomly selected from predator cue induction treatments, measured with calipers (length, width, and height). These linear measurements were later used to adjust predator-handling times relative to mussel size. After mussels were placed singly into a glass bowl with a sea star, recording began using a time-lapse video camera placed 50 cm below the rack of bowls. Each of these predation trials lasted at least 8 hrs, with no more than 2 trials in 24 hrs. The same 9 sea stars were used in all predation trials, and trials were repeated until each sea star had consumed 2-5 mussels from each predator cue treatment level. Later, upon viewing the videos, handling time was estimated for each sea star as beginning the moment it began opening the

mussel and ending when the mussel shell opened and the sea star relaxed and began digesting mussel tissue. Log transformed handling times were analyzed using an analysis of variance with *Asterias* identity as a random blocking factor and mussel shell height and width as covariates (shell length was removed because it was not significant, $p>0.20$). Interactions of the covariates and treatment were examined to determine homogeneity of slopes and discarded from the model if $p > 0.20$. *Asterias* identity was designated a random effect, thus the Restricted Maximum Likelihood (REML) technique rather than the traditional method of moments technique (i.e. Expected Mean Squares, EMS) was used to analyze its effect. REML was developed for unbalanced, incomplete blocks with random effects, and uses an iterative process in which the sample mean converges on the grand mean when computing variance components. As a result, F-statistics and P-values for the random effect are "shrunken" towards zero (SAS_Institute 2003). Subsequent *a priori* linear contrasts compared the handling times of mussels from each of the 3 predator induction treatment to controls.

For the *Carcinus* predation experiment, seven, 3.5 liter predation chambers (16 cm tall x 18 cm diameter) were placed 1 m beneath a video camera housed on a tripod such that each chamber could be viewed easily. Black plastic sheets hung around the tripod minimized visual disturbance of the chambers. One crab (approximately 4-5 cm carapace width) was placed in each chamber at the beginning of each trial. Mussels randomly selected from the four cue induction treatments were placed individually in predation chambers as

videotaping commenced (1 crab & 1 mussel chamber$^{-1}$). Each predation trial was recorded for 1.5 hours on video, with 8 hours between trials and no more than 2 trials in 24 hours. Between predation trials, the water in each chamber was changed and crabs were returned to the chambers. Videos were later examined and mussel handling time estimated as beginning when the crab picked the mussel up and ending when the crab discarded shell fragments and continued foraging. To improve sample sizes, data from a previous *Carcinus* predation experiment conducted in the fall of 2002 were added to the analysis; these predation experiments represented all treatments of predator-exposed mussels except the multiple predator-induced mussels (Freeman 2007). Thus, handling times from 9 crabs consuming 48 mussels from 3 treatments in 2002 were combined with handling times from 6 crabs consuming 30 mussels from 4 treatments. Combining these experiments was appropriate because year was a blocking factor and the effect of treatment was consistent between years for the 3 shared levels (i.e there was no interaction of year*treatment for control mussels, *Asterias* mussels or *Carcinus* mussels, $p >> 0.20$). The influence of predator cue treatment on handling time was analyzed using an analysis of variance with year as a blocking factor and crab identity as a random blocking factor, for which JMP used the REML function to estimate variance components, resulting in "shrunken" F-statistics and P-values for the random factor. The ANOVA was followed by *a priori* linear contrasts to compare the handling times of each predator treatment to control.

## Results

**Induction experiment** - An initial MANOVA of the 6 response variables revealed a significant effect of predator treatment (Table 7). Univariate ANOVAs indicated that predator cue had a significant effect on shell mass and shell surface area (Table 7a-b, Figure 5a-b). Specifically, linear contrasts revealed that mussels exposed to only *Asterias* or both *Asterias* and *Carcinus* had significantly lower shell growth (mass and surface area) than control mussels, but mussels exposed to only *Carcinus* did not differ from controls. Predator cue also had a significant effect on mussel shell thickness (Table 7c-d, Figure 5c-d). Linear contrasts indicated that only mussels exposed to *Carcinus* had significantly thicker shells than control mussels, while mussels exposed to only *Asterias* and mussels exposed to combined *Asterias* and *Carcinus* did not differ from controls. In addition, the type of predator cue had a significant effect on the adjusted total tissue weight (Table 7e, Figure 5e). Only in the presence of combined cues from both *Carcinus* and *Asterias* did mussels show significantly reduced tissue growth. Finally, adductor muscle weight (relative to final shell surface area) differed among treatments; adjusted adductor muscle weight was only significantly greater for mussels exposed to *Asterias* alone relative to control mussels (Table 7f, Figure 5f).

**Predation experiments** - Mussels raised with cues from *Asterias* for 4 months took significantly longer for the sea star to consume than control mussels (Table 8a, Figure 6a). In contrast, mussels exposed to *Carcinus* (alone or combined with *Asterias*) did not take longer than control mussels for the sea star

to consume. Mussels raised with cues from *Carcinus* took significantly longer for crabs to consume, as did *Asterias* exposed mussels (Table 8b, Figure 6b). However, mussels raised with cues from both predators (combined) did not subsequently deter predation by crabs.

Table 7. (M)ANOVA of mussel morphology - multiple predator experiment. Growth of *Mytilus edulis* in response to experimental predator treatments. These analyses were performed on the unadjusted means from each replicate bucket (b, c, & d) or the mean residuals from each replicate bucket adjusted to initial shell weight or final shell surface area (a, e & f). Initial measurements or scaling variables used to generate regressions for residuals appear in parentheses after the dependent variable. Linear contrasts represent *a priori* t-tests.

| Multivariate test | | | | |
|---|---|---|---|---|
| Source of variation | df | Wilks' λ | F | p |
| **Treatment** | **18,79.7** | **0.149** | **4.2457** | **<0.0001** |

| Univariate test | | | | |
|---|---|---|---|---|
| **Source of variation** | **df** | **MS** | **F** | **p** |
| a. Shell Weight (Initial Shell Wt.) | | | | |
| Treatment | 3 | 0.0223 | 6.5533 | 0.0013 |
| Error | 33 | 0.0034 | | |
| Linear contrasts: Control = *Carcinus* (p=0.482), Control > *Asterias* (p=0.028), Control > *Asterias* & *Carcinus* (p=0.005) | | | | |
| b. Change in Shell Surface Area | | | | |
| Treatment | 3 | 30047 | 6.9924 | 0.0009 |
| Error | 33 | 4297 | | |
| Linear contrasts: Control = *Carcinus* (p=0.7176), Control < *Asterias* (p=0.0152), Control < *Asterias* & *Carcinus* (p=0.0021) | | | | |
| c. Shell Thickness at Center | | | | |
| Treatment | 3 | 0.0049 | 5.8803 | 0.0025 |
| Error | 33 | 0.0008 | | |
| Linear contrasts: Control < *Carcinus* (p=0.045), Control = *Asterias* (p=0.239), Control = *Asterias* & *Carcinus* (p=0.098) | | | | |
| d. Shell Thickness at Lip | | | | |
| Treatment | 3 | 0.0060 | 7.0781 | 0.0008 |
| Error | 33 | 0.0008 | | |
| Linear contrasts: Control < *Carcinus* (p=0.004), Control = *Asterias* (p=0.241), Control = *Asterias* & *Carcinus* (p=0.803) | | | | |
| e. Total Tissue Weight mg (Initial Shell Weight mg) | | | | |
| Treatment | 3 | 75.079 | 2.9308 | 0.0479 |
| Error | 33 | 25.617 | | |
| Linear contrasts: Control = *Carcinus* (p=0.652), Control = *Asterias* (p=0.406), Control > *Asterias* & *Carcinus* (p=0.01) | | | | |
| f. Adductor Weight (Final Shell Surface Area) | | | | |
| Treatment | 3 | $9.37 \times 10^{-7}$ | 9.4897 | 0.0001 |
| Error | 33 | $9.88 \times 10^{-8}$ | | |
| Linear contrasts: Control = *Carcinus* (p=0.959), Control < *Asterias* (p=0.0002), Control = *Asterias* & *Carcinus* (p=0.7097) | | | | |

Table 8. Handling times for *A. vulgaris* and *C. maenas*. Laboratory handling times of a) *Asterias vulgaris* and b) *Carcinus maenas* consuming mussels raised under the 4 cue treatments.

| 8a. *Asterias* Handling times (min, log transformed) | | | |
|---|---|---|---|
| Source of variation | df | F | p |
| Treatment | 3 | 3.0958 | 0.0114 |
| Asterias ID&Random | 8 | - | Shrunk |
| Mussel Height | 1 | 18.6868 | <0.0001 |
| Mussel Width | 1 | 3.2361 | 0.0755 |
| Error | 88 | | |
| *Carcinus* = Control (p=0.3573), *Asterias* > Control (p=0.0142), *Asterias* & *Carcinus* = Control (p=0.4366) | | | |

| 8b. *Carcinus* Handling times (min, log transformed) | | | |
|---|---|---|---|
| Source of variation | df | F | p |
| Treatment | 3 | 5.3359 | 0.0026 |
| Crab ID&Random | 15 | - | Shrunk |
| Year | 1 | 4.3956 | 0.0405 |
| Length | 1 | 0.0966 | 0.7571 |
| Length*Treatment | 3 | 4.026 | 0.0115 |
| Error | 57 | | |
| *Carcinus* > Control (p=0.0045), *Asterias* > Control (p=0.0006), *Asterias* & *Carcinus* = Control (p=0.5224) | | | |

Figure 5. Morphological measurements – Multiple Predators. The relative growth and morphology of *M. edulis* when reared as Controls, with *Carcinus maenas* (Carcinus), *Asterias vulgaris* (Asterias), or *C. maenas* and *A. vulgaris* (Ast-Carc). Asterisks indicate when a level is significantly different from control using *a priori* t-tests ($P<0.05$). Data are means for each replicate of the change in shell surface area (5b), final shell thickness (5c-d), or residuals removing the effect of initial size (5a & 5e) or final shell surface area (5f; See text for details). Error bars indicate SE.

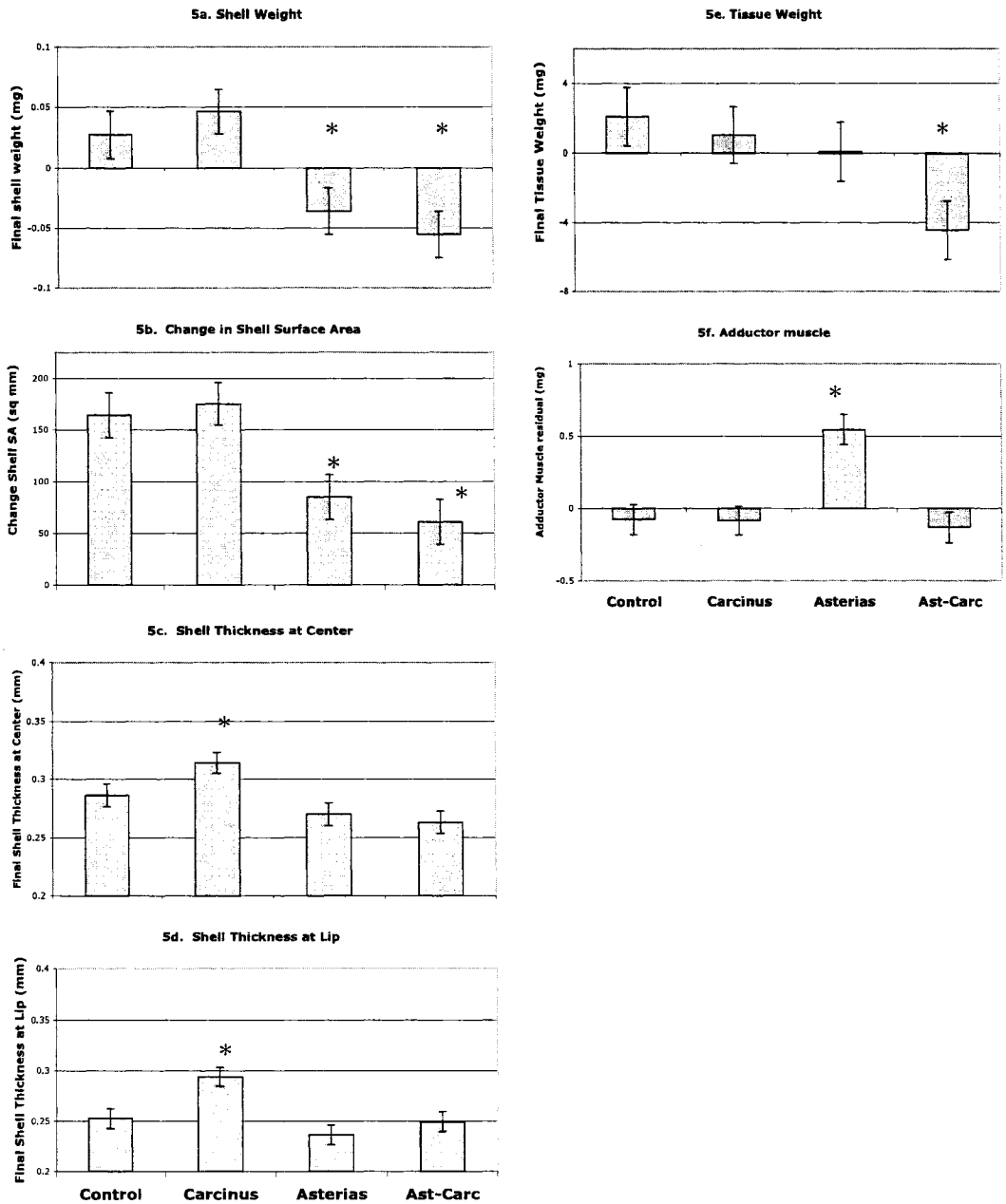
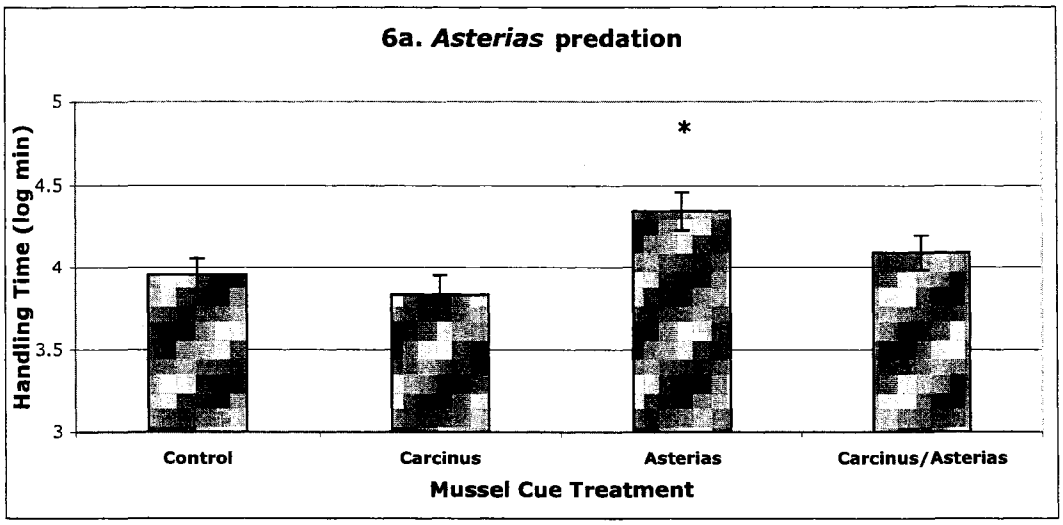
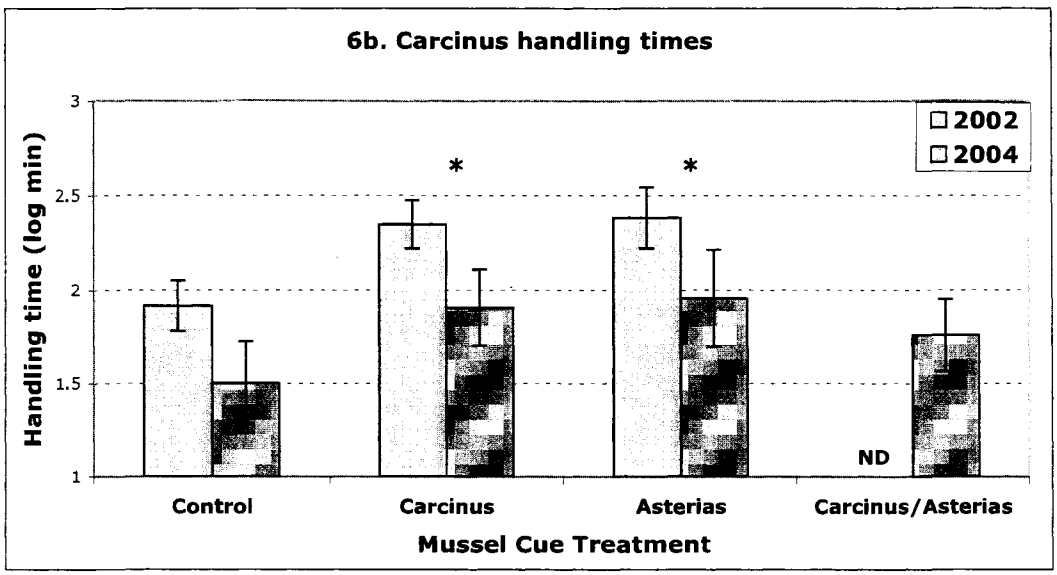


Figure 6. Handling times- Multiple Predator. Handling times (adjusted least square means) of a) *Asterias rubens* and b) *Carcinus maenas* when consuming mussels raised under various predator cue treatments for four months. Asterisks indicate treatment levels that are significantly different from controls using *a priori* t-tests (P<0.05). "ND" indicates no data. Note: handling times are log scale.



## Discussion

Consistent with previous studies, mussels developed larger adductor muscles in the presence of waterborne cues from the sea star *Asterias* alone, and thicker shells in the presence of cues from the crab *Carcinus* alone (Reimer and Harms-Ringdahl 2001, Freeman 2007). These induced traits affected the mussel's ability to deter these same predators: mussels exposed only to *Asterias* successfully hindered the sea star, while those exposed to *Carcinus* or *Asterias* successfully hindered crab predation. However, in response to combined cues from both *Asterias* and *Carcinus*, mussels developed neither larger adductor muscles nor significantly thicker shells. In subsequent predation trials, either predator consumed these latter mussels as easily as control mussels. In contrast to prior predictions, the mussels did not show an intermediate response to both predators (i.e. increasing both shell thickness and adductor muscle weight) or a predator specific response to the most threatening of the two predators. Thus, the combined effect of sea star and crab cues inhibited the appropriate expression of induced morphological defense to either predator, resulting in risk enhancement.

The inability of mussels to simultaneously express shell growth and adductor muscle growth (Figure 5a, b, c, d, & f) indicate that the induced characters are incompatible or that there is an energetic trade-off. That the characters are incompatible suggests poor phenotypic integration of the predator specific defenses (sensu Schlichting 1989), i.e. trait integration (DeWitt and

Langerhans 2003). In this case, mechanisms of linear shell growth may directly interfere with adductor muscle growth. Shell accretion occurs in the mussel's extrapallial space (near the shell margin), and progresses more rapidly at the shell margins than near the shell center (Wilbur and Saleuddin 1983). Thus, induced shell thickening and linear shell growth may be coupled. However, as a mussel shell grows, the adductor muscle does not retain the same attachment points on the interior of the shell; instead, as the shell grows linearly the adductor muscle migrates away from the shell hinge, toward the posterior shell margin. Thus, simultaneously maintaining linear shell growth and increasing adductor muscle may be in opposition. Indeed, in the *Asterias* alone treatment mussels reduced linear shell growth when increasing adductor muscle growth. Finally, integration of responses to *Asterias* and *Carcinus* together may be mechanistically undermined by direct interactions controlling their expression and development (i.e. hormones or other pleiotropic effects) (e.g. Cipollini 2004).

In addition, the incompatible response to both predators may indicate an energetic trade-off between adductor muscle and shell growth. An energetic trade-off is supported in the current experiment as mussels exposed to *Asterias* cues (alone) increased adductor muscle but showed reduced shell growth. In a separate induction experiment, mussels in the presence of cues from *Asterias* (alone) similarly increased adductor muscle mass and reduced linear shell growth relative to control mussels (Freeman 2007). However, mussels in that study also continued adding shell material (i.e. they did not alter shell weight change relative to control mussels), suggesting an energetic trade-off between

increasing adductor muscle and generally adding shell material is not invariant. In many mollusks, shell accretion is not metabolically expensive relative to other metabolic costs (Palmer 1983); in fact, starved mollusks can continue building shell material (Galstoff 1934, Palmer 1981). These facts suggest that the conflict between shell accretion and adductor tissue allocation is not solely an energetic limitation. In fact, under some circumstances it is possible that the two inducible traits can be limited by different resources; e.g., induced shell thickening is likely limited by water chemistry and temperature, while induced adductor muscle growth is likely limited by food intake (Rundle et al. 2004, Freeman in prep). However, under circumstances of food limitation the organic matrix of shells may limit allocation to shell material, especially when mussels also allocate to adductor muscle.

Finally, the mussels' inability to respond to both *Carcinus* and *Asterias* could be explained by reduced growth potential in the presence of both predators. Responses to predators can affect prey growth by reducing assimilation efficiency and/or increasing metabolic rate (Stoks 2001), potentially influencing the expression of inducible defenses and the costs of predator responses. Similarly, responses to particular waterborne cue combinations can result in lower tissue weights and diminished inducible defenses due to reduced feeding (Palmer 1990). In other well-studied systems of inducible defenses (e.g. anuran larvae) the mechanism underlying reduced growth in predator-exposed individuals has only recently been identified and linked to reduced digestive efficiency of the predator-exposed phenotypes (Relyea and Auld 2004).

However, observations of mussels feeding in the presence of cues from these predator combinations yielded no evidence of a behavioral response that reduces feeding, although mussels did reduce feeding in response to crushed conspecific cues (Freeman and Meszaros in prep) (but seeReimer et al. 1995).

In addition to the above constraints, there are at least three potential ecological explanations for the absence of integrated response to the two predators. First, the current and historical exposure of *Mytilus* to *Carcinus* and *Asterias* (individually and together) may influence natural selection of appropriate responses to the combined predators. For instance, in their current distribution crabs are less common at high flow sites (Leonard et al. 1999), while sea stars can be abundant in low intertidal, high flow sites (Lubchenco and Menge 1978). Hence, in many habitats mussels may only be exposed to one of the predators and, as a result diffuse (co)evolution of a response to both predators may not occur because the combined cue represents a rare environment (Moran 1992, Strauss et al. 2005). Second, even when *Asterias* and *Carcinus* co-occur, interference between them and other interaction modifications may reduce the realized threat to the mussels, altering subsequent selection pressure on traits (Inouye and Stinchcombe 2001).

Third, because *Carcinus* was introduced from Europe to New England less than 200 years ago, *Mytilus* in the NW Atlantic share relatively little evolutionary history with *Carcinus* (Wares and Cunningham 2001, Carlton and Cohen 2003). When prey have only limited recent evolutionary history with a predator, selection may not have had time to integrate the prey's response to

multiple predator scenarios involving the novel predator. Evolution of an optimal response to unique or rare environments (i.e. multiple predator combinations) likely occurs after selection has formed the response to the more common, single predator environment (Via and Lande 1985, Stearns 1989, Van Tienderen 1997, DeWitt and Langerhans 2003). Consequently, evolution may have successfully formed the mussel's specific response to either *Carcinus* or *Asterias*, but has not yet successfully formed an appropriate response (with minimal costs in terms of reduced tissue growth) to multiple predator situations including the invasive crab *Carcinus*. This explanation suggests the testable prediction that where both predators are native in Europe *Mytilus* should demonstrate better trait integration.

The predator specific responses exhibited by *Mytilus* provide some asymmetrical benefits in deterring the predators: only mussels exposed to *Asterias* are defended from the sea star while mussels exposed to either *Asterias* or *Carcinus* are defended from *Carcinus*. This may be due to a stronger adductor muscle making mussels more difficult for crabs to consume (Freeman 2007), particularly when crabs consume mussels by prying shells open instead of crushing them (Moody and Steneck 1993). Although the mussel's response to sea stars may appear to be a good universal response (because it defends against both crabs and sea stars), growth limitations imposed by the response to *Asterias* may detract from the benefit of this over-generalized response. Mussels experience reduced shell growth in response to *Asterias*. In fact, reduced shell size allows less surface area for sea stars to grasp and, in addition to increased adductor muscle size, may be a trait under selection as an induced response to

sea stars (Reimer and Tedengren 1996). However, reduced shell growth may represent a cost of expressing a "sea star response", because it reduces future reproductive output (i.e. an opportunity cost) and delays the attainment of a size refuge and ensuing protection from *Carcinus* (Elner and Hughes 1978). Mussels experience a size refuge from both crabs (Ebling et al. 1964) and sea stars (Paine 1976, Sommer et al. 1999, but seeSaier 2001). Thus, when not responding to sea stars, mussels may advantageously maximize shell growth to attain a size refuge, particularly in response to crabs (e.g. Crowl and Covich 1990).

Although this may be the first example of poor phenotypic integration of inducible morphological defenses to simultaneous exposure to multiple predators, there are several examples of poor phenotypic integration of behavioral responses to combinations of predators with differing foraging strategies or inducible morphological defenses to individual predators. For instance, some induced defenses can improve competitive ability or predator avoidance, but not both simultaneously (Smith and Van Buskirk 1995, Cipollini 2004, Relyea and Auld 2005). Similarly, wide or narrow snail apertures can defend against crushing or gape-entry predators (respectively), but these traits cannot be expressed simultaneously (DeWitt et al. 2000, Hoverman et al. 2005). Behaviorally, prey can switch habitats to avoid habitat-associated predators or alter life histories to avoid size-selective predators; but when multiple predators share complementary foraging strategies these alterations may make prey more vulnerable to either predator (Rahel and Stein 1988, Black 1993, Turner et al.

2000). Moreover, because poorly integrated traits are often not independent, correlations between plastic traits across environments can result in behavioral syndromes (Sih 2004) and trait correlations (Thompson 1997, DeWitt and Langerhans 2003) that are non-adaptive when expressed in inappropriate environments. Ecologically, these examples of poor phenotypic integration often result in risk enhancement. While many examples of risk enhancement are mediated by behaviors (Losey and Denno 1998, Sih et al. 1998), ours is the first example mediated by an induced morphological defense.

The patterns of multiple predator induced defenses observed in this study also illustrate how diffuse evolution may inform explorations of the adaptive value of inducible defenses. To explain why induced defenses are not always expressed, most models of the evolution of inducible defenses incorporate only two environments, i.e. the presence and absence of individual predators (Via and Lande 1985, Padilla and Adolph 1996, Van Tienderen 1997). Consistent with these models, studies of single predator systems have occasionally revealed trade-offs of induced defenses in terms of architectural constraints on growth (Lively 1986a, Trussell and Nicklin 2002), reduced competitive ability (Pettersson and Bronmark 1997), reduced growth rates (Harvell 1986), and trade-offs between acquiring energy and avoiding predators (Skelly 1992, Anholt and Werner 1995, Relyea and Werner 1999, Van Buskirk 2000, Relyea 2001). However, trade-offs of inducible defenses may become apparent if prey possess incompatible induced traits to multiple predators or if induced traits are disfavored under certain environmental conditions (Lima 1992, Agrawal and Karban 1999,

Relyea 2003, 2004a). For the mussels in the present study, continuously expressing elevated shell growth (the response to *Carcinus*) may preclude an induced response to *Asterias*. Similarly, maintaining high adductor muscle growth (the response to *Asterias*) may preclude an inducible response to *Carcinus*. These observations meet the criteria to explain the conditional expression of mussel-induced traits, particularly given the conspicuous absence of traditionally defined costs in single predator environments (Reimer et al. 1995, Smith and Jennings 2000, Frandsen and Dolmer 2002). Gaining a more realistic understanding of the selection pressures acting on inducible defenses may require assessing the trade-offs of inducible defenses in non-adaptive (Stearns 1989) and multiple predator scenarios (Sih et al. 1998, Relyea 2003, Hoverman et al. 2005), particularly when prey show specific and conflicting responses to predators with differing attack strategies.

Although there are numerous examples of induced defenses in marine systems, their trophic implications and expression under multiple predator conditions have been largely unresolved. Multiple predator effects are gaining recognition as essential in predicting numerous predator prey interactions, yet similar principles have not been applied to our understanding of the evolution of defensive responses. This study has shown that while mussels can express specific induced defenses to cues from *Asterias* or *Carcinus*, the combined exposure to cues from these two predators effectively negates the mussel's ability to respond to either predator appropriately. Thus, the adaptive value of

induced defenses may be influenced by diffuse evolutionary pressure from multiple predator species.

CHAPTER IV

# MULTIPLE-PREDATOR INDUCED DEFENSES IN *MYTILUS EDULIS*: A TEST OF THE FUNCTIONAL SIMILARITY OF TWO CRAB SPECIES


## Abstract

In this study, I compared the inducible defenses of blue mussels (*Mytilus edulis)* in response to cues from a sea star, *Asterias vulgaris (=rubens)*, and two predatory crabs, *Carcinus maenas* and *Cancer irroratus*. The mussels expressed predator specific inducible defenses in response to *Asterias* and *C. maenas*, increasing adductor muscle weight and shell thickness, respectively. However, when exposed to cues from both predators simultaneously, mussels express neither induced defense. Moreover, mussels did not thicken shells in response to combined cues from *C. maenas* and *C. irroratus*, or from *C. irroratus* alone; yet mussels did increase adductor muscle in response to combined cues from *Asterias* and *C. irroratus*, but not in response to *C. irroratus* alone. Thus, despite the functional similarity of these crabs their effects on mussel induced defenses were not substitutable and often interfere with the mussel's predator specific responses. These results are discussed in terms of cue specificity,

possible explanations for these differing responses, and resulting complexity of trophic interactions involving these inducible traits.

## Introduction

Inducible defenses, a type of phenotypic plasticity, allow organisms to express defensive phenotypes in response to predation threats that may vary within the organism's lifetime. Some examples of aquatic inducible defenses include spines on bryozoans, rotifers and cladocerans (Tollrian and Harvell 1999), morphologies affecting vulnerability to predators in fish and anurans (Bronmark and Miner 1992, Relyea 2001), shell morphology in gastropod mollusks (Appleton and Palmer 1988, Trussell 1996), and defended morphologies of barnacles (Lively 1986b). While there are numerous examples of inducible defenses, only a few studies have shown that prey can distinguish between water borne cues from predators with different foraging strategies and express unique responses appropriate to those strategies (Turner et al. 1999, Relyea 2001, Van Buskirk 2001, Teplitsky et al. 2005, Freeman 2007) and even fewer have examined the combined effects of multiple predators (Relyea 2003, Teplitsky et al. 2004)(Freeman and Byers in prep). The ability of prey to distinguish between predators with different foraging strategies can be ecologically important when prey respond to combined predators with conflicting, predator-specific defenses (Sih et al. 1998). Theoretically, the effects of responding to multiple predators may be predictable *a priori* in the absence of interactions between predator specific responses (Bolker et al. 2003); however,

prey responses to multiple predators may interact and be unpredictable if combined predators elicit poorly integrated responses (Sih et al. 1998, DeWitt and Langerhans 2003). Although predator specific behavioral responses to functionally redundant predators can be poorly integrated (Lawton and Brown 1993, Kurzava and Morin 1998) no studies have explored predator specific morphological defenses to functionally redundant predators.

In this study I compared the induced defenses of the marine, blue mussel (*Mytilus edulis*) in response to the predatory sea star (*Asterias*) and two functionally similar predatory crabs (*C. maenas* and *C. irroratus*), in isolation and together. *M. edulis* is a good study organism as it responds to several predators with induced morphological defenses highly specific to the predators foraging strategy (Smith and Jennings 2000, Reimer and Harms-Ringdahl 2001, Freeman 2007). In response to cues from *C. maenas,* mussels increase shell weight and thickness, and in response to cues from *Asterias* they increase adductor muscle size (Reimer and Harms-Ringdahl 2001, Freeman 2007). These induced traits increase handling time by the respective predators (Freeman 2007). However, the predator specific responses appear to be incompatible, as mussels raised with combined cues from both predators express neither induced defense and are no better defended than mussels raised without predator cues (Freeman and Byers in prep). Furthermore, mussels can distinguish between crab species (Freeman and Byers 2006), suggesting the possibility that different crab species may add further complexity to this collection of inducible traits.

While the sea star *Asterias* occupies a different functional group from *Cancer irroratus* (a brachyuran crab) and *Carcinus maenas* (a portunid crab), it is not clear if the mussel's response to various combinations of these predators depends in a straightforward way on the predator assemblage's functional groups. Seastars and crabs can be considered functionally distinct because *Asterias* pries mussels open to feed but normally does not break mussel shells, whereas the crabs must break mussel shells to access soft tissue. *C. irroratus* and *C. maenas* occupy similar trophic positions relative to mussels and have overlapping intertidal and subtidal distributions of the NW Atlantic (with *C. maenas* often migrating high in the intertidal)(Hunter and Naylor 1993). In addition, *C. irroratus* and *C. maenas* use dexterous chelae, whereas the two other decapod predators of mussels in the region, lobsters (*Homarus americanus*) and Jonah crabs (*Cancer borealis*), use their relatively larger claws solely for crushing mussel shells (Moody and Steneck 1993). An important distinction between these crabs may be that *C. irroratus* is native to the NE Atlantic but *C. maenas* was introduced in the mid-1800's (Carlton and Cohen 2003). Ironically, several studies have addressed the influence of *C. maenas* on the *M. edulis* inducible defenses in the NE Atlantic (Leonard et al. 1999, Smith and Jennings 2000)(Freeman in press), but none have examined induced defenses to *C. irroratus*. However, in Europe *M. edulis* does respond to a congener (*Cancer pagarus*) by increasing byssal thread width and count (Cote 1995). If the ecologically and functionally similarity of these crab's functional grouping corresponds to the inducible defense triggered in mussels, I expected

mussels would respond similarly to the crab predators, and responses to both crabs would be incompatible with a response to the sea star. However, if mussels distinguish between crab species they may respond differently to the two crabs (alone and in combination with *Asterias*) due to evolutionary history or current, nuanced ecological differences between the crabs. I have used a factorial experiment to address the following questions: 1) Do the mussels respond to *C. irroratus* with inducible defenses? 2) Do the mussels have different responses to these functionally similar crabs? 3) Are cues from the two crabs substitutable, i.e. do they elicit similar responses when the two crabs are together, or in combination with *Asterias*?

## Materials and Methods

**Collection and measurement** - In June 2004 I collected small (15-26 mm) mussels from Nubble Light, York (Maine). Mussels used in the induction experiment were initially measured and labeled as follows: Approximately 450 mussels were cleaned of epiphytes and divided into 10 size categories and mussels in each size category received similar color-coded dots on each valve. To improve durability, dots were covered with cyanoacrylate glue. All mussels were then combined into a common pool and, to insure each replicate received a similar size range of mussels, a single mussel from each color-coded group was haphazardly selected. The immersed mass of each mussel was taken with a below beam balance using a method similar to Palmer (1982). Care was taken

to ensure no bubbles in the mantle cavity would interfere with the immersed measurement. The length, width, and height of each mussel were taken using digital calipers. Ten mussels (each with distinct color dots) were measured in this way for each replicate and the process was repeated until there were 42 replicate mussel groups, all representing a similar size range (15-26 mm). In addition, I measured the immersed mass of several additional mussels for use in a destructive regression to determine the initial dry weight of the live experimental mussels at the beginning of the experiment. The immersed to dry weight of these mussels was highly correlated ($R^2$>0.999, dry weight = 1.557*(immersed weight)+0.0061, n=19).

**Experimental apparatus** - At the University of New Hampshire's Coastal Marine Lab (Newcastle, NH) I arranged 42, 3.5 liter buckets in a sea table. Each bucket was aerated with an airstone and supplied from an overhead manifold with flowing, unfiltered seawater. To protect mussels from possible escaped predators, groups of 10 pre-measured mussels were placed in stainless steel cages (5 cm x 5 cm x 8 cm, with 0.5 cm mesh size) in buckets. Each bucket also contained 2 mesh sided cue containers housing predators. Pairs of cue containers were randomly assigned to contain the following predator cue combinations: Control (no predator), 2 *C. maenas*, 2 *Asterias*, 2 *C. irroratus*, 1 *C. maenas* and 1 *C. irroratus* , 1 *C. maenas* and 1 *Asterias*, and 1 *C. irroratus* and 1 *Asterias*. Thus, 2 predators, in separate mesh-sided containers, resided in each replicate bucket. Predators were collected from the shallow subtidal and intertidal at Fort Stark (NH). The experiment ran for 118 days. Approximately

every 4 weeks buckets were cleaned and randomly rearranged in the sea table. When buckets were rearranged, predators were removed, fed crushed mussels, and returned to the apparatus within 6 hours. Each bucket also contained approximately 30 additional mussels (loose in the bucket) for use in a predation experiment reported elsewhere (Freeman and Byers in prep). At the end of the experiment, all mussels were re-measured for shell length, width, and height. After separating the posterior adductor muscle from remaining tissue and shell, all materials were dried in an oven at 60°C for at least 48 hrs and weighed.

**Statistical analysis** - I used a Shell Thickness Index (STI) as a measure of shell thickness at the beginning and end of the experiment. This STI is simply the shell weight divided by the surface area. Surface area was calculated using the equation: $SA=[L*(H^2 + W^2)^{0.5} * \pi/2]$. This surface area estimate correlated well with measures of mussel shell volume using an immersed-displacement technique (surface area$^{1/2}$ vs. volume $^{1/3}$: $P < 0.0001$, $R^2 = 0.97$, $n = 165$). Furthermore, in a multiple regression STI correlated well with actual measurements of shell thickness at 4 locations (left and right valves, center and lip thickness; $p<0.0001$, $R^2=0.911$, $n=48$). To compare shell thickness between cue treatments, I ran analysis of covariance of final STI with initial STI as a covariate. Similarly, to examine relative changes in adductor muscle between treatments I ran an ANCOVA of the final adductor muscle weight with shell surface area as a covariate. To test for homogeneity of slopes, I examined the covariate by treatment interaction and retained them if $p < 0.20$, although they were not significant if $p > 0.05$. Group (i.e. mussels in a replicate bucket) was

nested within treatment and designated a random variable, causing the denominator degrees of freedom to be estimated using Satterthwaite's approximation to test for the treatment effect. Residuals were visually inspected to insure homogeneity of variances. All statistical analyses were conducted in JMP IN 5.1.

## Results

An ANCOVA indicated that there was a nearly significant effect of cue treatment on shell thickness index ($p=0.06$; Table 9, Figure 7a). A priori post hoc comparisons revealed that only mussels exposed to cues from *C. maenas* thickened their shells relative to controls ($p=0.012$). Mussels did not thicken shells in response to either *C. irroratus* ($p > 0.90$) or the both crab species together ($p > 0.90$). An ANCOVA of the relative adductor muscle weight indicated a significant effect of predator cue ($p=0.0058$; Table 10, Figure 7b). Only mussels exposed to *Asterias* or both *Asterias* and *C. irroratus* developed significantly heavier adductor muscles relative to controls ($p=0.0103$ and $p=0.0036$, respectively).

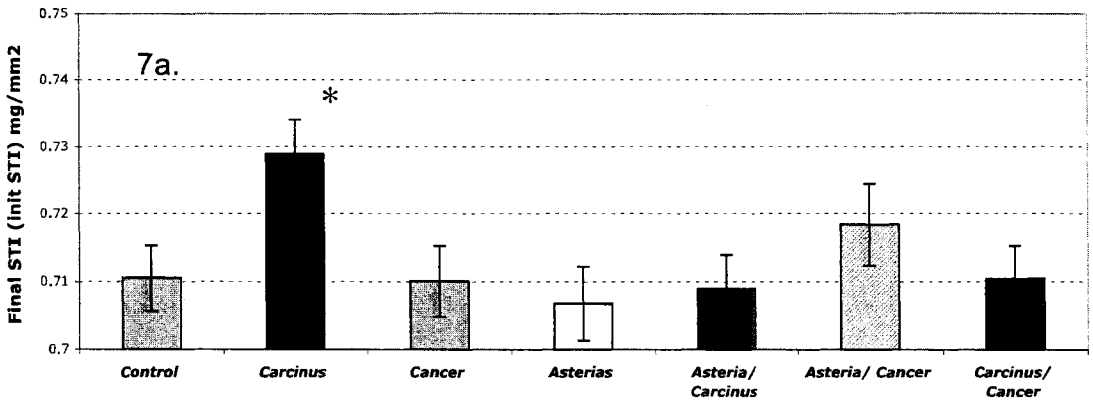
Table 9. ANCOVA Shell thickness index. Results of an analysis of covariance of sell thickness index (adjusted to initial shell thickness index) and a priori linear contrasts of each cue treatment to the control treatment.

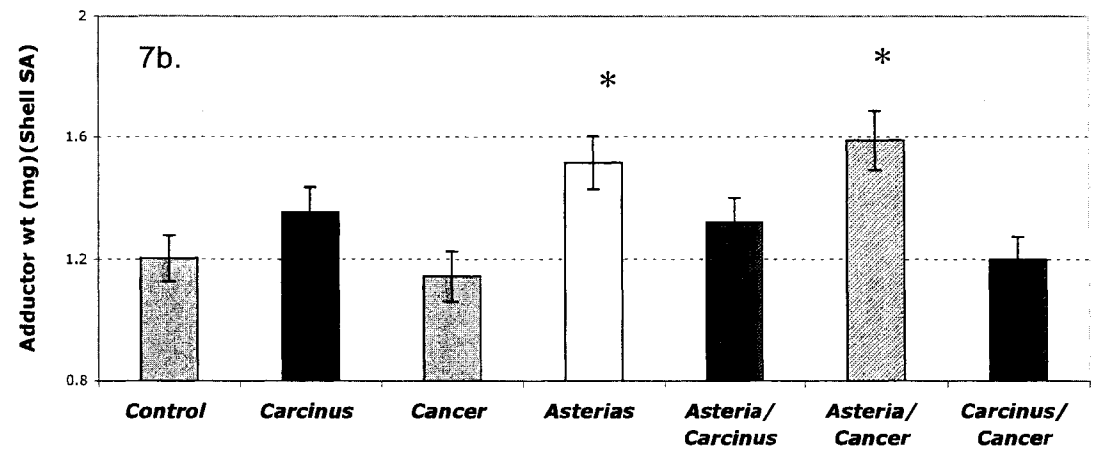
| Source of variation | df | MS Num | F | Prob > F |
|---|---|---|---|---|
| Cue Treatment | 6, 35.3 | 0.00269 | 2.2571 | 0.0601 |
| Group (Treatment) RANDOM | 35, 273 | 0.00122 | 4.6927 | <.0001 |
| Initial STI | 1, 273 | 3.53368 | 13634.04 | <.0001 |
| Initial STI * Treatment | 6, 273 | 0.00039 | 1.5101 | 0.1748 |
| Error | 273 | $2.59 \times 10^{-4}$ | | |
| linear contrasts: Control < ***C. maenas* (p=0.012)**, = *C. irroratus* (p=0.990), = *A. vulgaris* (p=0.652), = *C. maenas* and *C. irroratus* (p=0.990), = *C. maenas* and *A. vulgaris* (p=0.847), = *C. irroratus* and *A. vulgaris* (p=0.272). | | | | |

Table 10. ANCOVA Adductor muscle. Results of an analysis of covariance of final adductor muscle weight (adjusted to mussel shell surface area) and *a priori* linear contrasts of each cue treatment to the control treatment.

| Source of variation | Df | MS Num | F Ratio | Prob > F |
|---|---|---|---|---|
| Cue Treatment | 6, 35.7 | 1.1171 | 3.7025 | 0.0058 |
| Group (Treatment) RANDOM | 35, 273 | 0.3053 | 2.1567 | 0.0003 |
| Final SA | 1, 273 | 67.3478 | 475.7408 | <.0001 |
| Final SA * Treatment | 6, 273 | 0.2889 | 2.0404 | 0.0606 |
| Error | 273 | 0.1416 | | |
| linear contrasts: Control = *C. maenas* (p=0.1786), = *C. irroratus* (p=0.6088), **< *A. vulgaris* (p=0.0103)**, = *C. maenas* and *C. irroratus* (p=0.9675), = *C. maenas* and *A. vulgaris* (p=0.2877), **< *C. irroratus* and *A. vulgaris* (p=0.0036)**. | | | | |

Figure 7a. Final shell thickness index (STI, Adjusted Least Square Mean) adjusted to the initial STI. Mussels were raised for 118 days while exposed to cues from no predator (Control), *Carcinus maenas*, *Cancer irroratus*, *Asterias vulgaris*, *A. vulgaris* & *C. maenas*, *A. vulgaris* & *C. irroratus*, or *C. maenas* & *C. irroratus*. Asterisks (*) indicate treatments significantly different from controls. 7b. Final adductor muscle weight (ALSM) adjusted to the total shell surface area. Notations as in 7a.



## Discussion

In this experiment, mussels increased shell thickness in response to the crab *Carcinus maenas* alone, but did not respond to cues from *Cancer irroratus* alone. In addition, mussels increased adductor muscle in response to cues from *Asterias* alone or the combined cues of *Asterias* and *C. irroratus*, yet they did not express shell thickening in response to the combined cues of *C. maenas* and *C. irroratus.* Mussels also did not express induced shell thickening or adductor muscle growth in response to combined cues from *Asterias* and *C. maenas*. In other words, the simultaneous cues from various 2-predator combinations often interfered with predator specific response to each predator. This indicates that although both crabs share similar foraging strategies (Moody and Steneck 1993), cues from the two crabs did not induce similar responses in mussels and are not substitutable. The differing response to the two crabs is not unprecedented given that gastropod and bivalve mollusks can distinguish between crab species (Marko and Palmer 1991, Freeman and Byers 2006). However, the lack of induced shell thickening in response to cues from *C. irroratus* may be interpreted as maladaptive (e.g.Caudill and Peckarsky 2003) because thicker shells are an adaptive trait in mollusks to deter *Cancer* spp. predation (Palmer 1985). The lack of a response to *C. irroratus* may be related to the differing shared evolutionary histories of these crabs and mussels in the NW Atlantic or current ecological differences between crabs (not considered in their *a priori* categorization) that

suggest different requisite cues for induction of shell thickening (i.e. not just the crabs *per se*).

Shared evolutionary history between predators and prey can influence the expression of inducible traits (Case and Bolger 1991, Magurran et al. 1992, Freeman and Byers 2006) and may explain the mussels' differing response to these crabs. In the NW Atlantic *C. irroratus* is native, however *M. edulis* here have been exposed to the invasive, European green crab (*C. maenas*) for less than 200 years. Several trade-offs associated with inducible defenses may diminish their adaptive value, such as architectural constraints (Trussell and Nicklin 2002), time lags in the expression of inducible defenses (Padilla and Adolph 1996), and other costs associated with maintaining inducible defenses (DeWitt et al. 1998, Ernande and Dieckmann 2004). Over time, due to these constraints and costs of inducible defenses, selection may favor fixed or canalized shell thickness over an inducible defense (Van Tienderen 1991, Meiklejohn and Hartl 2002, Trussell and Nicklin 2002). It is also noteworthy that *C. maenas* cues interfere with the mussel's response to *Asterias* and responses to both predators are therefore not phenotypically integrated, whereas mussels in the presence of *C. irroratus* and *Asterias* can effectively respond to the sea star. Because the interaction of traits expressed in multiple predator environments influences selection on those traits (DeWitt and Langerhans 2003) poor phenotypic integration of inducible defense to *C. maenas* and *Asterias* may indicate a cost of induced responses to *C. maenas*. Thus, even if mussels once expressed induced shell thickening in response to *C. irroratus* this capacity may

have been lost if it was not integrated with the "seastar" response or any of the above trade-offs diminished its adaptive value, ultimately favoring canalized shell thicknesses.

Current differences in the mussels' response to the two crabs may also be related to habitat specific predation threat or the absence of required secondary predation cues. Several sources indicate that the *C. maenas* invasion has had a substantial impact on mollusks (Vermeij 1982, Seeley 1986), presumably greater than *C. irroratus*' historical impact. The greater threat of the invader may lead to greater required inducible defenses (Lima and Bednekoff 1999). Moreover, *C. maenas* often migrates high in the intertidal zone (Hunter and Naylor 1993) but *C. irroratus* normally forage in the low intertidal or subtidal zones (Ellis et al. 2005). Because mussels develop thicker shells in the low intertidal than in higher intertidal sites with or without predator cues (Freeman in prep), the adaptive value of induced shell thickening in response to *C. irroratus* may be lower than for *C. maenas*. Alternatively, although *C. irroratus* has a similar foraging mode to *C. maenas* and may be a less voracious predator, several *Cancer* spp. have far more formidable claws than the invader (Palmer et al. 1999) and can likely crush even mussels induced to thicken shells. Such formidable crushing predation by members the genus may overwhelm induced shell thickening in mussels, reducing its adaptive value. Finally, shell thickening in several molluscan prey can also be induced by crushed conspecifics (Appleton and Palmer 1988, Behrens et al. 1998, Leonard et al. 1999, Trussell and Nicklin 2002). In the present study, predators were fed in separate containers before being returned to

the experimental apparatus, minimizing any influence of consumed conspecifics. If the mussel's response to *Cancer* required the additional cue of crushed conspecifics an induced response to the crab would not be detected in my experimental arrangement. In contrast, the mussels' response to *C. maenas* may additively incorporate their response to crushed conspecifics with their response to the crab (e.g.Trussell and Nicklin 2002) creating a stronger defense against this more threatening, durophagous predator.

Finally, the absence of a response in mussels to *C. irroratus* may also be explained by an ability of the crabs to mask or break down cues detectable by mussels (e.g.Getty 1996, Adler and Grunbaum 1999). *C. irroratus* is native to the NW Atlantic and selection may have favored reduced cue emission from the crab. In contrast, selection has had less time to act on the invasive *C. maenas* to reduce cue emission; or lower genetic diversity of the invading population may constrain the evolution of reduced cue emission. However, consideration of the above explanation for the absence of a response to *C. irroratus* should be tempered by the facts that such selection: (1) is mediated by the mussel's time-lagged response to the crab (e.g.Padilla and Adolph 1996) and therefore can only weakly influence selection on the crab's cues, (2) is inherently weaker than the selection acting on the mussel's inducible defense, partly because the consequence of the crab being detected by the mussel is less severe than the mussel not detecting the crab (Brodie and Brodie 1999), (3) is likely also acting on *C. maenas* in Europe to limit detectable cues emanating from the invader, and

(4) would have to act on all *C. irroratus* individuals present in a mussel's vicinity to reduce cues.

The traits induced in this experiment largely agree with previous studies using a similar design (Freeman in prep), however mussels in previous experiments grew 31-49% more shell and 53-74% more tissue in 12-23% less time than mussels in the current experiment (Freeman pers obs). Reduced growth in the current experiment may have been due to the fact that in the current experiment mussels were housed in steel cages, whereas in previous experiments mussels were free in buckets and often climbed the bucket walls (Freeman pers obs). However, in the current experiment, mussels responded to two *Asterias* as well as a single *Asterias* (i.e. in the *Asterias/Cancer* treatment). This similar response to one or two sea stars suggest two things: 1) that predator cues were saturating the containers and were not reduced by cages in the current experiment; and 2) the mussels' lack of a response to the single *C. maenas* (i.e. in the *C. maenas* & *C. irroratus* treatment) is not due to reduced cues from *C. maenas*, assuming prey respond to similar predator cue thresholds; but more likely due to interference of cues from the two crabs. Thus, although the magnitude of growth in this experiment was reduced the relative expression of induced defenses was similar to previous experiments; only mussels exposed to *C. maenas* thickened their shells, and mussels exposed to *Asterias* increased their adductor muscle (Freeman and Byers in prep) as did mussels exposed to *Asterias* and *C. irroratus*. The concurrence of these induced traits with previous

experiments lends credence to the absence or shell thickening in response to *C. irroratus*, and the interaction of multiple predator cues.

While the evolutionary processes underlying the differing responses to these two crabs cannot be identified well from this study, the mussel responses suggest relatively clear ecological differences, individually and in conjunction with the seastar *Asterias*. Induced responses to *C. maenas* or *Asterias* increase handling time by *C. maenas* by 34-75%, and induced responses to *Asterias* increase handling times by *Asterias* by approximately 33% (Freeman 2007, Freeman and Byers in prep). In contrast, mussels exposed to *C. maenas* and *Asterias*, simultaneously, are not better defended from either predator than control mussels. Similarly, mussels exposed to *C. irroratus* (alone and with *C. maenas*) are not likely to be better defended from the crabs than controls (although the effects of any responses to *C. irroratus* have not been tested in predation experiments). Thus, *C. maenas* and *C. irroratus* may be functional equivalents (sensuLawton and Brown 1993), share similar foraging strategies on mussels (Moody and Steneck 1993), and have overlapping short term ecological effects on mussels. However, given time for the mussels to express induced responses, the two crabs likely initiate different indirect effects; *C. maenas* exposed mussels will be better defended than *C. irroratus* exposed mussels. Moreover, because the mussel's response to the seastar differs depending on the crab species, the indirect effects of multiple predators are difficult to predict based on predator's functional grouping.

Induced defenses in response to multiple predators can elicit responses not predicable from their individual effects (Relyea 2003, Teplitsky et al. 2004)(Freeman and Byers in prep). The effects of multiple predators can leave prey more vulnerable (i.e. risk enhancement)(Hixon and Carr 1997, Losey and Denno 1998, Sih et al. 1998, Meyer and Byers 2005) or make prey less vulnerable to predators (i.e. risk reduction)(Crowder et al. 1997, Sih et al. 1998, Vonesh and Bolker 2005, Griffen and Byers 2006a). Because many prey responses to multiple predators are mediated by chemical (or other) cues, they may reveal distinct trait-mediated interactions (TMIs) not readily apparent from the functional similarity of predators. As such, mutable traits that influence predation (such as inducible morphological defenses) will defy *a priori* classification schemes (Chalcraft and Resetarits 2003, Naeem and Wright 2003), unless they uniformly respond to predators of a functional group. In the current experiment, differing responses to the two crab cues (alone and with the seastar) undermine the utility of functional classification systems. In other examples,inducible traits are influenced by over-generalized prey responses (Langerhans and DeWitt 2002), predator attack strategies (Sih et al. 1998)(Hoverman et al 2005)(Teplitsky et al. 2005)(Schmitz (Barbosa & Castellanos)), or predator specific cues and prey alarm responses (Trussell and Nicklin 2002, LaFiandra and Babbitt 2004). When these traits have appreciable ecological effects the biologically relevant information lost by abstracting species into functional groups (Schmitz and Suttle 2001, Naeem and Wright 2003) draws into question the predictive power of functional groupings. However, the

accuracy of functional groupings may depend on the traits examined and their importance in transmitting indirect effect (Black 1993, Kurzava and Morin 1998, Nystrom et al. 2001, Bolker et al. 2003). For instance, in the current experiment mussel induced defenses depended on the initiating species of crab. In contrast, alterations in foraging behavior of two Pacific Ocean herbivores (a crab and a sea urchin) were identical, regardless of the initiating crab species (*Cancer productus* or *Cancer magister*)(Byrnes et al. 2006).

Currently, there is considerable debate over the importance of functional groups and the role of species identity (within and between functional groups) as it influences ecosystem function (Naeem and Wright 2003, Ives et al. 2005). This debate is clearly relevant to the integrity of ecosystems as extirpation is diminishing diversity, particularly in higher trophic levels (Duffy 2002), and these factors influence ecosystem function (Micheli and Halpern 2005). The emergent impacts of multiple predators not predictable from individual species functional groupings (Sih et al. 1998) is relevant to the debate of biodiversity and ecosystem function, particularly when trait mediated interactions influence community dynamics and are mediated by species specific cues.

CHAPTER V

# TEMPORAL AND SPATIAL VARIABILITY OF BLUE MUSSEL INDUCIBLE DEFENSES IN INTERTIDAL LANDSCAPES


## Abstract

Spatial and temporal variation in predation threat are theoretical underpinnings of inducible defenses, yet the influence of these factors on the expression of inducible defenses is largely unexplored. In this study, I exposed blue mussels (*Mytilus edulis*) to waterborne cues from the crab, *Carcinus maenas*, the seastar, *Asterias vulgaris* (=*rubens*) or both predators in mid and low intertidal heights on Appledore Island (Maine) during two years. After 3 months, these mussels generally increased shell thickness and adductor muscle more in the low intertidal than in the mid intertidal. However, the expression of predator specific induced defenses differed between mid and low intertidal: mid-intertidal mussels responded to *Carcinus* (but not *Asterias*) with induced shell thickening. Furthermore, mussels in low intertidal cages increased adductor muscle only in response to *Asterias*, but only during the year with high tissue growth. In an additional experiment testing the influence of ambient predator cues, mussels in low intertidal cages also responded to *Asterias* cues by increasing adductor muscle weight. These experiments show that ambient

predator cues influence induced defenses, but expression of these defenses may be limited by overriding environmental limitations.

## Introduction

A wide variety of organisms have been shown to adaptively alter their morphologies to accommodate changes in their local environment and predation threat (Tollrian and Harvell 1998). For instance, in response to cues from predators several prey species alter behaviors (Lima and Dill 1989) or defensive morphologies, such as test shape in barnacles (Lively 1986b), molluscan shell thickness and sculpting (Appleton and Palmer 1988, Trussell 1996, Leonard et al. 1999) and spines on cladocerans and bryozoans (Harvell 1984, Tollrian 1995). While there has been a proliferation of studies documenting phenotypic plasticity in the presence or absence of relevant predator cues in constant environments, the influence of variable environments on inducible defenses has been largely un-explored (but seeRelyea 2004a, Hoverman et al. 2005). Moreover, there is a paucity of studies exploring these factors outside of laboratory settings, in realistic/natural settings. Only by further exploring the influences of temporal and fine spatial variability on phenotypic plasticity (e.g.Huber et al. 2004, Miner and Vonesh 2004) can we understand the ecology and evolution of phenotypic plasticity (Miner et al. 2005) and the latter's role in processes such as invasions (Richards et al 2006).Fine spatially and temporally variable environmental factors (e.g in resources, predators, and competitors) are

likely a very important evolutionary influence on phenotypic plasticity of sessile organisms (Travis 1996, Huber et al. 2004).

Sessile organisms are greatly affected by habitat heterogeneity as each successive generation may be exposed to environmental conditions differing from the previous. Dispersal between heterogeneous environments can overwhelm traits locally favored by selection (Storfer and Sih 1998, Johannesson 2003) or lead to local adaptation to conditions (Bertness and Gaines 1993, Stachowicz and Hay 2000, Sotka 2005). Besides affecting selection on fixed traits dispersal can greatly favor the evolution of phenotypic plasticity (Moran 1992, Scheiner 1998, Sultan and Spencer 2002), ultimately allowing organisms to persist in these variable environments and altering interactions with competitors and predators (Travis 1996). Moreover, inducible responses to many predators have evolved within settings of heterogeneous environmental factors that may directly influence their expression.

The capacities to express predator sensitive behaviors and morphologies can be directly influenced by various environmental factors. For instance, when provided with more food, larval anurans can more effectively express predator avoidance behaviors (Anholt and Werner 1995). Similarly, limiting resources or stresses associated with strong abiotic and biotic gradients can modify or constrain the expression of inducible defenses ( Rundle et al. 2004, Wiackowski and Szkarlat 1996, (Relyea 2004a). Moreover, alterations of available resources for growth or abiotic stress can erode the adaptive advantages of induced defenses by altering costs and benefits of induced phenotypes (Dawidowicz and

Loose 1992, DeWitt et al. 1998, Wiackowski and Szarlat 1996). However, these patterns are rarely tested in natural conditions (Huber et al. 2004). To address temporal and spatial heterogeneity in the expression of inducible defenses I manipulated several factors that could influence the expression of inducible defenses in blue mussels (*Mytilus edulis*).

Marine mussels, such as *Mytilus edulis,* are sessile invertebrates and frequently subject to both temporal and fine spatial variability in factors affecting growth (Eckman and Duggins 1991, Mallet and Carver 1993, Frandsen and Dolmer 2002). Under these circumstances, mussels have also evolved morphological defenses specific to a predator's mode of feeding on mussels. The specificity of mussel's induced defenses have been well established in laboratory studies: when raised with cues from the seastar, *Asterias vulgaris* (=*rubens* (Wares 2001)), they increase adductor muscle growth, but when exposed to cues from the crab, *Carcinus maenas,* they increase shell thickness (Leonard et al. 1999, Smith and Jennings 2000, Reimer and Harms-Ringdahl 2001). However, when exposed to the combined cues from *Asterias* and *Carcinus* mussels allocate toward neither inducible defense (Freeman and Byers in prep). Although the genus *Mytilus* has figured prominently in intertidal ecology and a few studies have compared mussel morphology as it pertains to induced defenses and local predator assemblage (Theisen 1982, Kautsky et al. 1990, Leonard et al. 1999), none have induced morphological defenses in intertidal *Mytilus*. *In situ* observations may be essential as these organisms often occupy

an intertidal landscape where gradients in productivity may have considerable influence on the expression of several predator specific inducible defenses.

Intertidal gradients may directly influence the expression of inducible defenses. Many marine organisms in the low intertidal are immersed longer and have more feeding time than higher in the intertidal. As a result, filter feeders experience higher growth rates in the lower intertidal zone (Robles et al. 1990, Bertness et al. 1998) as well as greater predation pressure (Lubchenco 1980, Menge 1983, Robles et al. 1995). In addition, intertidal organisms can experience annual variation in resource availability and subsequent growth (Mallet and Carver 1993). Because inducible defenses in *Mytilus* require growth and resources, one could predict that in habitats with higher growth mussels will be better able to allocate to inducible defenses. Alternatively, different *Mytilus* induced defenses (e.g. adductor muscle and shell thickness) may be more easily expressed provided specific resources. Through the variable temporal and spatial expression of induced defenses in mussels, these factors may influence the evolution and ecological impacts of inducible defenses across intertidal landscapes.

To address temporal and spatial variability in the expression of mussel-induced defenses in response to predator cues, I raised mussels under differing habitat conditions, when exposed to non-lethal, caged predator cues. Mussels were raised for approximately 3 months in mid and low intertidal cages while exposed to cues from *Asterias*, *Carcinus*, both predators, or no predator. In addition, I raised mussels in intertidal cages at 10 sited in coastal Maine, New

Hampshire, and Massachusetts to address how they responded to ambient predator cues. Through these experiments, I found that annual variation in growth, intertidal gradients, and ambient predator cues affected species-specific induced defenses.

## Materials and Methods

**2004 Mid and Low Cages** - During the summer of 2004, I ran the first of two intertidal caged predator and mussel induction experiment at Shoals (i.e. Appledore Island, ME). I collected mussels (14.5-23.6 mm shell length) from the low intertidal zone at Nubble Light (York, ME) in late June 2004 and returned them to the laboratory for measurement. To quantify the dry shell weight of each live mussel, I used a technique describe by Palmer (1982). With each mussel suspended in seawater under a below-beam balance, I measured its immersed mass. I similarly obtained the immersed masses of an additional 25 mussels (drawn from the same pools of mussels) to create a destructive regression that could be used to calculate the dry shell weight of living mussels (Dry Shell Weight = Immersed*1.5794-0.000037, $R^2$>0.999, n=25) (Palmer 1982). In addition, using digital calipers (0.01mm), I measured the shell length of each mussel (the greatest anterior to posterior shell dimension), then separated the mussels into 48 replicate groups of 6, and marked each mussel with color-coded paint dots. I then transplanted mussels to mid and low intertidal cages on three rock ledges on both sides of Smith's Cove, Appledore Island (42.98573° N, 70.61910° W). Cages were constructed of stainless steel mesh (20cm x 20cm x

9cm, l x w x h: 0.5 cm mesh opening) with a large "arena" for crabs and seastars and a small (7 cm x 10 cm) stainless steel mesh internal compartment housing and protecting the pre-measured mussels. Cages were bolted to the rock substrate with mid intertidal cages at approximately +1.7m, and low intertidal cages at approximately +0.75m (above MLW).

Cages were randomly assigned to contain 2 *C. maenas*, 2 *A. vulgaris*, 1 of each, or no predator (controls), such that a random series of the 4 treatments was repeated every 4 cages, i.e. 6 times at each tidal height. In addition, each cage in the mid intertidal was paired with an adjacent low intertidal cage directly below. This was done to allow pairs of cages sites in the mid and low to remove some variability in mussel growth associated with cage placement. Although, I removed rockweed (*Ascophyllum nodosum*) in order to attach cages, adjacent rockweed often rested on and covered mid-intertidal cages (low intertidal cages were below the rockweed zone). I also added a large handful of rockweed (*Ascophyllum nodosum*) to the large "arena" in each cage to mitigate predator desiccation. Predators were added to cages on July 4, 2004 and every two weeks afterward cages were monitored and any dead predators replaced. Ambient *Asterias* or *Carcinus* near these cage sites were removed with each visit. Cages were removed 76 days later and mussels frozen for later morphological measurements. All mussels in 5 mid and 13 low intertidal cages were dead at the end of the experiment (many due to predation by small whelks, *Nucella lapillus*). In addition, two cages from the low intertidal zone washed away. Tidbit data loggers (Onset Corporation, Bourne, MA) attached to the

inside, bottom of one mid and one low recorded the air and water temperature inside the cage at 15 minute intervals.

**2005 Mid and Low Cages** - To determine patterns of temporal variability, in 2005 I repeated cage transplants as in 2004. In May 2005, I collected mussels (15.3-25 mm shell length) from the low intertidal zone at Nubble Light (York, Maine) and maintained them in non-flowing seawater until measured. Initial measurements included: initial dry shell weight (estimated as in 2004) and shell length. To insure repeated estimates of spatial factors in the second year, I attached the same 48 cages used in 2004 in mid and low intertidal zones, using the same bolt-holes used in 2004. Cages were also randomly assigned to contain 2 *C. maenas*, 2 *A. vulgaris*, 1 of each, or no predator (controls). Predators and 10 mussels $cage^{-1}$ were added in mid-June and removed 81 days later. In this iteration of the experiment, the internal cages housing mussels were positioned with >1 cm from the exterior cage wall to prevent ambient *Nucella* from attacking mussels. During the experiment, cages were checked approximately every 2 weeks and any dead predators replaced. Ambient *Asterias* or *Carcinus* near these cage sites were removed with each visit. Mussel loss to *Nucella* predation was again substantial; all mussels were consumed in15 low intertidal cages and 1 high intertidal cages and were not used in the analyses. Tidbit data loggers (Onset Corporation, Bourne, MA) in one mid and one low intertidal cage recorded the temperature inside cages at 15 minutes intervals.

**Statistical Analysis of Shoals Cages** - I summarized the following temperature data from the mid and low intertidal cages in 2004 and 2005:

average daily maximum temperature, average daily temperature, and average daily temperature range (maximum – minimum temperature). These summary temperatures were then analyzed using a two-way analysis of variance with year and tidal height as factors. In this model, date was nested within year because temperature measurements in high and low intertidal cages were not independent on any given day, relative to the temperature measurements made in different years. I used Tukey tests to make post hoc comparisons.

After the experiments, I measured all mussels from 2004 & 2005 mid and low intertidal cages (shell length, width and height; as in Freeman in press) then separated and dried adductor muscle tissue and remaining tissue. Linear shell measurements provided a good estimate of shell surface area using the equation: $L^2 \times (W^2 + H^2)^{-2} \times 1/2$ pi (Reimer and Tedengren 1996, Freeman and Byers 2006). In order to examine how shell thickness was influenced by tidal height, year, and predator cue treatment I used a nested analysis of covariance (ANCOVA) of final shell thickness with initial shell thickness as a covariate to adjust for initial shell thickness. For this analysis a shell thickness index (STI= dry shell weight/ shell surface area) was the response variable with an estimate of shell thickness (initial shell weight$^{-2}$/ shell length) as a covariate. In addition, to examine how year, treatment, and tidal height affected adductor muscle growth I used an ANCOVA of final adductor muscle weight with final shell surface area as a covariate (to adjust for mussel overall size). Lastly, I determined if final mussel tissue weight (adductor and remaining tissues) were affected by these factors, I used a 3-way, ANCOVA with initial shell weight as a covariate. Initially, these

models included all higher order interactions of fixed factors (Year, Treatment, and Tidal Height) with the covariate. All interactions of these factors were first tested against the covariate to insure homogeneity of slopes and then discarded if their P> 0.20. Because the paired placement of mid and low intertidal cages had a substantial effect on several growth parameters, I used cage pair ("Placement") as a blocking factor across tidal height and years. To recognize cage as a replicate, cue treatment, tidal height, and year were nested within cage (i.e. Cage (Treatment, Tidal Height, Year)) and designated a random effect. For this random, nested factor the statistical program JMP use the REML technique to estimate variance components, thus F-statistics and P-values are "shrunken" to 0. To determine which predator cue combination had an effect on mussel induced defenses, I then compared each predator cue treatment to controls using *a priori* contrasts. I also compared tissue growth between tidal heights and years using a Tukey test.

**Ambient Predator Cue Effects** - To examine the influence of un-manipulated, ambient predator cues and spatial variability in abiotic factors on the expression of induced defenses, I raised mussels at 10 intertidal sites with differing predator assemblages. In July 2005, I collected small mussels (15 – 23 mm) from an exposed shore in the rocky, low intertidal zone near Nubble Light (ME). I then labeled and measured the mussels using the following technique: 500+ mussels were haphazardly divided into 10 groups of similar sized mussels. Mussels were weighed while suspended from a below beam balance to obtain immersed shell weights (later used to estimate initial shell weights; see below)

and measured for shell length, width, and height and individually marked with colored dots. Mussels for a total of 50 cages were measured in this manner. At each of 10 sites (Table 11), I placed 5 cages (each containing 10 mussels) at least 2 meters apart on large rocky ledges approximately 0.5m above MLW. Cages containing mussels were enclosed within a second cage, and both were bolted to the rock substrate. The cage-within-a-cage arrangement better protected the mussels from actual predation, particularly by *Nucella lapillus* that can reach through single cages and drill mussel shells (Freeman pers. obs.). A single temperature logger (Tidbit) was placed in the middle cage at each site and logged temperature every 15 minutes during the experiment.

Cages were in place for approximately 88 days beginning in late-July 2005. Three times during the experiment (at the beginning of the experiment and once a month thereafter), I surveyed the sites during low tide and counted all mobile fauna (mostly *Nucella*) within a 1 $m^2$, circular quadrat centered on each cage. I also surveyed each site twice while snorkeling just prior to high tide. During each immersed survey of the sites, I counted the number of large mobile fauna (crabs and sea stars) within a 1 $m^2$, circular quadrat centered on each cage. At the end of the experiment, I collected the surviving 167 mussels and froze them for later morphological measurements. By the end of the experiment all mussels in 13 of the 50 cages had been killed by small *Nucella* recruits, and were not used in analyses.

**Ambient Predator Cue Analysis** - In order to analyze mussel morphological changes in response to ambient predator cues, I categorized each

of the 37 cages based on predator presence, temperature, and wave exposure. I categorized 19 cage locations as having no *Carcinus* if *Carcinus* was not found within a meter$^2$ during any survey of cages, and the remaining 18 cages were categorized as having been exposed to *Carcinus*. Similarly, 21 cages were categorized as having no *Asterias,* and 16 cages were categorized as exposed to *Asterias*. Because only 8 cages had no *Nucella* within the meter$^2$ during surveys, I used the median *Nucella* density (4.5 Nucella meter$^{-2}$) as an objective cut-off for considering *Nucella* present. As such, 19 cages were categorized as having negligible *Nucella* present (i.e. <5 individuals m$^{-2}$) and 18 were categorized as having *Nucella* present (i.e. 5 to 30 individuals m$^{-2}$). By categorizing the cage sites in this way, each treatment level conveniently subdivided other treatment levels. For instance, of the 18 cage locations with *Nucella* present, 12 also had *Carcinus*; and of the these 12 cages with both *Carcinus* and *Nucella,* 7 also had *Asterias*. Using this categorization scheme 5 of the 6 predator combinations were represented by at least 3 cages, however a single cage was exposed to both *Asterias* and *Nucella* but no *Carcinus*. Thus, most predator categories were represented within each other predator category allowing comparisons of the influence of each predator's presence on the expression of inducible defenses.

Using the above predator categories I examined the influence of ambient predator cues on mussel morphology. To determine initial shell dry weights from immersed weights I used the same destructive regression as in 2004 Shoals cages. I removed adductor muscle tissue and remaining tissue and dried shells and tissues in a drying oven overnight. I then obtained dry adductor muscle

weight, dry tissue weight, final dry shell weight, shell length, width, and height of mussels and used the shell dimensions to calculate their shell thickness index (see above). Initial and final shell length, width, height, and weight were used to calculate initial and final STI (see above equation). The morphological data from mussel growth was analyzed using an analysis of variance (ANOVA) of the mean residuals for each cage of the shell thickness index, adjusted to initial STI, and the mean residual for each cage of the adductor muscle weight, adjusted to shell surface area. Because predator assemblages were quantified for each cage, I considered cages to be independent replicates when analyzing mussel morphological data and predator assemblage. Moreover, when I incorporated predator assemblage nested within the 10 sites (i.e. Site (*Asterias*, *Nucella*, *Carcinus*)) as a random factor it was not significant ($P>0.20$.).

I also incorporated temperature in the above Analysis of Variance using the mean for each site of the daily minimum temperature, daily average temperature, daily temperature range, and daily maximum temperature. I categorized these site temperatures "high" if they were at or above the median of that metric (for all sites combined), or "low" if they were below the median of that metric. I also subjectively categorized the wave exposure of each site as "high" or "low". I then individually added each of these 5 abiotic factors to the above analysis of variance with ambient predator assemblages to determine if they influenced shell thickness index or adductor muscle weight. Because each abiotic factor was measured once for each site, I included a random nested level (site nested within the abiotic factor category). The statistical program JMP used

the random nested level to generate the denominator mean squares and degrees of freedom when testing the abiotic factor, however, second-order interactions were tested over the error term.

## Results

**Shoals Mid and Low Intertidal Cages** - Induction of shell thickening, adductor muscle growth, and overall tissue growth showed differing patterns in Shoals mid and low intertidal cages. Shell thickness index showed a (nearly) significant interaction of tidal height and cue treatment ($F_{1,56}$=2.65, P=0.0572; Table 12). While mussels in the low intertidal developed generally thicker shells (Figure 8), only mussels in the mid intertidal thickened shells in response to cues from *Carcinus* (*a priori* contrast, P<0.05). In addition, mussels in 2005 had consistently thicker shells than in 2004 ($F_{1,56}$=125.53, P<0.0001; Table 12, Figure 8). In contrast, adductor muscle weight was influenced by an interaction of cue treatment, tidal height, and year ($F_{1,48}$=3.997, P=0.0127; Table 13, Figure 9). Mussels responded to cues from *Asterias* with increased adductor muscle growth relative to controls, but only in low intertidal cages in 2005 (Table 13, Figure 9). However, because of high mortality, the latter results are based on only 3 mussels (2 cages) in low intertidal *Asterias* cages in 2004 and 4 mussels (1 cage) in 2005. None of the mussels in mid intertidal cages allocated more towards their adductor muscles than controls. In fact, in 2004, mussels in low and mid intertidal cages had significantly reduced adductor muscle growth when they were raised with cues from *Carcinus,* as did mussels in mid-intertidal cages

in 2005 when raised with *Asterias* (Figure 9, $P<0.05$). At no point did mussels exposed to both *Asterias* and *Carcinus* cues increase their adductor muscle or shell thickness. Mussel tissue growth was influenced by the interactive effect of tidal height and year ($F_{1,54}=5.41$, $P=0.0236$) but not predator cue treatment (Table 14, Figure 10). Mussel tissue increased similarly in mid and low intertidal cages in 2004, but in 2005 mussels in the low intertidal grew faster than those in the mid intertidal. (I observed similar tidal height and year effects on shell weight.). Thus, in spite of relative lower tissue (and shell) growth in the mid-intertidal, mussels responded positively to cues from *Carcinus* by increasing shell thickness only in mid intertidal cages (Table 12, Figure 8). However, mussels responded to *Asterias* with increased adductor muscle growth but only in the low intertidal in the year with high tissue growth rates (Tables 13 & 14, Figures 9 & 10).

**Mid and Low Intertidal Cages, Temperatures** - Average Daily Temperatures, Average Maximum Daily Temperatures and Average Daily Temperature Ranges were influenced by interactions of year and tidal height ($F_{1,174}=4.623$, $P=0.0329$, $F_{1,174}=20.192$, $P<0.0001$ and $F_{1,174}=21.792$, $P<0.0001$, respectively, Table 15). In 2004, the temperature differences between mid and low cages were more pronounced than in 2005. For instance, the average daily temperatures were warmer in the mid than the low intertidal and warmer in 2005 than in 2004, but these differences were more pronounced in 2004 (Figure 11). Moreover, the maximum daily temperature and daily temperature range did not differ between mid and low intertidal cages in 2005, but they did differ in 2004

(Figure 11). Thus although average temperatures were consistently warmer in mid than low intertidal cages, animals in the mid intertidal cages did not consistently experience greater stress (as indicated by the daily maximum and range in 2005) than low intertidal mussels.

**Ambient Predator Cues** - In response to ambient predator cues in low intertidal cages, mussels responded to *Asterias* cues, but showed little effect of *Carcinus* or abiotic factors. A 3-way analysis of variance of residual adductor muscle weight (adjusted to total shell surface area) indicated that there was a significant interaction of *Asterias* and *Carcinus* presence (Table 16, $P=0.0062$). *A priori* contrasts indicated that *Asterias*/No *Carcinus* had significantly larger relative adductor muscles than all other cue exposures (Figure 12; $P<0.002$). Moreover, mussels in cages exposed to *Nucella* cues had significantly larger adductor muscles than those not exposed to *Nucella*, but there was no interaction of *Nucella* presence and *Carcinus* or *Asterias* presence (Table 16, Figure 12). A 3-way ANOVA of shell thickness (STI) of mussels exposed to ambient predator cues indicated there was an interaction of *Asterias* and *Carcinus* cues on caged mussels (Table 17, Figure 13). However, a Tukey test indicated that none of these predator categories had a significant effect. Finally, none of the abiotic factors (wave exposure or temperature metrics) or interactions of abiotic factors and predator cues influenced induction of shell thickness index (all $Ps>0.15$) or adductor muscle (all $Ps>0.18$).

Table 11. Transplant sites. Transplant sites examining ambient predator cue effects. Exposure indicates the subjective categories of wave exposure. Max/Min of temperature and Average/Range of temperature indicate categories of Low and High if the average of the daily temperatures were below or above the median of all sites for that metric.

| *Site* | Latitude (°N) Longitude (°W) | Exposure | Max/Min Temp. (°C) | Average/Range Temp. (°C) |
|---|---|---|---|---|
| Nahant Dive Beach East | 42.41990 70.90240 | High | Low/High | High/Low |
| Nahant Dive Beach West | 42.41980 70.90340 | Medium | Low/Low | Low/Low |
| Nahant Pump House Beach | 42.41710 70.90570 | High | High/High | High/Low |
| Odiorne North | 43.05275 70.71664 | High | High/High | High/High |
| Odiorne South | 43.03658 70.71373 | High | High/High | High/High |
| Fort Stark East | 43.05920 70.71290 | Medium | Low/Low | Low/High |
| Fort Stark West | 43.05820 70.71150 | Medium | Low/Low | Low/Low |
| Shoals Appledore Ledges | 42.98583 70.61174 | High | High/High | High/Low |
| Shoals Larus North | 42.99117 70.61680 | Medium | Low/Low | Low/High |
| Shoals Smith's North | 42.98573 70.61910 | Medium | High/Low | High/High |

Table 12. ANCOVA STI of Shoals experiment. Nested ANCOVA of final shell thickness index (STI) with initial shell weight$^{-2}$/ shell length as a covariate for 320 mussels raised in mid and low intertidal cages at Shoals. Factors include Treatment (Control, *Carcinus*, *Asterias*, and both), tidal height (mid and low), year (2004 &2005), and placement (low cages paired with cages in the mid-intertidal zone). Higher order interactions were excluded if they were not significant (P>0.11). "a" indicates factors that have variance components shrunken to zero using the REML technique.

| Source of variation | DF | SS | F-Ratio | P |
|---|---|---|---|---|
| Treatment | 3, 56 | 0.0122 | 1.1634 | 0.3319 |
| Tidal Height | 1, 56 | 0.1033 | 29.465 | <.0001 |
| Tr*TH | 3, 56 | 0.0279 | 2.6560 | 0.0572 |
| Year | 1, 56 | 0.4402 | 125.53 | <.0001 |
| Cage [Tr,Y,TH]&Random | 55, 59 | 0.0022 | . | a |
| placement | 23, 172 | 0.1164 | 1.4427 | 0.0972 |
| Initial Shell Weight/Length | 1, 59 | 3.8894 | 1109.09 | <.0001 |
| CageX ISW/L [Tr,Y,TH]&Random | 59, 172 | 0.00819 | . | a |

Table 13. ANCOVA Adductor muscle mass of Shoals experiment. Nested ANCOVA of final adductor muscle weight adjusted to the surface area of each mussel. Higher order interactions excluded if P> 0.16. Factors and notations as in Table 12.

| Source | DF | SS | F Ratio | P |
|---|---|---|---|---|
| Treatment | 3, 49 | 0.00001208 | 6.4819 | 0.0009 |
| Tidal Height | 1, 49 | 0.00000783 | 12.605 | 0.0009 |
| Tr*TH | 3, 49 | 0.00000339 | 1.8171 | 0.1563 |
| Year | 1, 49 | 0.00000115 | 1.8589 | 0.1790 |
| Tr*Y | 3, 49 | 0.00000694 | 3.7263 | 0.0172 |
| TH*Y | 1, 49 | 0.00000363 | 5.8444 | 0.0194 |
| Tr*TH*Y | 3, 49 | 0.00000745 | 3.9969 | 0.0127 |
| Cage[Tr,Y,TH]&Random | 48, 52 | 0.00000216 | a | a |
| placement | 23, 173 | 0.00004195 | 2.9365 | <.0001 |
| Final Shell Surface Area | 1, 52 | 0.00010436 | 168.04 | <.0001 |
| Tr*FSSA | 3, 52 | 0.00000463 | 2.4875 | 0.0707 |
| TH*FSSA | 1, 52 | 0.00000196 | 3.1618 | 0.0812 |
| Year*FSSA | 1, 52 | 0.00000353 | 5.6813 | 0.0208 |
| TH*Y*FSSA | 1, 52 | 0.00000079 | 1.2684 | 0.2652 |
| Cage*FSSA[Tr,Y,TH]&Random | 52, 173 | 0.00002239 | a | a |

Table 14. ANCOVA Tissue mass of Shoals experiment. Nested ANCOVA of final tissue dry weights with initial shell dry weights as a covariate of 320 mussels raised in mid and low intertidal cages at Shoals. Factors and notations as in Table 12. *A posteriori* Tukey tests indicated that in 2005 mussels raised in the low intertidal grew faster than those in the mid intertidal ($P<0.05$), but not in 2004.

| Source of variation | DF | SS | F-Ratio | P |
|---|---|---|---|---|
| Treatment | 3, 57 | 0.00043 | 1.0288 | 0.3868 |
| Tidal Height | 1, 57 | 0.00130 | 9.283 | 0.0035 |
| Year | 1, 57 | 0.00442 | 31.625 | <0.0001 |
| TH*Year | 1, 57 | 0.00076 | 5.410 | 0.0236 |
| Cage*[Y,Tr,TH] random | 58, 57 | 0.00209 | . | a |
| placement | 23, 173 | 0.00379 | 1.190 | 0.2696 |
| Initial Shell Dry Weight | 1, 57 | 0.01848 | 132.23 | <0.0001 |
| Y*ISDW | 1, 57 | 0.00124 | 8.8812 | 0.0042 |
| Cage* ISDW[Tr,Y,TH] random | 57, 173 | 0.00047 | . | a |

Table 15. Shoals temperatures. Comparisons of temperatures in Shoals mid and low intertidal cages for 2004 and 2005. ANOVAs of 3 temperature metrics. See Figure 11 for post-hoc Tukey-tests.

| | | Average Daily Temperature | | Maximum Daily Temperature | | Daily Temperature Range | |
|---|---|---|---|---|---|---|---|
| **Source** | **DF** | **F** | **P** | **F** | **P** | **F** | **P** |
| Year | 1, 174 | 39.7697 | <.0001 | 44.5845 | <.0001 | 18.6820 | <.0001 |
| Tidal Height | 1, 174 | 149.9656 | <.0001 | 57.2284 | <.0001 | 35.7328 | <.0001 |
| Yr*TH | 1, 174 | 4.6232 | 0.0329 | 20.1921 | <.0001 | 21.7922 | <.0001 |
| Date[Yr]&Rnd | 174 | 21.6827 | <.0001 | 4.1329 | <.0001 | 3.7452 | <.0001 |

Table 16. ANCOVA Adductor muscle mass- Ambient Cues. Ambient predator cue effects on transplanted mussels; ANOVA of adductor muscle weights (residuals adjusted to shell surface area). Site (*Asterias*, *Nucella*, *Carcinus*) was excluded because it was not significant (P>0.20).

| Source | DF | SS | F-Ratio | P |
|---|---|---|---|---|
| Nucella | 1, 32 | 1.31E-05 | 9.462 | 0.0043 |
| Asterias | 1, 32 | 2.05E-05 | 14.803 | 0.0005 |
| Carcinus | 1, 32 | 8.20E-06 | 5.914 | 0.0208 |
| Asterias *Carcinus | 1, 32 | 1.19E-05 | 8.593 | 0.0062 |
| Error | 32 | 3.91E-05 | | |

Table 17. ANCOVA STI- Ambient Cues. Ambient predator cue effects on transplanted mussels; ANOVA of final shell thickness index (STI). Final STI values are the residuals of a regression of initial STI (x-axis) against final STI (y-axis). Site(Asterias, Nucella, Carcinus) was excluded because it was not significant (P>0.20).

| Source | DF | Sum of Squares | F-Ratio | P |
|---|---|---|---|---|
| Nucella | 1,32 | 2.06E-04 | 0.0633 | 0.8029 |
| Asterias | 1,32 | 0.00494 | 1.5195 | 0.2267 |
| Carcinus | 1,32 | 0.00548 | 1.6843 | 0.2036 |
| Carcinus*Asterias | 1,32 | 0.0203 | 6.2427 | 0.0178 |
| Error | 32 | 0.10404 | | |

Figure 8. Final STI- Shoals Experiment. Final shell thickness index (STI) of mussels raised in middle and low intertidal cages with various (non-lethal) predators. Years are graphed separately to depict annual differences, however contrasts are done on both years together (no factor showed higher order interactions with year). Cue treatments significantly different from controls in *a priori* contrasts (after pooling the effect of Year) are indicated by an "*".

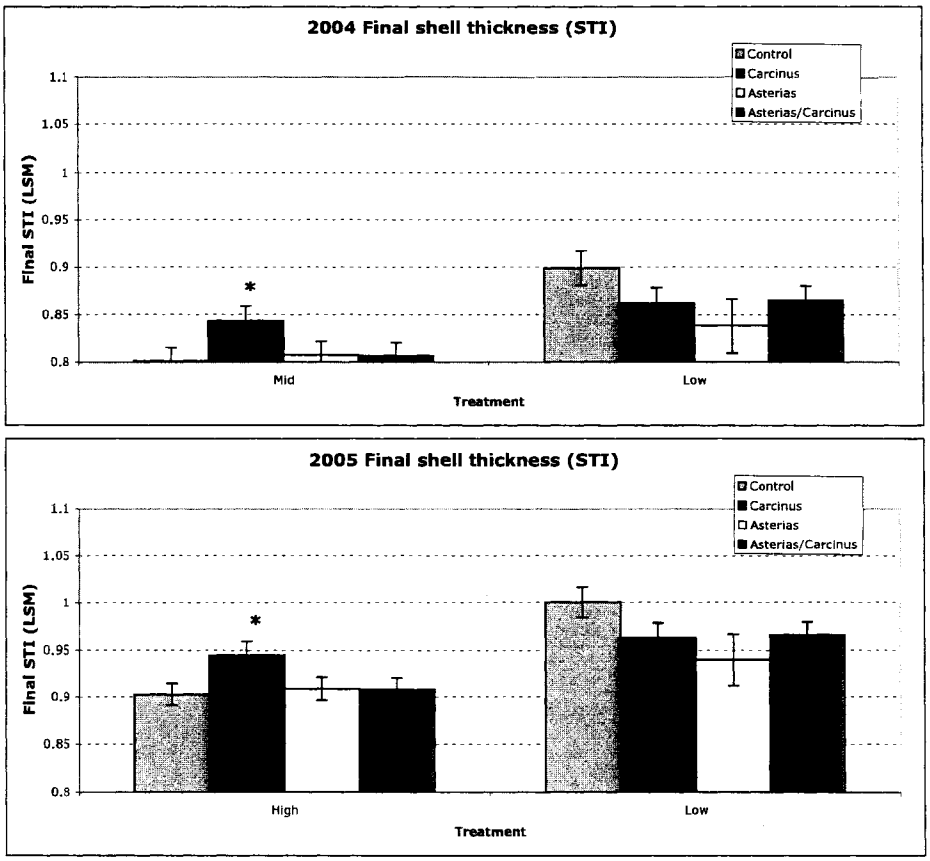


Figure 9. Final Adductor Muscle Mass- Shoals Experiment. Final adductor muscle weight of mussels raised in intertidal cages at Shoals with various (non-lethal) predators. Values are adductor muscle weights adjusted to the total shell surface area. A "*" indicates a treatment level that is significantly different from the control for that year and tidal height in *a priori* contrasts ($P<0.05$).

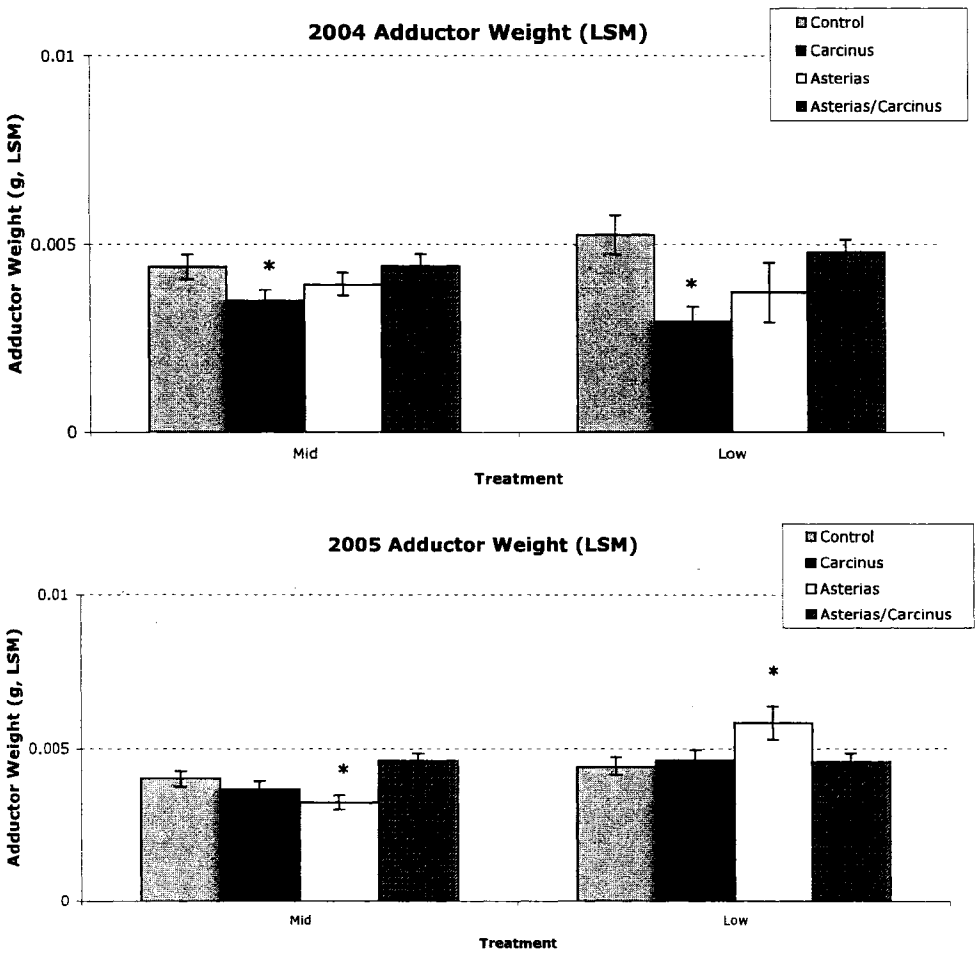


Figure 10. Final Tissue weight- Shoals Experiment. Tissue growth of mussels transplanted to the mid and low intertidal in cages at Shoals. Final tissue weight is the least square mean (LSM) adjusted to the initial shell weight. Predator cue treatment had no effect on growth. Mussels in 2005 grew significantly faster than in 2004, and there was an interactions between year and tidal height. Bars sharing letters were not significantly different in a Tukey test.

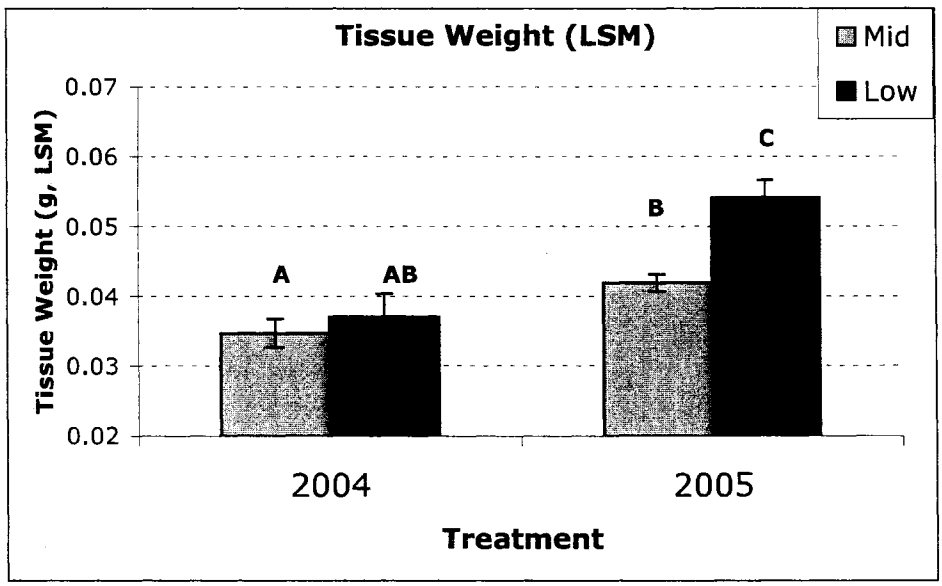


Figure 11. Temperatures- Shoals Experiment. a) Average Daily Temperature, b) Maximum Daily Temperature, and c) Daily Temperature Range for mid and low intertidal cages at Shoals. Results are means (± SE). Bars sharing letters were not significantly different in a Tukey test.

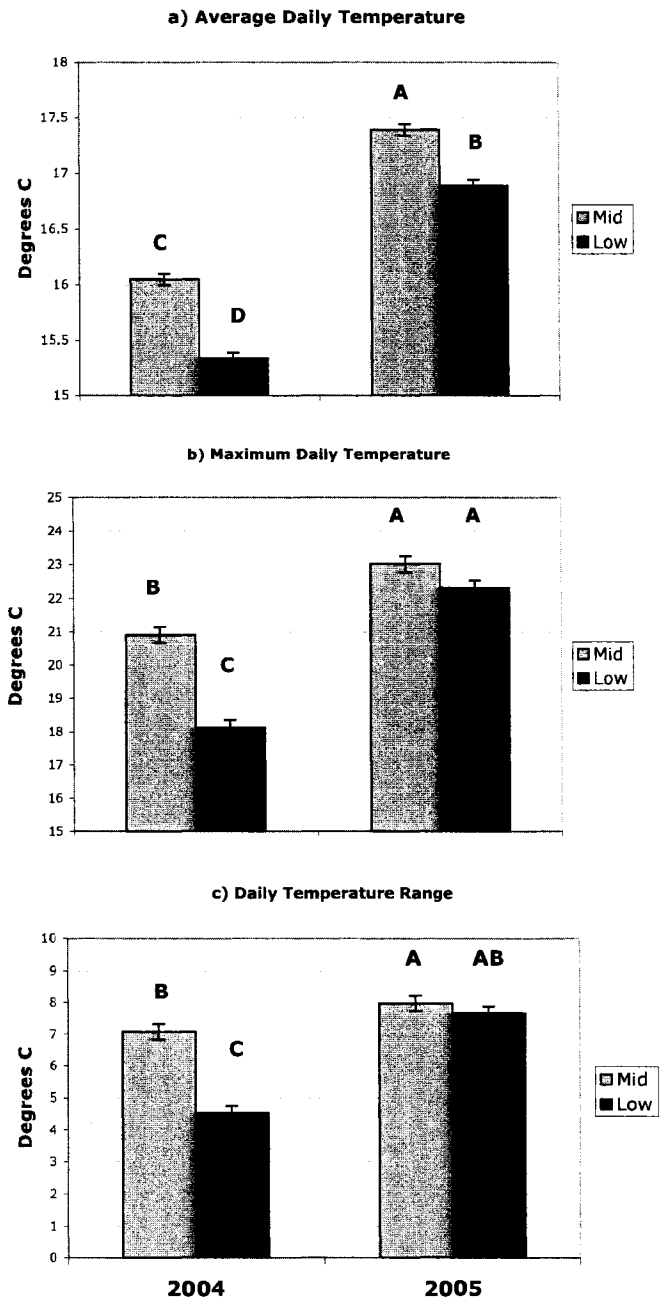


Figure 12. Adductor Muscle Weight-Ambient Predators. Adductor muscle weight (residuals adjusted to total shell surface area) of mussels raised in 37 *in situ* cages, with and without *Carcinus* and *Asterias* and with *Nucella* (a) or without *Nucella* (b). Numbers above bars indicate the number of cages in each treatment level at the end of the experiment.

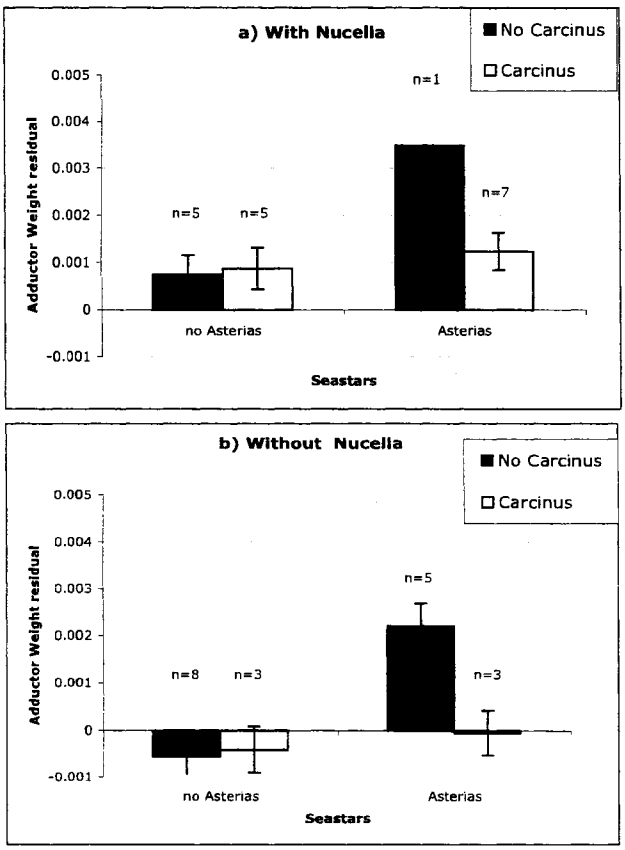
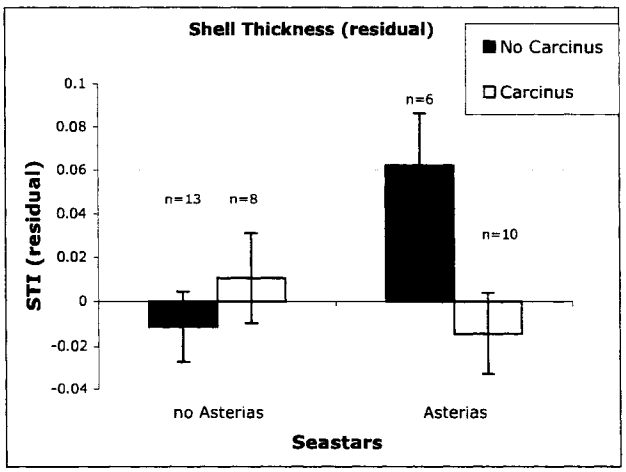


Figure 13. Final STI - Ambient Predators. Values are the residuals of a regression against initial STI (Shell thickness index). Notations as in Figure 12.



## Discussion

In this series of experiments, the capacity of mussels to express predator specific responses in an intertidal landscape was influenced by proximity to predator cues, but induced defenses were only expressed under specific habitat conditions. In mid and low intertidal cages at Shoals, the mussels responded to *Carcinus* in the mid intertidal but not in the low intertidal. In addition, these patterns of shell thickening in mid and low intertidal cages were very similar between years despite annual differences in temperature (Table 15, Figure 11) and tissue growth rates (Table 14, Figure 10). In contrast, mussels only expressed induced adductor muscle increase in response to *Asterias* in low intertidal cages and during the year that they exhibited high growth rates (Figures 9 and 10). Similarly, mussels raised in low intertidal cages in sites exposed to ambient cues from both *Carcinus* and *Asterias* expressed neither predator specific induced defense, however mussels did increase adductor muscle in response to *Asterias* alone. In addition, mussels in sites with *Nucella* consistently increased adductor muscle. However, in these low intertidal cages mussels did not thicken shells in response to ambient *Carcinus* cues. These patterns of differential expression of inducible defenses may be related to phenotypic integration, differing requirements of induced shell thickening and adductor muscle growth, background cues, and the relationship of these inducible traits across immersion and productivity gradients.

**Phenotypic Integration** - In Shoals cages, mussels exposed to *Asterias* in the low intertidal allocated more towards their adductor muscles but did not increase their shell thicknesses (STI), while mussels exposed to *Carcinus* in the mid intertidal increased shell thickness but showed evidence of reduced adductor muscle growth. However, mussels did not express these predator specific responses to combined cues from *Asterias* and *Carcinus*. This disruption of opposing predator specific responses suggests poor phenotypic integration of these defenses (sensu Schlichting 1989), i.e. that increasing shell thickness in response to *Carcinus* is not compatible with increasing adductor muscle in response to *Asterias*. Although reduced feeding in the presence of either predator could explain some patterns (i.e. reduced adductor muscle in response to *Carcinus* in the mid and low or to *Asterias* in 2004 mid intertidal cages), there was no predator cue treatment effect on tissue growth. If the lack of this response was due to reduced feeding (Palmer 1990) or "lowered assimilation efficiency and/or a higher metabolic rate" (e.g.Stoks 2001), one would expect that mussels with higher growth rates (i.e. low intertidal mussels with more available food) could express both defenses simultaneously (e.g.Andersson et al. 2006), yet the mussels did not. Moreover, mussels do not appear to reduce feeding behavior in response to *Asterias* and *Carcinus* (individually or together), but they do reduced feeding in response to crushed conspecifics (Meszarros and Freeman in prep) further suggesting poor phenotypic integration of these defenses.

**Shell Growth** - The occurrence of induced shell thickening in response to *Carcinus* may be overridden by interactive effects of relative tissue and shell growth, but also influenced by factors directly affecting shell deposition (temperature, immersion time etc.). Molluscan shell growth is often maintained at constant shell accretion rates resulting in a trade-off between linear shell growth and shell thickening. But this trade-off is regulated by tissue growth; conditions of high tissue growth result inhigher linear shell growth, and reduced shell thickening (Kemp and Bertness 1984, Trussell 2000). High tissue (and shell) growth rates in low intertidal mussels may have obscured any apparent induced shell thickening in response to *Carcinus*. Moreover, fast growing mussels have lower risk of predation as they reach a size refuge quickly (Mallet and Carver 1993), suggesting and adaptive advantage for low intertidal mussels to maintain high growth. However, for a given size, mid-intertidal mussels may have thicker shells than low intertidal mussels (e.gBeadman et al. 2003); this pattern reflects the limited tissue growth of high intertidal mussels that results in thick-shelled phenotypes (e.g.Kemp and Bertness 1984).

In addition to interactions with tissue growth, changes in shell thickness during this experiment were likely influenced by factors directly influencing shell growth, i.e. organic resources to build shell, temperature, and immersion time. About 50% of carbon incorporated in bivalve shells is contributed from metabolic carbon (Tanaka et al. 1986), suggesting that factors increasing metabolism may increase shell deposition. However, shell accretion can often proceed when food is low or absent (Palmer 1990, Alluno-Bruschia et al. 2001) and because mussel

shells are < 5% organic this ionic process is influenced by temperature. Shell calcification rates are facilitated by increases in temperature (Malone and Dodd 1967) and impeded by greater dissolution rates in colder water due to lower $CaCO_3$ saturation (Trussell 2000). Through these mechanisms, higher average daily temperatures may have contributed to greater increases in shell thickness in 2005 (Figures 8 & 11). Yet despite annual temperature difference (Figure 11) mussels in the mid intertidal in both years responded to *Carcinus* with increased shell thickness, suggesting that detection of induced shell thickening in response to *Carcinus* may have been obscured in low intertidal mussels by greater dissolution due to longer immersion times in cold water. Collectively, shell growth may be facilitated by higher food availability and interact with tissue growth rates, but detection of induced defenses may be limited by interactions of water temperature, immersion times, and overall growth rates.

**Productivity and Somatic Induced Defenses** - In intertidal Shoals cages, there were clear differences in the expression of induced defenses related to tidal height (immersion time) and annual differences in growth rate. Although induced shell thickening in low intertidal mussels may have been obscured partly by higher growth rates, increased growth rates due to higher food availability may have facilitated the expression of induced defense involving somatic growth (i.e. an increase in adductor muscle due to cues from *Asterias* sp.). In mussels, tissue synthesis precedes shell growth (Mallet and Carver 1993, Alluno-Bruschia et al. 2001) but is largely limited by quantity and quality of food and seston (Page and Richard 1990, Ross and Nisbet 1990, Lesser et al. 1994). Low intertidal

mussels in Shoals cages in 2005 had higher tissue growth rates likely related to higher chlorophyll a levels during the experiment in 2005 than 2004. Estimates of Chla $m^3$ based on Modis satellite images indicated average Chla levels of 5.0 mg $m^3$ in 2005 and 3.5 mg $m^3$ in 2004, during the experiments. However, higher growth rates only translated to larger adductor muscles for mussels raised with cues from *Asterias*. These factors suggest there is considerable potential for fine spatial and temporal variation in the expression of induced defenses and their influence on trophic interactions.

Although tidal height, annual differences in growth and ambient predator cues clearly affected the mussel's induced responses, the lack of significant site-to-site differences in abiotic factors may be partly due to lower replication or insufficient range of these abiotic factors. Mussel growth can vary regionally and seasonally (Alluno-Bruschia et al. 2001) and locally with supplements from kelp fragments (Duggins and Eckman 1994). Areas with higher coastal productivity can support higher growth rates of suspension feeders (Menge 1992, Sanford and Menge 2001, Phillips 2005, Blanchette et al. 2006). Results in the present study indicate that high productivity alone does not result in increased adductor muscle, but may facilitate the expression of induced adductor muscle increase in responses to *Asterias*. However, *in situ*, patterns of productivity and prey's induced responses to predators may be difficult to disentangle. For instance, near-shore circulation patterns may promote higher recruitment and result in greater competitive interactions between sessile adults (Connolly and Roughgarden 1998). Increased mussel density influences both the effectiveness

of predators (Dolmer 1998) and competition for available seston can vary considerably within patches of filter-feeders (Bertness et al. 1998). A combination of high growth rates and high recruitment may allow mussel beds to persist despite sea star predation, if growth rates allow mussels to attain a size refuge (Mallet and Carver 1993, Reusch and Chapman 1997). Finally, predators may aggregate in areas with high prey abundances resulting in stronger top-down control of this bottom-up process (Robles et al. 1995). These influences may obscure many ecological effects of induced responses to predator presence.

While the responses of prey to predators are informative of their strategies for a particular set of environmental conditions, gaining a more complete picture of the evolutionary pressures on predator sensitive inducible defenses requires exploring the expression of these defenses across the various environments prey populations experience. For instance, numerous studies have demonstrated *Mytilus'* induced responses in homogeneous laboratory (Leonard et al. 1999, Smith and Jennings 2000) or subtidal settings (Reimer and Tedengren 1996, Reimer 1999, Reimer and Harms-Ringdahl 2001, Freeman and Byers 2006), but detection of similar induced responses was influenced by tidal height and annual growth. Interactions between resources and predator induced behaviors and defenses can clearly alter the impacts of predator sensitive behaviors (Turner 2004). If active foraging by prey increases predation, mortality of prey can be reduced at higher resource levels (Anholt and Werner 1995), or if reduced foraging by prey increases resources prey can have increased growth (Peacor 2002). Although, mussel induced defenses largely appear to be independent of

predator induced reductions in feeding (Meszaros and Freeman in prep), in general resource availability can clearly affect the expression of inducible defenses (Peckol et al. 1996), shape of the reaction norm (Pigliucci 2001, DeWitt and Scheiner 2004), and costs of induced defenses (Dawidowicz and Loose 1992). Finally, several studies suggest that at low resource levels costs of induced defenses can be more pronounced than at high resource levels (Dawidowicz and Loose 1992, Turner 2004). By altering the adaptive advantage of induced defenses, environmental factors likely influence their selection, particularly in sessile, intertidal organisms.

**Background Cues** - Although ambient predator cues from all 4 predators appear to influence induced defenses in mussels, these patterns likely have differing implications for the observed patterns in the two experiments. In field surveys, ambient predator density estimates were likely more accurate for slower predators (i.e. *Asterias* and *Nucella*) than for more mobile predators (i.e. *Carcinus*). This difference in accuracy may partly explain the lack of shell thickening in response to ambient *Carcinus*. However, low intertidal Shoals caged mussels also did not respond to caged *Carcinus* by thickening their shells. While these coincident patterns further suggest that induced shell thickening is difficult to detect in the low intertidal (due to high growth), interference from ambient cues may have been a factor for several reasons. First, although I removed *Asterias* and *Carcinus* from this Shoals site, *Carcinus* likely migrated to the site at appreciable numbers (approx. 2-3 $m^2$; Freeman pers obs; Ellis et al. 2005). Ambient *Carcinus* cues may have influenced the mussel's response to

*Asterias* in the low intertidal in 2004, but it did not negate a response to *Asterias* in the low intertidal in 2005. In addition, cues from *Carcinus* may have affected all low intertidal treatments and diminished any difference between Controls and *Carcinus* exposed mussels, particularly if crabs congregated around cages. Background cues from mobile decapod predators are more likely to affect low intertidal cages than higher shore cages, simply because these predators must pass through the low intertidal and are less likely to make it into the higher intertidal. Finally, *Nucella* was present among these low intertidal cages at Shoals, but ambient *Nucella* cues did not have an interactive, or overriding effect on the observed response to *Asterias* cues (Tables 16 & 17).

**Environment Frequency and Selection on Induced Defenses** - Clearly, the exposure of a prey population to predation pressure will influence the evolution of novel inducible morphological defenses (Freeman and Byers 2006) and behaviors (Magurran et al. 1992); and the canalization of these defenses to fixed defenses (Trussell and Nicklin 2002, Dalziel and Boulding 2005). Because each successive generation of sessile mussels settles in the subtidal zone or along an intertidal gradient, local selection in these habits will influence the level of plasticity for the whole population. While the frequency at which prey encounter predator environments affects selection for plasticity (Van Tienderen 1991, Tufto 2000, Sultan and Spencer 2002), selection can only act on inducible defense when environmental factors allow defenses to be expressed (Ernande and Dieckmann 2004). Thus, the ability of mussels to express induced defenses under environmental conditions where predators are encountered will affect their

ecological and evolutionary effects. These environmental factors can alter the costs and benefits of an inducible phenotype (Dawidowicz and Loose 1992, Huber et al. 2004, Turner 2004) or directly limit their expression (Rundle et al. 2004). While it is advantageous for an organism to only express an induced defense when a predator is present (de Jong and van der Meijden 2000), if local environmental factors reduced costs or limitations of inducible defenses it may be advantageous for prey to spontaneously allocate towards these defenses, even when predators are not present. Although it is unlikely that intertidal and subtidal mussels are locally adapted, more rapid shell thickening in low intertidal mussels, regardless of predator cues, may be a favored strategy if costs of induced defenses are reduced or mussels there predictably experience more intense predation.

The vast majority of studies of plasticity have placed organisms in a single environment and observed responses. However, unlike the locally reproducing organisms that have received much attention regarding inducible defenses in marine systems (i.e. *Nucella* spp. (Appleton and Palmer 1988) and *Littorina obtusata* (Trussell 1996)), *Mytilus* has widely dispersing larvae that recruit into locations with different predator assemblages, tidal heights, and coastal productivities. Like many sessile organisms with pelagically dispersed larvae, these mussels experience highly variable environments between generations, which favors phenotypic plasticity (Sultan and Spencer 2002), but underscores the importance of spatial and temporal variability in the expression of these defenses. Ultimately, exogenous restrictions on the ability to express induced

defenses will impact the ecological effects of similar inducible defenses across these habitats. Variable control of the expression of induced defenses by abiotic factors will influence the evolution and maintenance of inducible defenses within (meta)population of mussels.

# APPENDIX

Table A1. Unadjusted STI (Nahant 2002). Initial and final unadjusted treatment mean Shell Thickness Indexes used in the ANCOVA of Nahant (MA) 2002 laboratory induction experiment. Site abbreviations as in Table 1. Mussels grew in all treatments.*

| | **Initial STI Control** | **Initial STI *Carcinus*** | **Initial STI *Hemi-grapsus*** | **Final STI *Control*** | **Final STI *Carcinus*** | **Final STI *Hemi-grapsus*** |
|---|---|---|---|---|---|---|
| **Source, Site** | **Mean (SE)** | **Mean (SE)** | **Mean (SE)** | **Mean (SE)** | **Mean (SE)** | **Mean (SE)** |
| **South, PJRI** | 0.591 (0.027) | 0.673 (0.036) | 0.633 (0.028) | 0.614 (0.028) | 0.685 (0.038) | 0.655 (0.029) |
| **South, SCT** | 0.741 (0.027) | 0.728 (0.036) | 0.705 (0.03) | 0.678 (0.028) | 0.671 (0.038) | 0.682 (0.031) |
| **South, NCT** | 0.687 (0.028) | 0.726 (0.04) | 0.648 (0.031) | 0.708 (0.029) | 0.770 (0.041) | 0.698 (0.033) |
| **South, MNY** | 0.593 (0.031) | 0.656 (0.036) | 0.612 (0.028) | 0.616 (0.033) | 0.708 (0.038) | 0.669 (0.029) |
| **South, CNY** | 0.680 (0.027) | 0.763 (0.044) | 0.665 (0.028) | 0.687 (0.028) | 0.772 (0.046) | 0.661 (0.029) |
| **South, PLNY** | 0.561 (0.028) | 0.710 (0.04) | 0.584 (0.036) | 0.594 (0.029) | 0.752 (0.041) | 0.643 (0.038) |
| **North, LME** | 0.562 (0.031) | 0.577 (0.04) | 0.578 (0.03) | 0.608 (0.033) | 0.653 (0.041) | 0.649 (0.031) |
| **North, CME** | 0.595 (0.031) | 0.681 (0.04) | 0.650 (0.03) | 0.660 (0.033) | 0.753 (0.041) | 0.680 (0.031) |
| **North, JME** | 0.566 (0.027) | 0.633 (0.051) | 0.545 (0.027) | 0.664 (0.028) | 0.760 (0.053) | 0.635 (0.028) |
| **North, MME** | 0.552 (0.027) | 0.575 (0.04) | 0.531 (0.028) | 0.581 (0.028) | 0.644 (0.041) | 0.592 (0.029) |
| **North, PHME** | 0.618 (0.03) | 0.611 (0.04) | 0.589 (0.034) | 0.646 (0.031) | 0.682 (0.041) | 0.619 (0.035) |
| **North, BME** | 0.559 (0.026) | 0.598 (0.036) | 0.533 (0.028) | 0.613 (0.027) | 0.689 (0.038) | 0.594 (0.029) |

*The following are mussel shell weight changes [g LSM (SE)] from an ANOVA of the Nahant (MA) 2002 laboratory induction experiment: North Control = 0.064 (0.004), South Control = 0.048 (0.004), North Carcinus = 0.077 (0.006), South Carcinus = 0.065 (0.006), North Hemigrapsus = 0.065 (0.004), South Hemigrapsus 0.059 (0.004).

Table A2. Unadjusted STI (Woods Hole 2003). Initial and final (unadjusted) treatment mean Shell Thickness Index used in the ANCOVA of Woods Hole (MA) 2003 *in situ* induction experiment. Mussels grew in all treatments.*

| | **Initial STI Control** | **Initial STI *Carcinus*** | **Initial STI *Hemi-grapsus*** | **Final STI *Control*** | **Final STI *Carcinus*** | **Final STI *Hemi-grapsus*** |
|---|---|---|---|---|---|---|
| **Source, Site** | **Mean (SE)** | **Mean (SE)** | **Mean (SE)** | **Mean (SE)** | **Mean (SE)** | **Mean (SE)** |
| **South, JRI** | 0.766 (0.032) | 0.730 (0.032) | 0.725 (0.034) | 0.713 (0.032) | 0.752 (0.032) | 0.766 (0.033) |
| **South, PJRI** | 0.767 (0.036) | 0.794 (0.032) | 0.773 (0.034) | 0.732 (0.035) | 0.789 (0.032) | 0.764 (0.033) |
| **South, APCT** | 0.820 (0.032) | 0.822 (0.032) | 0.798 (0.032) | 0.751 (0.032) | 0.805 (0.032) | 0.807 (0.032) |
| **South, SCT** | 0.770 (0.032) | 0.720 (0.034) | 0.764 (0.032) | 0.762 (0.032) | 0.747 (0.033) | 0.807 (0.032) |
| **South, NCT** | high mortality - all discarded | | | | | |
| **North, SME** | 0.655 (0.032) | 0.644 (0.032) | 0.657 (0.032) | 0.733 (0.032) | 0.734 (0.032) | 0.724 (0.032) |
| **North, JME** | 0.586 (0.051) | 0.547 (0.051) | 0.642 (0.042) | 0.696 (0.05) | 0.786 (0.05) | 0.781 (0.041) |
| **North, BME** | 0.638 (0.042) | 0.602 (0.036) | 0.607 (0.032) | 0.705 (0.041) | 0.700 (0.035) | 0.698 (0.032) |
| **North, PHME** | 0.686 (0.034) | 0.718 (0.032) | 0.644 (0.039) | 0.741 (0.033) | 0.798 (0.032) | 0.738 (0.038) |
| **North, WME** | 0.557 (0.034) | 0.587 (0.036) | 0.582 (0.032) | 0.706 (0.033) | 0.798 (0.035) | 0.704 (0.032) |

*The following are mussel shell weight changes [g LSM (SE)] from an ANOVA of the Woods Hole *in situ* induction experiment: North Control = 0.254 (0.033), South Control = 0.201 (0.031), North Carcinus = 0.332 (0.032), South Carcinus = 0.280 (0.030), North Hemigrapsus = 0.259 (0.030), South Hemigrapsus 0.290 (0.031).

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