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Poor phenotypic integration of blue mussel inducible defenses in environments with multiple predators

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Aquatic prey encounter an array of threat cues from multiple predators and killed conspecifics, yet the vast majority of induced defenses are investigated using cues from single predator species. In most cases, it is unclear if odors from multiple predators will disrupt defenses observed in single-predator induction experiments. We experimentally compared the inducible defenses of the common marine mussel *Mytilus edulis* to waterborne odor from pairwise combinations of three predators representing two attack strategies. Predators included the sea star, *Asterias vulgaris* (= *Asterias rubens*), and the crabs *Carcinus maenas* and *Cancer irroratus*. The mussels increased adductor muscle mass in response to cues from unfed *Asterias* (a predatory seastar that pulls mussel shells open) and increased shell thickness in response to unfed *Carcinus,* a predatory crab that crushes or peels shells. However, the mussels did not express either predator specific response when exposed to the combined cues of *Asterias* and *Carcinus*, and mussels did not increase shell thickness when exposed to cues from *Cancer* alone or any pairwise combination of the three predators. Shell closure or 'clamming up' did not occur in response to any predator combination. These results suggest that predator-specific responses to the *Asterias* and *Carcinus* are poorly integrated and cannot be expressed simultaneously. Simultaneous cues from multiple predators affect the integration of predator specific defenses and predator odors from functionally similar predators do not necessarily initiate similar defenses. Ultimately, the degree that prey can integrate potentially disparate defenses in a multiple predator environment may have ecological ramifications and represent a seldom explored facet of the evolution of inducible defenses.

Many prey organisms advantageously alter their defensive behaviors or morphologies in response to threat cues from predators. Inducible morphological defenses can take the form of increased spines on cladocerans and bryozoans (Tollrian and Harvell 1998), bent morphologies in barnacles (Lively 1986), thickened shells of mollusks (Palmer 1990, Trussell 1996, Brookes and Rochette 2007, Freeman 2007), induced defensive morphologies and behaviors in anuran larvae (Smith and Van Buskirk 1995, Relyea 2004), and defensive chemicals in plants (Karban and Baldwin 1997). The vast majority of these studies have investigated induced responses to the presence or absence of cues from single predator species (reviewed by Lima and Dill 1990, Tollrian and Harvell 1998, Relyea 2004). However, an understanding of the ecology and evolution of induced defenses based on single predator systems may be incomplete as most predator–prey systems involve multiple predators (reviewed by Sih et al. 1998, Relyea 2003). Prey responses to multiple predators can often interact to affect prey mortality (e.g. risk enhancement or risk reduction; Sih et al. 1998, DeWitt and Langerhans 2003, Teplitsky et al. 2004, Meyer and Byers 2005), thus environments with multiple predators must be considered in the evolution and ecology of induced defenses, particularly when combined predators elicit poorly integrated responses.

Inducible defenses allow organisms to adjust phenotypes and maintain a higher average fitness across dissimilar environments than organisms with constitutive, 'non-plastic' phenotypes (Via and Lande 1985). The ability of prey to integrate distinct defensive phenotypes will directly influence the overall 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). For instance, predator specific inducible defenses that integrate well in multiple predator environments may be more favored than predator specific responses that do not integrate well 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 isolated interactions of prey with a single predator species (DeWitt and Langerhans 2003, 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 2004).

To examine the impact of multiple predators on induced defenses we used the common marine bivalve, *Mytilus edulis* and three important predators on this mussel: 1) *Asterias rubens* (=*Asterias vulgaris*; Wares 2001), a native sea star, 2) *Carcinus maenas*, an introduced, but long-established, crab in the northwest Atlantic, and 3) *Cancer irroratus*, a native crab in the northwest Atlantic. *Mytilus* is a common species in many intertidal and shallow subtidal, hard-substrate marine communities and ideal for investigating the impacts of multiple predators on inducible 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).

Different species of predators may simultaneously induce morphological defenses, but the defensive responses may be similar and integrate well if the predators belong to a similar functional group (Laforsch and Tollrian 2004), or the defenses may be dissimilar and not integrate well if the predators belong to different functional groups (Benard 2006, Hoverman and Relyea 2007). If prey respond to species belonging to functional groups with similar induced traits then induced defenses may be more tractably incorporated in theoretical models (Bolker et al. 2003). Because *Mytilus* responds to predators with different foraging strategies with appropriate morphological defenses (Smith and Jennings 2000, Reimer and Harms-Ringdahl 2001, Freeman 2007) and can distinguish between crab species (Marko and Palmer 1991, Freeman and Byers 2006), mussels are a fitting system to address generalizations about responses to functional groups. We compared *Mytilus'* response to *Cancer* and *Carcinus* as these crabs may be considered a functionally similar group (Moody and Steneck 1993). While *Mytilus* in Europe respond to *Cancer pagurus* by increasing byssal threads (Cote 1995), it is not clear if mussels express morphological defenses to *C. irroratus*, and if *Cancer* and *Carcinus* initiate similar morphological responses in *Mytilus* (individually and in combination with *Asterias*).

Behavioral responses to predators can influence the appearance of induced defenses (Brookes and Rochette 2007), we therefore explored how shell closure behavior, or 'clamming up', in *Mytilus* is influenced by various waterborne cues. The behaviors and morphologies of marine mollusks can be modified by waterborne cues from unfed predators (i.e. predator odor) (Marko and Palmer 1991, Trussell and Nicklin 2002, Freeman 2007), predators fed killed prey conspecifics (Reimer and Tedengren 1996, Trussell 1996, Leonard et al. 1999, Smith and Jennings 2000, Trussell and Smith 2000, Trussell and Nicklin 2002), and predators feeding on unrelated prey (Leonard et al. 1999, Smee and Weissburg 2006). Often responses to these threat cues may have similar underlying selection pressures and ecological outcomes, yet the kinds of cues may induce different behaviors in prey (Smee and Weissburg 2006) and lead to differing capacities to recognize invasive predators (Chivers et al. 2001). For instance, if prey respond to killed conspecifics but not predator odor, evolutionarily novel, invasive predators would go unrecognized unless they are feeding on prey conspecifics. Thus, because behavioral responses may affect the manifestation of inducible defenses (Palmer 1990, Trussell 2000, Brookes and Rochette 2007) it is essential to understand if behavioral responses are occurring and if responses to predator odors and killed conspecifics differ.

In this study, we addressed the following questions, 1) can predator specific inducible defenses be integrated when predator cues are combined? 2) Are responses to the crabs (*Cancer* and *Carcinus*) similar and substitutable, individually and with *Asterias*? 3) Do mussels show behavioral reductions in feeding elicited by predator odor or crushed conspecifics that might interfere with induced morphological defenses?

Material and methods

Induction experiments

In June 2003, a laboratory induction experiment compared mussels' induced morphological response to pairwise combinations of *Carcinus* and *Asterias*. Mussels were collected from a floating dock at the Univ. of New Hampshire's Coastal Marine Laboratory (Newcastle, NH). Mussel shells were measured with digital calipers (length, width and height; ± 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 individual mussel shell was estimated by measuring the immersed mass of the live mussel using a below beam balance while the mussel was suspended in seawater on a mesh net. Prior to weighing, mussels were kept submerged to allow their gaping shells to expell any bubbles that would interfere with the immersed measurement. Forceps were then used to close the gaping valves and transfer mussels to the weighing apparatus. Because the mussel tissue is neutrally buoyant, this technique isolates the mass of the mussel shell. The dry shell mass of experimental mussels was then accurately estimated from the immersed mass using a separately quantified relationship of immersed mass and dry shell mass. This regression relationship was generated through destructive sampling of immersed and dry shell mass of a group of mussels subsampled from the experimental source pool (in 2003, mussel dry shell mass = $1.599 \times$ immersed mass $+0.002$, $R^2 > 0.999$, $n = 28$; in 2004, mussel dry shell mass = $1.557 \times$ immersed mass $+ 0.006$, $R^2 > 0.999$, $n = 19$). Finally, the pre-measured, experimental mussels were marked with small color-coded dots using paint pens, and dots covered with cyanoacrylate glue to increase the mark's durability.

Forty replicate buckets (3.5 l) were arranged in a sea table at the Univ. of New Hampshire's Coastal Marine Laboratory. Each bucket was independently supplied with flowing, unfiltered seawater drawn from the shallow subtidal (6–7 m depth) approximately 100 m from shore (1.5–1.9 l min^{-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 four predator cue treatments: Control (i.e. no predators in two empty containers), two *Carcinus*, two *Asterias*, or one *Carcinus* and one *Asterias*. The perforated containers allowed cues from predators to permeate the bucket but prevented access of the predators to the mussels or interactions between predators. Twice weekly, excess sediments were siphoned from each bucket, and every four weeks the buckets were cleaned and randomly rearranged on the sea table to diminish any effect of irregular air or water flow. At each four-week cleaning, predators were removed to a separate container and fed crushed mussels. Within eight 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 of 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 airflow. These replicates were not used in analyses.

In order to determine if responses to *Carcinus* and *Asterias* were robust and responses to *Carcinus* were similar to the native *Cancer irroratus*, a second induction experiment was initiated, incorporating *Cancer* crabs in the pairwise predator combinations. In June 2004, small (15–26 mm) mussels were collected from Nubble Light, York (Maine) and, following the protocol used in 2003, were measured, labeled, randomly selected, and divided among 42 replicate buckets (10 mussels bucket^{-1}). Mussels were housed in wire cages. Pairs of cue containers were randomly assigned to contain the following predator cue combinations: Control (no predator), two *Carcinus*, two *Asterias*, two *Cancer*, one *Carcinus* and one *C. irroratus*, one *Carcinus* and one *Asterias*, or one *Cancer* and one *Asterias*. Thus, two predators, in separate mesh-sided containers, resided in each replicate bucket. Predators were collected from the shallow subtidal and intertidal at Fort Stark, NH. Replicates were maintained as in 2003 and experiment ran for 118 days. At the end of the experiment, all mussels were frozen for later morphological measurements.

Morphological statistics

To determine if mussels expressed predator specific responses the following traits were compared between treatments: the shell thickness (adjusted to initial shell thickness for individual mussels), adductor muscle dry mass (adjusted to final shell surface area), and change in tissue mass. Final dry shell mass, adductor muscle mass, and tissue mass were obtained after samples were dried at 70°C for 36 h. Shell thickness index (STI) was used 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: $SA = [L \times (H^2 + W^2)^{0.5} \times \prod/2]$. 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 four locations (left and right valves, center and lip thickness; $p < 0.0001$, $R^2 = 0.911$, $n = 48$). An analysis of covariance (ANCOVA) of final STI with initial STI as a covariate was used to compare shell thickness between cue treatments. An ANCOVA of final adductor muscle weight was used to examine relative changes in adductor muscle between treatments. Shell surface area was used as a covariate because it correlates well with mussel volume and is the surface against which seastars must pull to open the mussel. Because water retained in the mantle cavity obscures tissue mass of live mussels, tissue mass at the beginning of each experiment was estimated from the initial, immersed mass of each mussel using a destructive regression of mussels subsampled from the source pool. In 2003 initial tissue mass = $0.0968 \times$ immersed mass $+ 0.0032$, $R^2 = 0.9778$; and in 2004 initial tissue mass $= 0.1588 \times$ immersed mass $- 0.0029$, $R^2 = 0.9172$, $n = 19$. The standard test for homogeneity of slopes was used; the covariate by treatment interactions were examined and retained if $p < 0.20$, although they were not significant if $p > 0.05$. Group (i.e. all 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 6.0.

Behavioral observations

A series of behavioral observations examined the responses of *Mytilus* to waterborne cues from *Asterias*, *Carcinus*, *Asterias* and *Carcinus* together, and crushed conspecifics. The crushed conspecific cue illustrates a treatment commonly applied in inducible defense experiments in which predators are fed conspecifics and served as a positive control for a behavioral response. Medium (26–55 mm) and small (9–25 mm) *Mytilus* were collected from the intertidal zone on Appledore Island in the Gulf of Maine. Mussels were separated into groups of 20 and randomly assigned to test for behavioral responses to one of the following cue treatments: *Carcinus* (three individuals), *Asterias* (three individuals), *Asterias* and *Carcinus* together (three individuals of each), three crushed medium mussels, or no predator (control). Aquaria (7.5 l) were built as flow-through systems with a drain hole at one end, unfiltered sea water input at the other end, and plastic grating separating the tank into two chambers. Prior to trials mussels were placed in the bottom of respective aquariums and allowed to acclimate for 20–30 min. After a record was made of the initial ($t = 0$) number of mussels with papillae extended or shells closed (i.e. feeding or not feeding), predator cue treatments were started by gently placing predators in the upstream compartment in respective aquaria. At intervals of 5, 10, 20, 30, 60, 120, 240 and 360 min, the number of mussels with shells closed was recorded. At the end of each behavioral trial, mussels and predators were discarded and aquaria thoroughly cleaned. A block of the five cue treatments for each size class of mussels was run within a two-day period, with medium and small mussels tested in alternating two day blocks. This process was repeated a total of 10 times for each mussel size class.

In statistical analyses of feeding-related behaviors, to control for the initial condition of each mussel group the change in number of mussels with closed shells was used as a response variable [i.e. $C_{tx} = B_{tx} - B_{t0}$; where C_{tx} is the change in number of mussels with closed shells, B_{t0} is the number of closed shells at time = 0 (prior to cue addition), and B_{tx} is the unadjusted, observed number of mussels with closed shells at time = x]. Most mussels were open before cues were added; at time = 0, 62% of replicates had 0 mussels closed, 28% had 1 mussel closed, and 10% had 2–3 mussels closed. Results were analyzed in JMP 6.0 using a repeated measures analysis of variance with Treatment and Time as fixed factors, and the repeatedly sampled Group nested within Treatment and treated as a random factor. Finally, trials conducted within two days were classified as a Block and analyzed as a fixed blocking factor. Because there was no replication of mussel group identity nested within treatment (i.e. Group [Treatment] random), the restricted/residual maximum likelihood (REML) function used by JMP shrank the variance component estimate to zero. To determine when mussel behaviors differed from Controls, a priori linear contrasts ($\alpha = 0.05$) were used to compare the relative number of mussels with closed shells in each cue treatment to Controls within each time interval.

Results

Induction experiments

Mussels raised with pairwise combinations of *Asterias* and *Carcinus* in 2003 only developed significantly thicker shells when exposed to cues from *Carcinus* (Fig. 1). In addition, mussels only increased adductor muscle mass in response to *Asterias* (Fig. 1). Mussels did not increase shell thickness or adductor muscle mass in the presence of both *Asterias* and *Carcinus*. In addition, mussels reduced tissue growth rates when raised with cues from both *Carcinus* and *Asterias*, but not when exposed to each predator individually (Fig. 1).

Mussels raised with pairwise predator combinations incorporating *Cancer* crabs in 2004 only developed thicker shells when exposed to cues from *Carcinus* (Fig. 2). However, mussels did increase adductor muscle weight in response to either *Asterias* or *Asterias* and *Cancer* (Fig. 2). Finally, mussels in 2004 did not change tissue growth rates in response to cues from the pairwise predator combinations (Fig. 2).

Behavior results

Small mussels exposed to cues from crushed conspecifics were significantly more likely to be closed (and not feeding) than controls at 5, 10, 20 and 60-min time observations during the 6 h experiment (Fig. 3A). Similarly, medium mussels only closed in response to cues from crushed conspecifics at t = 5, 10, 0 and 30 min (Fig. 3B). At no time did mussels close in response to predator odors.

Discussion

In this series of experiments mussels developed thicker shells in response to predator odor from the crab *Carcinus* alone,

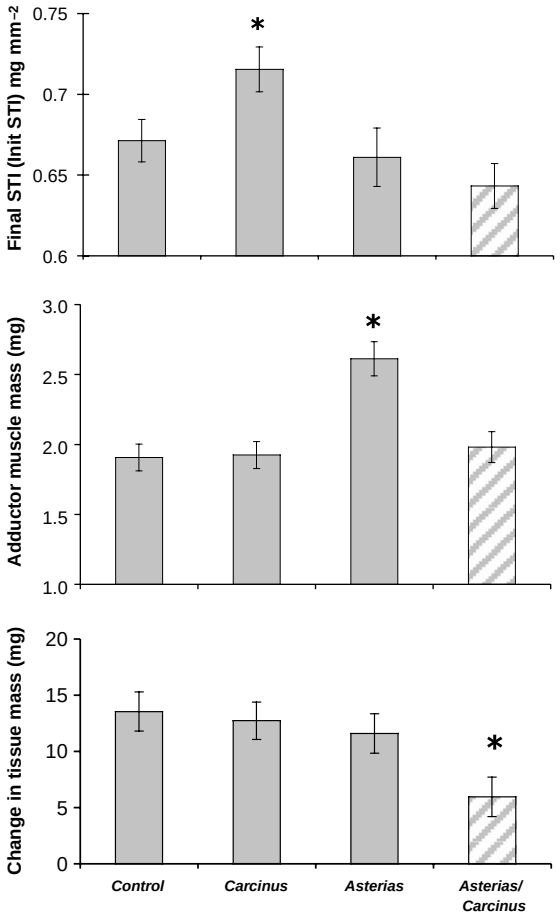

Figure 1. The relative shell thickness (STI), adductor muscle growth, and change in tissue mass of *Mytilus edulis* when reared under the following predator cue treatments during the 2003 induction experiment: Control (no predator), *Carcinus* (alone), *Asterias* (alone), or *Asterias*/*Carcinus* (both predators). Solid bars are single predator odor treatments and striped bars are pairwise predator combinations. Asterisks indicate cue treatments that are significantly different from control using a priori t-tests ($p < 0.05$). Error bars indicate SE. (STI ANCOVA: $F_{Treatment} = 4.77$, DF = 3,32, $p = 0.0068$; $F_{Group\ (Treatment)} = 3.77$, DF = 32, 84 $p < 0.0001$; $F_{Initial\ STI} = 212.85$, DF = 1, 84 $p < 0.0001$) (Adductor muscle ANCOVA: $F_{Treatment} = 8.49$, DF = 3,32, $p < 0.0001$; $F_{Group\ (Treatment)} = 1.14$, DF = 32,84 $p = 0.31$; $F_{Final\ shell\ surface\ area} = 78.27$, DF = 1, 84, $p < 0.0001$) (Tissue mass change ANOVA: $F_{Treatment} = 3.61$, DF = 3,32, $p = 0.0229$; $F_{Group\ (Treatment)} = 6.74$, DF = 32,85, $p < 0.0001$).

and larger adductor muscles in the presence of predator odor from the sea star *Asterias* alone or *Asterias* paired with *Cancer*. However, a shell thickening, 'crab' response was not elicited by *Cancer*, any pairwise combination including *Cancer*, or any pairwise combination of predators with *Carcinus*. These results indicate that the frequent occurrence of multiple predator species in nature may often lead to poorly integrated responses to pairwise combinations of predators. In addition, the lack of a consistent 'crab' response indicates that predator functional groups do not elicit consistent responses and the expression of inducible defenses is contingent on predator species identity. Finally, although there was no apparent reduction in feeding behavior in response to predator odor, mussels did 'clam

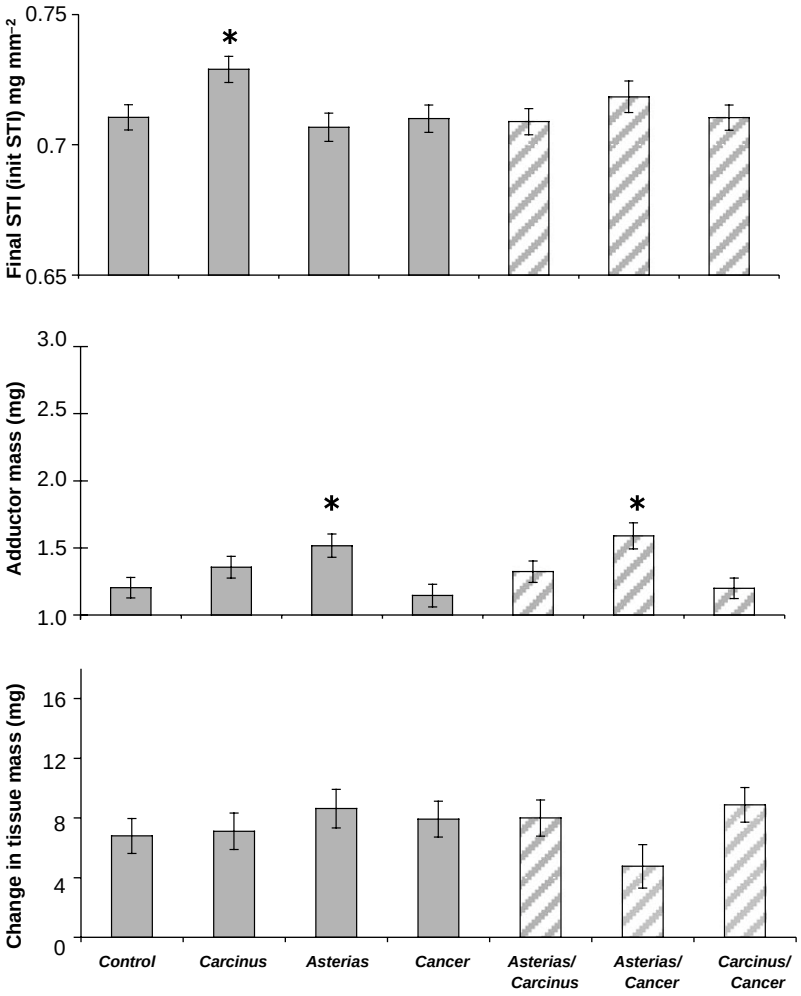

Figure 2. The relative shell thickness (STI), adductor muscle growth, and change in tissue mass of *Mytilus edulis* when reared under the following predator cue treatments during the 2004 induction experiment: no predator (Control), each predator alone (*Carcinus*, *Asterias* or *Cancer*), or pairwise combinations of predators (*Asterias*/*Carcinus, Asterias*/*Cancer* or *Carcinus*/*Cancer*). Details as in Fig. 1. (STI ANCOVA: $F_{Treatment} = 2.25$, DF = 6,35, $p = 0.0604$; $F_{Group\ (Treatment)} = 4.65$, DF = 35,279, $p < 0.0001$; $F_{InitialSTI} = 14071.09$, DF = 1,279, $p < 0.0001$) (Adductor muscle ANCOVA: $F_{Treatment} = 3.70$, DF = 6,35, $p = 0.0058$; $F_{Group\ (Treatment)} = 2.16$, DF = 35,278, $p < 0.0003$; $F_{Finalshell\ surfacearea} = 475.74$, DF = 1,278, $p < 0.0001$; $F_{Treatment \times Final\ SSA} = 2.04$, DF = 6,278, $p = 0.0606$) (Tissue mass change ANOVA: $F_{Treatment} = 1.6208$, DF = 6,35, $p = 0.1696$; $F_{Group(Treatment)} = 1.2587$, DF = 35,280, $p < 0.1591$).

up' in response to crushed conspecifics. Shell closing in response to crushed conspecifics (but not predator odor) may interfere with the expression of inducible defenses and the interpretation of many inducible defense studies. Thus, *Mytilus* shows poor phenotypic integration of predator specific defenses, functionally similar predators do not elicit similar responses, and these responses are not due to 'clamming up' induced by predator odor.

In both experiments (2003 and 2004), mussels did not simultaneously increase shell growth and adductor muscle growth, suggesting these induced traits require an energetic tradeoff, or are phenotypically incompatible. The process of induced shell thickening appears to be mediated by an increase in shell calcification (Brookes and Rochette 2007, Freeman 2007); a process that may be energetically unrealizable when combined with increased adductor muscle growth. Responses to predators can reduce assimilation efficiency or increase metabolic rate (Relyea and Auld 2004) and may ultimately limit resources available for induced defenses (Palmer 1990, DeWitt et al. 1998). In general, mussels in 2004 had lower growth than in 2003 but still expressed predator specific induced defenses, suggesting that these individual responses are not energetically prohibitive. Under field conditions mussels also respond to predator cues and wave action by increasing byssus production (Cote 1995, Farrell and Crowe 2007) and likely have higher energetic demands.

Integration of responses to combined *Asterias* and *Carcinus* may also be mechanistically undermined by direct interactions controlling their expression and development (i.e. signaling hormones or other pleiotropic effects) (Cipollini 2004), or a variety of poor trait/phenotypic integration processes (Schlichting 1989, DeWitt and Langerhans 2003). For instance, 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). However, as a mussel shell grows, the adductor muscle must migrate away

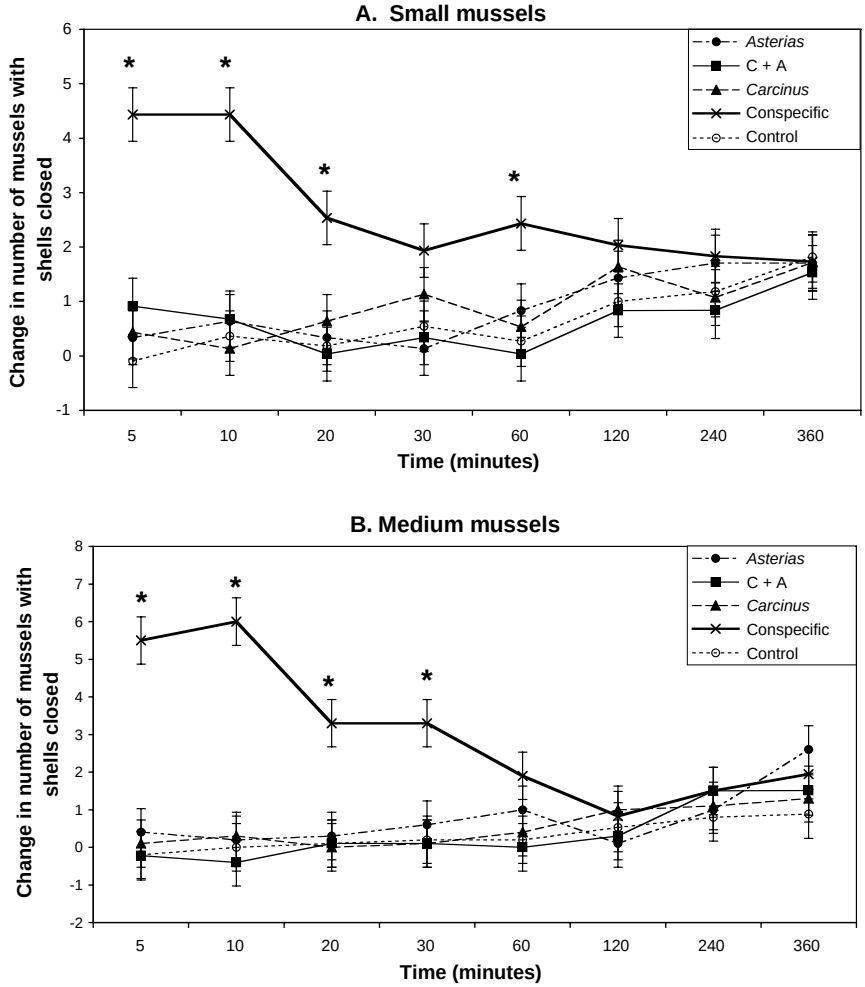

Figure 3. Change (from time = 0) in number of mussels with shells closed for small (9–25 mm) and medium (26–55 mm) mussels after cue treatment commenced. Asterisks indicate when times and treatments differed significantly from control mussels in a priori linear contrasts. "C+A" indicates both predators (*Carcinus* and *Asterias*, combined). (Small mussels ANOVA: $F_{Treatment} = 10.34$, DF = 4,46, $p < 0.0001$; $F_{Time} = 2.98$, DF = 7,304, $p = 0.0049$; $F_{Time \times Treatment} = 2.77$, DF = 28, 304, $p < 0.0001$; $F_{Block} = 2.38$, DF = 10,304, $p = 0.01$)(Medium mussels ANOVA: $F_{Treatment} = 6.29$, DF = 4,45, $p < 0.0004$; $F_{Time} = 2.79$, DF = 7,300, $p = 0.0079$; $F_{Time \times Treatment} = 5.11$, DF = 28, 300, $p < 0.0001$; $F_{Block} = 1.36$, DF = 9,304, $p = 0.20$).

from the shell hinge, toward the posterior shell margin. Accordingly, while *Carcinus* exposed mussels increase shell weights, *Asterias* exposed mussels reduce linear shell growth (Freeman 2007), responses that may be incompatible. In contrast, the physiological mechanisms in mussels controlling induced defenses and the waterborne cues initiating responses have received little attention (but see de Vooys 2003).

A variety of responses to threats can lead to poor trait integration and alter the adaptive value of induced traits across dissimilar environments. Although the underlying mechanism of this poor phenotypic integration cannot be elucidated from this study, it is clear that the combined predator odor from *Carcinus* or *Asterias* disrupted predator specific responses that were expressed with these predators individually. Handling times in subsequent predation trials reflect the expression of these defenses: mussels raised with combined cues from *Carcinus* and *Asterias* are not as well defended as those raised with cues from each predator individually (Freeman 2007). Although similar examples of poor phenotypic integration also result in risk enhancement, most are mediated by behavioral responses (Sih et al. 1998) with few examples mediated by induced morphological defenses. In many cases, multiple predators induce similar morphological defenses (Van Buskirk 2001, Laforsch and Tollrian 2004), but poorly integrated defenses to multiple predators often lead to hierarchical or intermediate responses (Relyea 2003, Teplitsky et al. 2004), likely leaving prey more vulnerable to at least one of the predators.

Despite the mosaic of phenotype and environment matches, 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). A more realistic understanding of the selection pressures acting on inducible defenses may require assessing the adaptive value of inducible defenses in non-adaptive and multiple predator scenarios (Sih et al. 1998, DeWitt and Langerhans 2003, Relyea 2003, Hoverman et al. 2005), particularly when

prey show specific and conflicting responses to predators with differing attack strategies. For instance, studies of single predator systems have revealed tradeoffs of induced defenses in terms of architectural constraints on growth (Lively 1986, Trussell and Nicklin 2002), reduced competitive ability (Pettersson and Brönmark 1997), reduced growth rates (Harvell 1986), and tradeoffs between acquiring energy and avoiding predators (Skelly 1992, Anholt and Werner 1995, Relyea and Werner 1999, Van Buskirk 2000, Relyea 2001). Among studies of induced defenses in mussels there is a conspicuous absence of traditionally defined costs in single predator environments (Reimer et al. 1995, Smith and Jennings 2000, Frandsen and Dolmer 2002), however as we show here in environments with multiple predators, mussels are often incapable of expressing appropriate, single-predator responses (and may further have some reduced tissue growth) suggesting some costs or tradeoffs of predator-specific induced defenses. These patterns suggest that tradeoffs of inducible defenses may become apparent if prey possess incompatible induced traits to multiple predators or if induced traits are disfavored under adverse environmental conditions (Lima 1992, Agrawal and Karban 1999, Relyea 2003, 2004).

Although *Carcinus* and *Cancer* appear similar in ecological direct effects, given time for the mussels to express induced responses, the two crabs likely influence different community-wide indirect effects; *Carcinus* exposed mussels will be better defended than *Cancer* exposed mussels. Distinctive responses to crabs may be common as several gastropod and bivalve mollusks can distinguish between crab species (Marko and Palmer 1991, Freeman and Byers 2006). Thus, classifying crabs as a functional group is complicated by ecologically significant indirect effects mediated by inducible defenses and may overlook biologically relevant interactions (Schmitz and Suttle 2001). Although *Cancer* and *Carcinus* have a similar foraging mode and use dexterous chelae to manipulate prey (Moody and Steneck 1993) several *Cancer* spp. have far more formidable claws than *Carcinus* (Palmer et al. 1999) and can likely crush even mussels induced to thicken shells; reducing the adaptive value of this induced trait. In many habitats mussels may only be exposed to one of the predators due to predator-predator interaction modification or environmental factors (Leonard et al. 1999). Consequently, 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).

Shared evolutionary history may also contribute to mussels' different responses to *Cancer* and *Carcinus*. There is little genetic exchange between *Mytilus* in the northwest Atlantic and populations in Europe (Wares and Cunningham 2001), indicating that *Mytilus* in the northwest Atlantic have a long evolutionary history with the two native *Cancer* species, but less than 200 years of interactions with *Carcinus*, which is not native to North America (Carlton and Cohen 2003). Our results are in contrast to Nicastro et al. (2007) who found that native mussels recognized and responded to a native predator, but invasive mussels did not. The limited shared evolutionary history of *Carcinus*, with *Asterias* and *Mytilus* in the northwest Atlantic, may have hampered integration of the mussel's inducible defense to these two predators. Furthermore, *Mytilus* may not respond to *Cancer* if the crab can camouflage chemical cues detectable by the mussels (Getty 1996, Adler and Grunbaum 1999), if *Cancer's* crushing strength is sufficient to overwhelm any induced shell thickening, or if any response of mussels to the crab has become canalized as a fixed defense. Previous studies in the northwest Atlantic have explored *Mytilus'* response to the invasive *Carcinus* (Leonard et al. 1999, Smith and Jennings 2000, Freeman and Byers 2006), but none had examined induced defenses to native *Cancer* crabs.

In these induction experiments, predators were fed outside of the cue containers and the mussels were only exposed to predator odors (not crushed conspecific cues). Thus, in these experiments 'clamming up', as seen in response to crushed conspecifics, did not influence the expression of induced defenses. 'Clamming up' appears to be a general response in bivalves to reduce vulnerability to and detection by predators, but it often results in reduced growth (Reimer et al. 1995, Nakaoka 2000, Smith and Jennings 2000, Reimer and Harms-Ringdahl 2001, Smee and Weissburg 2006). Indeed, the 'clamming up' response may be reserved for when predators are actively consuming conspecifics to minimize the costs of reduced foraging and allowing mussels to maintain growth unless the predator threat is extreme (Smee and Weissburg 2006). But, 'clamming up' may interfere with expression of induced defenses and the interpretation of many studies. Because, shelled mollusks often show strong tradeoffs between shell and tissue growth, reduced tissue growth may lead to shell thickening (Palmer 1981, Brookes and Rochette 2007). Consequently, different mechanisms may underlie the appearance of induced defenses in *Mytilus* in response to crushed conspecifics (Leonard et al. 1999), predators fed mussels (Reimer and Tedengren 1996, 1997, Smith and Jennings 2000, Reimer and Harms-Ringdahl 2001), simple predator odors (i.e. unfed predators; Cote 1995, Freeman and Byers 2006, Freeman 2007), or predators fed fish (Leonard et al. 1999). In short, when threat cues can initiate reduced feeding and growth, as well as induced defenses, caution is warranted in interpreting the evolutionary significance of defenses such as shell thickening.

Although mussels can express specific induced defenses to cues from *Asterias* or *Carcinus*, the combined exposure to cues from *Carcinus* with another predator negates the mussel's ability to respond to either predator appropriately. The functionally similar crab, *Cancer irroratus*, did not have similar effects to *Carcinus*. Finally, 'clamming up' did not seem to be responsible for poor phenotypic integration of the *Carcinus* and *Asterias* responses and furthermore suggests that reduced feeding may interfere with experimental detection of predator specific responses per se when predators are fed mussels. Future studies of the evolution and ecological implications of inducible morphological defenses should consider the effects of multiple predators and potential interactions of cues from killed conspecifics.

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