Stony Brook University

Predator-induced behavioral and morphological plasticity in marine snails, *Nucella* spp.

A Dissertation Presented

by

Paul Eugene Bourdeau

to

The Graduate School

in Partial Fulfillment of the

Requirements

for the Degree of

Doctor of Philosophy

in

Ecology and Evolution

Stony Brook University

May 2009

Stony Brook University

The Graduate School

Paul Eugene Bourdeau

We, the dissertation committee for the above candidate for the

Doctor of Philosophy degree, hereby recommend

acceptance of this dissertation.

Dianna K. Padilla – Dissertation Advisor
Professor, Ecology and Evolution

Jeffrey S. Levinton - Chairperson of Defense
Distinguished Professor, Ecology and Evolution

Massimo Pigliucci
Professor, Ecology and Evolution

Geerat J. Vermeij
Geology, University of California, Davis

This dissertation is accepted by the Graduate School

Lawrence Martin
Dean of the Graduate School

Abstract of the Dissertation

Predator-induced behavioral and morphological plasticity in marine snails, *Nucella* spp.

by

Paul Eugene Bourdeau

Doctor of Philosophy

in

Ecology and Evolution

Stony Brook University

2009

Predator-induced changes in the defensive traits of prey organisms are widespread in nature and have important ecological consequences. Currently, our understanding of inducible defense comes from studies focused on single trait responses to single predators that often fail to link traits to patterns of co-occurrence with predators in nature. Consequently, we have poor understanding of how prey express multivariate phenotypes in the presence of multiple predators and which traits are important for allowing prey to coexist with predators. This dissertation explores the ecology and evolution of inducible defenses, using marine snails as a model system. Specifically I investigate predator-induced behavior and morphology in three sympatric intertidal snails, (*Nucella* spp.) in response to the presence of two predators with different attack modes (crab and seastar). My objectives were to determine: 1) whether snails exhibit adaptive responses to different types of predation risk, 2) how prey respond to combined predators, 3) what mechanisms underlie inducible morphological defenses, and 4) whether snails in the genus *Nucella* exhibit species-specific responses to predators. Snails were extremely plastic in their ability to respond to different predator cues. Responses to a given predator were dependent on the predator's diet and prey prioritized their response to the most dangerous predator when exposed to multiple predators simultaneously. I also found that consideration of single traits alone can lead to erroneous interpretations about how prey respond to combined predators. Growth rates were slowed in the presence predators indicating that snails are paying a cost for defending themselves. However, reduced feeding activity rather than an active

physiological response appears to be the mechanism underlying predator-induced shell thickening, suggesting that the cost is associated with lost feeding opportunity rather than the production of structural defenses. Statistical analyses of shell strength indicate that the accumulation of new shell material, rather than architectural or material properties changes in the shell was responsible for increased structural strength. Lastly, all species altered their feeding behavior and shell morphology in the presence of predators, but exhibited species-specific differences that can be linked to their distribution across a predation gradient.

To the little breeze off the water (or the little bird that sings) that helped me along the way

Table of Contents

List of Tables……...viii
List of Figures…….. x
Acknowledgements……xii
Chapter 1: Introduction……...1
References…….. 5
Chapter 2: Cue reliability, risk sensitivity and inducible morphological defense in a marine snail
Abstract……9
Introduction…….. 10
Materials and methods……...13
Results……...16
Discussion…… 17
Tables……... 24
Figures……..26
References……..... 29
Chapter 3: Specificity of cues that induce shell defenses in marine snails: Is a crab just a crab?
Abstract…… 35
Introduction…….. 35
Methods…… 37
Results……..41
Discussion…… 42
Figures……..48
References…….. 49
Chapter 4: Prioritized phenotypic responses to combined predators in a marine snail
Abstract……56
Introduction…….. 57
Methods…… 60
Results……..68
Discussion…… 71
Figures……..81
References……...85
Chapter 5: An inducible morphological defence is a passive by-product of behaviour in a marine snail
Abstract……92
Introduction…….. 93
Methods…… 96
Results……..101

Discussion…… 104
Figures……..108
References……...113
Chapter 6: Variation in inducible defenses in a marine snail from habitats with different predation regimes
Introduction…….. 118
Methods…… 120
Results……..125
Discussion……127
Tables…….. 132
Figures…… 136
Refereces…… 142
Chapter 7: Predator-induced plasticity and habitat partitioning in congeneric marine snails distributed along a vertical intertidal gradient.
Introduction……. 147
Materials and methods…….. 149
Results…… 155
Discussion…… 160
Tables……166
Figures……..174
References…….. 179
Chapter 8: Conclusions…… 184
References…….. 186
Bibliography…….. 187
Appendices
Appendix 1……227
Appendix 2……228
Appendix 3……229
Appendix 4……230
Appendix 5……231
Appendix 6……232

List of Tables

Chapter 2:
Table 1. Analyses of defensive shell responses and linear shell growth of *Nucella lamellosa* to the presence of injured con- and hetero-specific snails and *Cancer productus* fed different diets…….. 24
Chapter 6:
Table 1. Factorial ANCOVA for the effects of origin, treatment, size and their interactive effects on *Nucella lamellosa* shell shape……132
Table 2. Factorial ANCOVA for the effects of origin and treatment and their interactive effects on *Nucella lamellosa* apertural lip thickening……133
Table 3. Factorial ANCOVA for the effects of origin and treatment and their interactive effects on *Nucella lamellosa* shell mass gain…… 134
Table 4. Factorial ANCOVA for the effects of origin and treatment, and their interactive effects on *Nucella lamellosa* shell strength change……..135
Chapter 7:
Table 1. Results of factorial ANOVA on activity of three species of *Nucella* raised in the presence and absence of cues associated with the risk of predation…….. 166
Table 2. Analysis of growth of three species of *Nucella* raised in the presence or absence of cues associated with the risk of predation…..167
Table 3. Results of nested analyses of covariance on morphological traits for three species of *Nucella* raised in the presence or absence of cues associated with the risk of predation …… 168
Table 4. Differences in the allometeric growth rates of three species of *Nucella* raised in the presence or absence of cues associated with the risk of predation……170
Table 5. Analyses of allometric scaling relationships among three species of *Nucella*…….171
Table 6. Results of nested analyses of covariance on shell strength for three species of *Nucella* raised in the presence or absence of cues associated with predation risk…….. 173
Appendix 2:
Analyses of morphological responses to predators by *Nucella lamellosa* after 70 days of exposure to experimental conditions…… 228
Appendix 3:
Results of MANCOVA on shell shape variables in *Nucella lamellosa* ……..229
Appendix 4:
Analysis of shell and body growth variation in *Nucella lamellosa* ……..230
Appendix 5:
Final size ranges of reference samples of *Nucella* species raised in the

presence or absence of chemical cues from crabs…… 231
Appendix 6:
Results of analyses of covariance on shell strength for experimental and reference samples of *Nucella* species raised in the presence or absence of cues associated with predation risk…… 232

List of Figures

Chapter 2:
Figure 1. Schematic of predation cue induction experiment…….. 26
Figure 2. Morphological responses of *Nucella lamellosa* to the presence of risk cues……27
Figure 3. Linear shell growth of *Nucella lamellosa* in the presence of risk cues……...28
Chapter 3:
Figure 1. Shell mass and soft tissue mass of *Nucella lamellosa* raised in the presence of different crab scents…….. 48
Chapter 4:
Figure 1. Diagram showing the linear measurements used in this study and the position of 10 landmarks used in the geometric morphometric analysis…… 81
Figure 2. Morphological responses and avoidance behavior of *N. lamellosa* to different predator treatments…….. 82
Figure 3. Relative warp plot for *N. lamellosa*.…… 83
Figure 4. Variation in body weight and spiral shell growth of *N. lamellosa* in response to different predator treatments……...84
Chapter 5:
Figure 1. Number of barnacles eaten by *Nucella lamellosa* under different experimental treatments…… 108
Figure 2. Relation between growth and amount eaten for *Nucella lamellosa* across all experimental treatments…….109
Figure 3. Variation in (a) final shell length and (b) body mass in *Nucella lamellosa* under different experimental treatments……110
Figure 4. Relation between final body mass and final shell mass of *Nucella lamellosa* under different experimental treatments……. 111
Figure 5. Variation in force to fracture of *Nucella lamellosa* under different experimental treatments…….112
Chapter 6:
Figure 1. Source habitats on San Juan Island, Washington, USA ….136
Figure 2. Typical shell forms of *Nucella lamellosa* from sites without and with abundant crabs……137
Figure 3. Shell shape in response to experimental treatment for *Nucella lamellosa* from habitats with and without abundant crabs……138
Figure 4. Variation in apertural lip thickening in response to experimental treatment for *Nucella lamellosa* from habitats with and without abundant crabs……..139
Figure 5. Variation in shell mass gain in response to predation treatment for *Nucella lamellosa* from habitats with and without abundant crabs ……140

Figure 6. Variation in shell strength change in response to experimental treatment for *Nucella lamellosa* from habitats with and without abundant crabs……. 141
Chapter 7:
Figure 1. Intertidal distributions and mean densities of three species of *Nucella* in relation to tidal immersion……174
Figure 2. Activity of three species of *Nucella* in the presence and absence of cues associated with predation risk ……... 175
Figure 3. Adjusted growth and plasticity for whelks of three species of *Nucella* in the presence and absence of cues associated with predation risk……. 176
Figure 4. Morphological scaling relationships in three species of *Nucella* in the presence and absence of cues associated with predation risk….177
Figure 5. Interspecific comparisons among three species of *Nucella* raised in the presence or absence of cues associated with predation risk...178
Appendix 1:
Pseudo-allometry plot of centroid size and relative warp 1 for experimental and wild-caught *Nucella lamellosa*…….. 227

Acknowledgements

I owe a large debt of gratitude to the many people who helped me during my dissertation. First, I would like to thank my advisor, Dianna Padilla, for her ever-present enthusiasm for scientific research, for always challenging me to ask questions, and for urging me to put my work in a larger context. I owe most of my development as a professional scientist to her. I am particularly grateful for her pushing me to complete my experiments, analyze my data and write up my results rather than just thinking about them. She also put up with an extremely stubborn graduate student, who didn't often listen, and I thank her for not gloating too much when saying 'I told you so'. I would also like to thank my committee; Jeff Levinton, Massimo Pigliucci and Geerat Vermeij. Each of them influenced my work before I even met them, and now each of them have changed the way I view the natural world.

The Department of Ecology and Evolution (E&E) and Friday Harbor Laboratories (FHL) were extraordinary places with extraordinary people, and I am sad to be leaving both. I am particularly grateful to the director and staff at FHL for providing almost the entirety of the financial and logistical support I needed to complete my dissertation. The ladies in the office, particularly Martha Nolan and Iris Roth in E&E, and Aimee Urata at FHL, made my life as a graduate student easier and better. I especially thank Megan Mach for her support, encouragement and companionship over the last few years – I could not have finished my dissertation without her. I am grateful to all of the amazing friends I have made at E&E and FHL, most of all: Melissa Mark for the many years of love and friendship; My cohort in E&E, Adam Ehmer, JMatt Hoch, Chris Noto and Eliza Woo, and Fernanda Oyarzun at FHL – thank you for the motivation and inspiration (there is nothing like being surrounded by people five years younger than you and ten times smarter than you to motivate you to be a better graduate student and there is nothing like being surrounded by people this interesting, kind, and generous to make you want to be a better person); y officemates, Bengt Allen, Isabel Ashton, Sarah Gray and Stephen Sabatino – thank you for making it fun to come to work everyday. A most special thank you to Emily Thompson, whose love, patience and understanding has gotten me through the toughest part of the process.

Finally, without the love, support, and encouragement of family, none of this would be possible. My parents, Nancy and Jean-Paul Bourdeau have, from the beginning, always encouraged me to pursue my goals, and have willingly made every sacrifice to allow me to obtain them. My brother, Daniel, although six years younger, has always been the responsible one, and became a normal, contributing member of society long before I will. Its not too often that one can look up to a younger brother, I thank him for the inspiration. Mom, Dad and Dan – thank you – every one should be so luck as to have a family like you.

Chapter 1 - Introduction

Phenotypic plasticity, especially the environmentally-contingent expression of phenotypes, is widespread in nature (West-Eberhard 2003a, Pigliucci 2005). Numerous species of plants and animals can change behavioral, morphological, physiological and life history traits in response to different environmental conditions. These trait changes can have important ecological and evolutionary implications (Miner et al. 2005, Fordyce 2006). Phenotypic plasticity often involves ecologically relevant traits that can alter a species' interaction with its environment. In addition to influencing an individual's fitness, plastic responses can also have consequences at higher levels of ecological organization (Miner et al. 2005). In recent years, phenotypically plastic trait changes have been shown to affect species interactions, population dynamics, community structure and even ecosystem function. Moreover, phenotypic plasticity may play a role in the diversification of phenotypes and even speciation (West-Eberhard 2003a).

Much of what we know about phenotypic plasticity in animals comes from studies of predator-induced plasticity (Tollrian and Harvell 1998). So-called inducible defenses involve changes in behavior, morphology, and life history in response to perceived changes in predation risk. Inducible defenses are predicted to evolve when defenses are costly (Lively 1986, Moran 1992, Tufto 2000), and an effective defense can be produced in a relatively short time compared to the time scale of environmental change (Padilla and Adolph 1996). Inducible defenses are particularly common in terrestrial plants and aquatic

animals. Plant defense against herbivores is often achieved through the induction of chemical defenses (Karban and Myers 1989). In aquatic animals, inducible defenses often take the form of behavioral and morphological responses. Such defenses include reduced activity and increase refuge use in the presence of predators, which is seen in many species (Lima and Dill 1990), spines in bryozoans in response to predatory sea slugs (Harvell 1992), neckteeth and helmets in cladocerans (Spitze 1992, Tollrian 1993) in response to predatory midges, the thickening of shells in molluscs in response to shell-breaking predators (Palmer 1990, Trussell 1996, Leonard et al. 1999) and changes in body shape in fish (Bronmark and Miner 1992) and larval anurans in response to gape-limited predators (Smith and Vanbuskirk 1995, Relyea 2001).

Currently, much of our understanding of inducible defenses comes from studies that focus on changes in single traits in response to a single inducing agent (direct attacks or cues associated with specific predators). Consequently, we do not have a very good understanding of multiple integrated phenotypic responses in organisms, or how organisms will respond when exposed to multiple environmental cues or predators simultaneously. Organisms are an amalgam of multiple traits. In nature animals inhabit complex environments and are likely faced with multiple inducing agents, including predators with different attack modes, which may pose a conflict for the most appropriate defense (Sih 1987).

I used marine gastropods in the genus *Nucella* to examine inducible phenotypic responses to predators, including cues that trigger these responses. Species of *Nucella* are marine intertidal zone whelks in the family Muricidae (Collins et al. 1996). These whelks can vary enormously in shell form among populations within a single species. This variability is a conspicuous feature of species from rocky shores of the northeastern Pacific (*Nucella canaliculata*, *N. lamellosa*, *N. ostrina*), and includes variability in shell thickness, shape and sculpture (Kincaid 1957, Kitching 1976, Crothers 1984). Much of the intraspecific variation in the shells of whelks appears to be adaptive. Thicker shells, or those with smaller apertures and apertural teeth, are less vulnerable to predation by shell-breaking crabs (Palmer 1985). Thinner shells, on the other hand, are thought to be energetically less expensive to produce and less likely to limit the maximum rate of shell and tissue growth (Palmer 1992). Two species of *Nucella*, *N. lamellosa* and *N. lapillus,* exhibit predator-induced plasticity for shell thickness and apertural teeth in response to water-borne effluent from predatory crabs feeding on conspecific snails (Appleton and Palmer 1988, Palmer 1990).

To address some of the gaps in our knowledge about predator-induced plasticity, I exposed field-collected snails to chemical cues from different predators and/or cue sources, with no physical contact with the cue source. The cue sources included predatory and non-predatory crabs, a predatory seastar and cues from injured snails. The response variables included multiple

morphological and behavioral traits known or previously hypothesized to influence susceptibility to predation. The specific questions I addressed were: What are the effects of cues from predatory crabs and cues from injured snails in inducing shell defenses in *N. lamellosa*? (Chapter 2)

Are the chemical cues released from crabs that induce shell defenses in *N. lamellosa* species-specific? (Chapter 3)

Can *N. lamellosa* distinguish between cues from predators with different attack modes, and what types of responses result from simultaneous exposure to cues from those predators? (Chapter 4)

What is the proximate mechanism underlying predator-induced shell thickening in *N.lamellosa*? (Chapter 5)

Does the magnitude of predator-induced responses differ among populations from habitats with and without abundant crabs? (Chapter 6)

Do predator-induced responses differ among species of eastern North Pacific *Nucella*? (Chapter 7).

References

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

Bronmark, C. and J. G. Miner. 1992. Predator-induced phenotypical change in body morphology in crucian carp. Science **258**:1348-1350.

Collins, T. M., K. Frazer, A. R. Palmer, G. J. Vermeij, and W. M. Brown. 1996. Evolutionary history of northern hemisphere *Nucella* (Gastropoda, Muricidae): Molecular, morphological, ecological, and paleontological evidence. Evolution **50**:2287-2304.

Crothers, J. H. 1984. Some observations on shell shape variation in Pacific Nucella. Biological Journal of the Linnean Society **21**:259-281.

Fordyce, J. A. 2006. The evolutionary consequences of ecological interactions mediated through phenotypic plasticity. Journal of Experimental Biology **209**:2377-2383.

Harvell, C. D. 1992. Inducible defenses and allocation shifts in a marine bryozoan. Ecology **73**:1567-1576.

Karban, R. and J. H. Myers. 1989. Induced plant-responses to herbivory. Annual Review of Ecology and Systematics **20**:331-348.

Kincaid, T. 1957. Local races and clines in the marine gastropod *Thais lamellosa* Gemlin. The Calliostoma Co., Seattle, WA, USA.

Kitching, J. A. 1976. Distribution and changes in shell form of *Thais* spp (Gastropoda) near Bamfield, BC. Journal of Experimental Marine Biology and Ecology **23**:109-126.

Leonard, G. H., M. D. Bertness, and P. O. Yund. 1999. Crab predation, waterborne cues, and inducible defenses in the blue mussel, *Mytilus edulis*. Ecology **80**:1-14.

Lima, S. L. and L. M. Dill. 1990. Behavioral decisions made under the risk of predation - a review and prospectus. Canadian Journal of Zoology-Revue Canadienne De Zoologie **68**:619-640.

Lively, C. M. 1986. Canalization versus developmental conversion in a spatially-variable environment. American Naturalist **128**:561-572.

Miner, B. G., S. E. Sultan, S. G. Morgan, D. K. Padilla, and R. A. Relyea. 2005. Ecological consequences of phenotypic plasticity. Trends in Ecology & Evolution **20**:685-692.

Moran, N. A. 1992. The evolutionary maintenance of alternative phenotypes. American Naturalist **139**:971-989.

Padilla, D. K. and S. C. Adolph. 1996. Plastic inducible morphologies are not always adaptive: The importance of time delays in a stochastic environment. Evolutionary Ecology **10**:105-117.

Palmer, A. R. 1985. Adaptive value of shell variation in *Thais lamellosa* - effect of thick shells on vulnerability to and preference by crabs. Veliger **27**:349-356.

Palmer, A. R. 1990. Effect of crab effluent and scent of damaged conspecifics on feeding, growth, and shell morphology of the Atlantic dogwhelk *Nucella lapillus* (L). Hydrobiologia **193**:155-182.

Palmer, A. R. 1992. Calcification in marine mollusks - how costly is it. Proceedings of the National Academy of Sciences of the United States of America **89**:1379-1382.

Pigliucci, M. 2005. Evolution of phenotypic plasticity: where are we going now? Trends in Ecology & Evolution **20**:481-486.

Relyea, R. A. 2001. Morphological and behavioral plasticity of larval anurans in response to different predators. Ecology **82**:523-540.

Sih, A. 1987. Predators and prey lifestyles: an evolutionary and ecological overview. Pages 203-224 *in* W. C. Kerfoot and A. Sih, editors. Predation: Direct and Indirect Impacts on Aquatic Communities. University Press of New England, London.

Smith, D. C. and J. Vanbuskirk. 1995. Phenotypic design, plasticity, and ecological performance in 2 tadpole species. American Naturalist **145**:211-233.

Spitze, K. 1992. Predator-mediated plasticity of prey life-history and morphology - Chaoborus americanus predation on *Daphnia pulex*. American Naturalist **139**:229-247.

Tollrian, R. 1993. Neckteeh formation in *Daphnia pulex* as an example of continuous phenotypic plasticity - morphological effects of *Chaoborus*

kairomone concentration and their quantification. Journal of Plankton Research **15**:1309-1318.

Tollrian, R. and C. D. Harvell, editors. 1998. The Ecology and Evolution of Inducible Defenses. Princeton University Press, Princeton, NJ.

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

Tufto, J. 2000. The evolution of plasticity and nonplastic spatial and temporal adaptations in the presence of imperfect environmental cues. American Naturalist **156**:121-130.

West-Eberhard, M. J. 2003. Developmental Plasticity and Evolution. Oxford University Press, New York.

Chapter 2 - Cue reliability, risk sensitivity and inducible morphological defense in a marine snail

Abstract

Reliable cues that communicate current or future environmental conditions are a requirement for the evolution of adaptive phenotypic plasticity, yet we often do not know which cues are responsible for the induction of particular plastic phenotypes. I examined the single and combined effects of alarm cues from injured prey and predator kairomones and the effect of predator diet on the induction of plastic shell defenses in the marine snail *Nucella lamellosa*. Snails were subjected to six treatments: injured conspecific snails, injured heterospecific snails, crabs fed fish, crabs fed heterospecific snails, crabs fed conspecific snails, and a no cue control. Water-borne cues from injured conspecific and heterospecific snails did not affect plastic responses in shell weight, shell thickness or apertural teeth in *N. lamellosa*. Kairomones released by feeding predators, independent of diet, had significant effects on some shell traits, but only crabs fed conspecific snails induced a full suite of shell defenses and induced the greatest response in all traits. Results indicate that alarm cues from conspecifics alone do not trigger a response, but, in combination with kairomones, act synergistically to signal predation risk and trigger inducible

defenses in this species. The ability to 'label' predators as dangerous may decrease predator avoidance costs and highlights the importance of the feeding habits of predators on the expression of inducible defenses.

Introduction

The adaptive value of phenotypic plasticity depends on the recognition of reliable cues that accurately predict or are closely correlated with environmental conditions (Lively 1986, Moran 1992). For inducible defenses, some of the best-studied and ecologically important forms of phenotypic plasticity (Tollrian and Harvell 1998, Miner et al. 2005), the reliability of cues are crucial because the cost of responding inappropriately or not responding at all can be very high. In aquatic systems, chemical cues induce plastic defenses that not only influence performance and survival (McCollum and VanBuskirk 1996, Benard 2006) but can also can alter population dynamics and modify species interactions (Turner et al. 2000, Trussell et al. 2002, Verschoor et al. 2004). Despite their importance, we know surprisingly little about which chemical cues induce plastic defensive traits in many systems.

Previous work has shown that both scent emanating from predators (i.e.,

kairomones) and odors from injured or consumed prey (i.e., alarm cues) can provide information about predation risk. For example, some prey can discriminate among predators and produce predator-specific defenses that reflect the risk due to that predator and that predator's attack mode (Relyea 2001, Freeman 2007, Bourdeau 2009b). Prey can also respond directly to damaged or injured con- and heterospecifics, or to chemical 'labels' in the predator's diet (Chivers and Smith 1998, Jacobsen and Stabell 2004).

Much of what we know about the roles of alarm cues and kairomones in inducing defenses comes from studies of prey behavior. Behavioral responses to alarm cues from injured conspecifics have been documented in a number of taxa (Jacobsen and Stabell 1999, Kristensen and Closs 2004, Smee and Weissburg 2006). However, for other species, isolated alarm cues do not induce behavioral responses or induce only weak responses (Slusarczyk 1999, Hagen et al. 2002, Griffiths and Richardson 2006). Frequently, a combination of alarm cues and kairomones is required to induce a full behavioral response in prey (Smith and Belk 2001, Brown and Dreier 2002).

Less is known about the role of alarm cues in inducing morphological defenses, although many prey species produce morphological defenses in response to chemical cues associated with risk. Thus far, evidence for the importance of alarm cues in the induction of morphological responses is equivocal (Bronmark and Pettersson 1994, Stabell and Lwin 1997, Chivers and Smith 1998, Schoeppner and Relyea 2005, Laforsch et al. 2006). Conflicting

results have been attributed to the difficulty of detecting weak responses to alarm cues. However, just as for behavioral traits, some prey may only alter their morphological traits when they are exposed to the simultaneous presence of alarm cues and kairomones (Chivers and Smith 1998). The necessity of a combination of cues would be particularly likely if the simultaneous presence of alarm cues and kairomones are a more reliable indicator of risk than either cue alone. For example, kairomones, while accurately indicating the presence of the predator, may not provide reliable information about its feeding activity. Additionally, the scent of injured conspecifics may be due to many factors other than injury from predators, and may provide a less reliable indication of risk (Kats and Dill 1998). Such unreliable cues, in isolation, may indicate risk but fail to indicate the absence of risk, or cause the excessive or unnecessary production of costly defenses. However, the combination of these cues should reliably indicate both mortality risk and the presence of a predator.

I conducted an experiment to assess the separate and combined effects of alarm cues and kairomones and their relative importance for inducing morphological defenses in *Nucella lamellosa,* a marine gastropod known for its plastic shell morphology. Previous studies have demonstrated that *N. lamellosa* produce more predator-resistant shells in response to water-borne stimuli released by predatory crabs and damaged prey (Appleton and Palmer 1988, Bourdeau 2009b). Snails produce a stronger morphological response when exposed to crabs fed conspecific snails than when exposed to crabs fed fish,

suggesting that snails produce defenses in response to both the scent of injured conspecifics and the scent of crabs (Appleton and Palmer 1988). Whether alarm cue and kairomone effects contribute additively or synergistically to morphological responses is unknown. To determine the role of these cues in the induction of morphological defenses I tested the separate and combined effects of injured heterospecific and conspecific snail prey and kairomones on growth and induction of shell defenses known to increase resistance to crab predation.

Materials and methods

Study organisms and collection sites

One hundred and eighty juvenile (< 25 mm shell length) *Nucella lamellosa* were collected from the intertidal zone at Cantilever Pier, a moderately exposed point near the University of Washington's Friday Harbor Laboratories (FHL) on San Juan Island, WA, USA (48°33'N, 123°0l'W). Before the start of the experiment I marked individual snails with colored numbered labels (bee tags) affixed to the leading edge of main body whorl with cyanoacrylate adhesive. After collection and prior to the beginning of the experiment, snails were kept in flow-through seawater tables for 48 hrs without exposure to predator cues and fed *ad libitum* barnacles (*B. glandula*, their preferred prey). Predators, (red rock crabs, *Cancer productus*) were trap-collected from the FHL pier. Additional conspecific snails

used for crab prey and alarm cues were collected from the same location as the experimental snails. Co-occurring heterospecific snails (*Littorina sitkana*) were collected from boulders at False Bay, on the west side of San Juan Island. Snails were selected from False Bay because snails from this site are relatively large and more similar in size to *N. lamellosa*. Frozen fish (Dover sole) was obtained from a local supermarket.

Experimental Design

I used covered plastic aquaria (30.5W X 19.1D X 20.3H cm) as the experimental unit for the induction experiment. Each aquarium was fitted with a plastic tub (1892 ml), attached to the underside of the aquarium cover. Cue sources (e.g., crushed snails, crabs fed different diets) were added to these tubs. Experimental aquaria received seawater from a header tank fed by the flow-through seawater system at FHL (~ 0.9 L/s). Seawater flowed from a header tank into the tubs through an opening in the aquarium cover and then overflowed into the aquarium. Holes cut just below the level of the tub allowed seawater to drain out of each aquarium. This set-up created a flow-through system that facilitated the movement of water-borne chemical cues through the aquaria without any physical contact between the snails and the cue source (Fig. 1). Aquaria were randomly assigned to one of six predation risk cue treatments: a control (no cue), injured heterospecific snails, injured conspecific snails, crabs fed fish, crab fed heterospecific snails, and crab fed conspecific snails. Treatments were

replicated three times. Small stones encrusted with barnacles that served as food for the experimental snails were added to each aquarium. At the beginning of the experiment, ten marked snails were placed among the stones in each aquarium. Stones were replaced as the barnacle supply became depleted; snails always had access to a surplus of barnacles. To keep the amount of cue similar among injured snail treatments I used either 2 medium (25-35 mm shell length) *N. lamellosa,* or 8 large (6-8 mm shell height) *L. sitkana*; approximately 2-3 g of snail soft tissue wet weight. Snails were either coarsely crushed by hand and homogenized with mortar and pestle in the alarm cue treatments or presented pre-cracked to predators in the predator diet treatments to ensure that crabs could access the snail soft tissues. Crabs always consumed all of the offered prey. Cue sources were refreshed 3 times per week. After 48 d, the morphological responses of the snails were assessed.

Morphological Measurements

I measured shell length, apertural lip thickness and apertural tooth height with digital calipers and a computer-assisted image analysis system (Image J, 1.36b, National Institutes of Health). Arpertural teeth and thickened apertural lips function as predator deterrents by reducing vulnerability to shell-peeling predators and decreasing the area of the aperture through which crabs insert their claws (Vermeij 1987). Shell mass, which is a good indicator of overall shell thickness and resistance to crushing (Trussell and Nicklin 2002, Avery and Etter

2006), was measured nondestructively following the protocol of Palmer (Palmer 1982).

Statistical Analysis

I examined the effects of cue type (treatment) on log-transformed morphological data with nested analyses of covariance (ANCOVA). Depending on which morphological variable was being analyzed, final shell length or final shell mass was used as a covariate to control for snail size. Replicate aquaria were nested within treatment because snails sharing an aquarium may not be independent. Initial analyses indicated that the two covariates, final shell length and final shell mass, had no effect on apertural tooth height, so they were removed from the final model for the analysis of that trait. The effect of cue type on log-transformed linear shell growth, calculated as the log of the difference between final shell length and initial shell length, was analyzed with single-classification ANOVA. Post-hoc pair-wise comparisons were made with Fisher's Least Significant Difference (LSD) test to determine which treatments were different. Statistica (v. 6.1, StatSoft) was used for all statistical analyses.

Results

I found a significant difference among cue treatments for all of the defensive shell

traits I measured (Table 1). Alarm cues from injured conspecific or heterospecific snails and kairomones from crabs fed fish or crabs fed heterospecific snails failed to induce thickened apertural lips or apertural teeth in experimental snails. Neither of these traits differed among these treatments nor were they different from the no cue control. However, crabs fed conspecific snails induced significantly greater responses than the no cue treatment or the crab-fish treatment for both of these traits (Fig. 2a, b). Kairomones from crabs fed all three diets did increase overall shell weight relative to no cue controls, but alarm cues from injured heterospecifics or injured conspecifics did not (Fig. 2c).

Cue treatment also had a significant effect on linear shell growth (Table 1; Fig. 3). Only snails exposed to crabs fed conspecific snails grew slower than those in the no cue controls. Neither alarm cues from injured conspecifics or heterospecifics nor kariomones from crabs fed alternative prey reduced linear growth rate relative to controls.

Discussion

Results from my study suggest that a combination of cues, crab kairomones and alarm cues from injured conspecifics, is required to induce the full suite of shell traits that *N. lamellosa* uses for defense. Snails failed to produce any shell defenses in response to alarm cues from either injured conspecific or

heterospecific snails alone, but were able to make heavier shells in response to chemical cues released by crab predators, independent of the crabs diet. However, only snails exposed to predatory crabs feeding on conspecific snails exhibited full deployment of all defenses: heavier shells, thickened apertural lips and apertural teeth.

These results support the general findings of Appleton and Palmer (1988) who found that cues from snail-fed crabs induce taller apertural teeth than fish-fed crabs. However, Appleton and Palmer (1988) did not test for the effect of alarm cues from injured snails alone, which were tested here. Interestingly, alarm cues alone do not induce plastic defenses, even though cues from injured prey should reliably indicate mortality risk. This may be because risk cannot be attributed to a particular predator when only alarm cues are detected. As such, predator-specific responses to general alarm cues could be costly or even hazardous, particularly in environments inhabited by predators with contrasting attack strategies and prey selectivities (e.g., shell crushing and shell entry predators) where a response to one predator may make prey more vulnerable to the alternative predator (Bourdeau 2009b). In such environments, only alarm cues associated with kairomones would provide the information necessary to assess predation risk and respond appropriately to a given predator (Van Buskirk 2001, Teplitsky et al. 2004). Moreover, the absence of alarm cues from injured prey does not necessarily indicate the absence of risk (e.g., nonfeeding predators could be present in the area). Reliance on these cues would therefore increase

the number of incorrect assessments of the predator environment and the unnecessary deployment of costly defenses. Consequently, selection for a plastic response to injured conspecifics alone should be weaker than selection for a response to combinations of scents (e.g., the scent of feeding predators), if both the presence and absence of these scents is informative regarding risk.

If snails were only detecting and responding to kairomones and injured conspecifics, the combination of these cues (i.e., the crab-conspecific treatments) should have contributed additively to the expression of the morphological responses. However, the effects of combined risk cues were highly non-additive for all morphological traits among experimental treatments. There are two possible explanations for the non-additivity: 1) a synergistic interaction when both alarm cues and kairomones are detected simultaneously; or 2) predators produce a qualitatively different kairomone when consuming conspecific prey. The fact that injured conspecifics elicited no detectable response suggest that the synergism is not simply the result of encountering both cues simultaneously, but rather a consequence of prey consumption. One possibility is that conspecific alarm cues are chemically altered during passage through the predator's gut, (Hagen et al. 2002, Stabell et al. 2003, Jacobsen and Stabell 2004), and snails have evolved the ability to detect chemical 'labels' in the predator's diet (Jacobsen and Stabell 2004).

That *N. lamellosa* does not respond to cross-species predator labels is somewhat surprising given the overlap in habitat and predators. *C. productus* is

a voracious predator on *L. sitkana* in the low intertidal zone (Yamada and Boulding 1996) where these two snail species coexist (Bourdeau, personal observation). Two species occupying similar habitats that are exposed to the same predators may associate predation risk with heterospecific alarm stimuli (Chivers et al. 1996). However, my data suggest that *N. lamellosa* are able to distinguish between cues released from conspecific and heterospecific snails from the crabs' diet. The stronger effect of crabs fed conspecifics is not likely to be a food concentration effect because I provided roughly equal prey biomass and all food was consumed. Thus the amount of alarm cue should have been nearly identical in both treatments. It is also unlikely that differences in source location of snail prey are what produced these results as *L. sitkana* is commonly preyed upon by *C. productus* at the site where *N. lamellosa* were collected for the experiment (Yamada and Boulding 1996).

The observed variation in the morphological responses to crabs fed different diets likely reflects sensitivity to predation risk (Helfman 1989). Before deploying a complete anti-predator response, prey may require information about both predator species and predator diet, particularly if defensive responses are costly. For *N. lamellosa* the shell-thickening response is a consequence of reduced feeding (Bourdeau 2009). This reduced feeding incurs a somatic growth cost, thus snails would benefit if they adjust their response according to the level of risk in the environment. If snails can fine-tune their feeding behavior to the risk indicated by different cues, as is seen in many other organisms (Sih 1987, Lima

and Dill 1990), then the magnitude of morphological responses, which is linked to behavior, should match the degree of risk indicated by the different cues.

The phylogenetic distance between *N. lamellosa* and *L. sitkana* could also explain the weaker response to crabs fed heterospecific snails. Recently, phylogenetic relationships among species have been shown to account for observed patterns of cross-species alarm responses. For example, in larval anurans and freshwater snails, the intensity of cross-species responses decreases as the prey species and cue species become more distantly related (Schoeppner and Relyea 2005, Dalesman et al. 2007). As members of different families (Muricidae and Littorindae respectively) in the order Caenogastropoda, *N. lamellosa* and *L. sitkana* are not closely related (Colgan et al. 2007). Any generalized alarm substances, if present in a common ancestor, may have diverged to the point where they are unrecognizable to the extant signal receiver. Alternatively, muricids may have independently evolved a distinct signal-detection system that is incompatible with littorinid alarm cues. Future studies that compare the responses of snails to crabs fed different molluscan species representing different degrees of phylogenetic relatedness and ecological overlap are needed to elucidate the reason for the observed patterns of cross-species responses.

Sensitivity to risk cues depended on which trait was considered. Both apertural lip and tooth development were more sensitive to risk cues than shell weight. Shells equipped with thickened apertural lips and well-developed

apertural teeth represent a more extreme defensive response than simply heavier shells because thickened apertural lips not only reinforce resistance to crushing, but also prevent shell-peeling, and, in association with apertural teeth, act to restrict apertural entry (Vermeij 1987). Theory suggests that prey should exhibit more extreme phenotypic responses in the presence of more dangerous predators (Lima and Dill 1990), and this prediction can easily be extended to risk cues, in that prey should be expected to produce more extreme phenotypes in the presence of cues that indicate greater danger. Here the simultaneous presence of crab and conspecific cues likely represent greater risk than crab scent alone. Consequently, snails fine-tuned their phenotypic response according to the heightened risk. This result highlights the importance of considering multiple traits when testing hypotheses about how prey respond to risk, as one would arrive at different conclusions based on which trait was measured. Consideration of single traits in studies of induction may be misleading or provide an incomplete picture of the phenotypic response to risk.

We know very little about the actual nature of kairomones, or chemical cues from injured prey. Chemical labels are probably present in crab urine, which is released during feeding as a strategy to adjust for short-term internal volume changes due to ingestion (Taylor and Taylor 1991). Other information molecules, such as pheromones, are known to be a constituent of crab urine (Bamber and Naylor 1997, Hardege et al. 2002). Pheromones and kairomones are thought to be the secondary function of some crustacean structural polymers. Studies using

synthetic mimics of these polymers have shown that they can modify physiological and or behavioral responses in crabs, molluscs and a variety of other marine organisms (Rittschof and Cohen 2004). Further experimentation is needed to identify the molecular structure of these info-chemicals and the precise mechanism responsible for their synergistic effects with alarm cues on snail inducible defenses.

The fact that a specific combination of risk cues is required for the deployment of a complete anti-predator response has important ecological implications. The defensive responses of prey can have strong effects on both prey population dynamics and community structure. If predators switch diets often or modulate their feeding activity regularly, as with seasonal, generalist predators such as crabs, prey may not consistently encounter the chemical cues that induce the most extreme defenses. Such variation will undoubtedly alter the magnitude and/or increase the variability of plasticity-mediated effects in natural systems (Miner et al. 2005). Given the important role of predator behavior in determining the magnitude of the risk-induced plastic responses of prey, future studies aimed at creating ecologically realistic plasticity experiments should account for the variability of their experimental predator's diet and feeding habits.

Tables

Table 1. Analyses of defensive shell responses and linear shell growth of *Nucella lamellosa* to the presence of injured con- and hetero-specific snails and *Cancer productus* fed different diets (fish, conspecific and heterospecific snails) after 48 days of exposure to experimental conditions, with final shell mass as a covariate for lip thickness and final shell length as a covariate for shell mass. Replicates aquaria were nested within treatments. All data were $\log_{10}$-transformed prior to analyses.

Dependent variable	Source	Df	MS	*F*	*P*
Lip thickness	Shell mass	1	0.147422	62.9890	0.000000***
	Treatment	5	0.262399	7.5698	0.002016*
	Replicate(Treatment)	12	0.034762	14.8529	0.000000***
	Error	157	0.002340		
Apertural teeth	Treatment	5	0.036603	35.47666	0.000001***
	Replicate(Treatment)	12	0.001031	0.61104	0.830673
	Error	158	0.001687		

Shell mass	Shell length	1	1.335533	831.0485	0.000000***
	Treatment	5	0.022200	3.2264	0.044059*
	Replicate(Treatment)	12	0.007052	4.3885	0.000005***
	Error	157			
Linear shell growth	Treatment	5	0.77167	3.7417	0.028369*
	Replicate(Treatment)	12	0.20634	15.6090	0.000000***
	Error	159	0.01322		

Figures

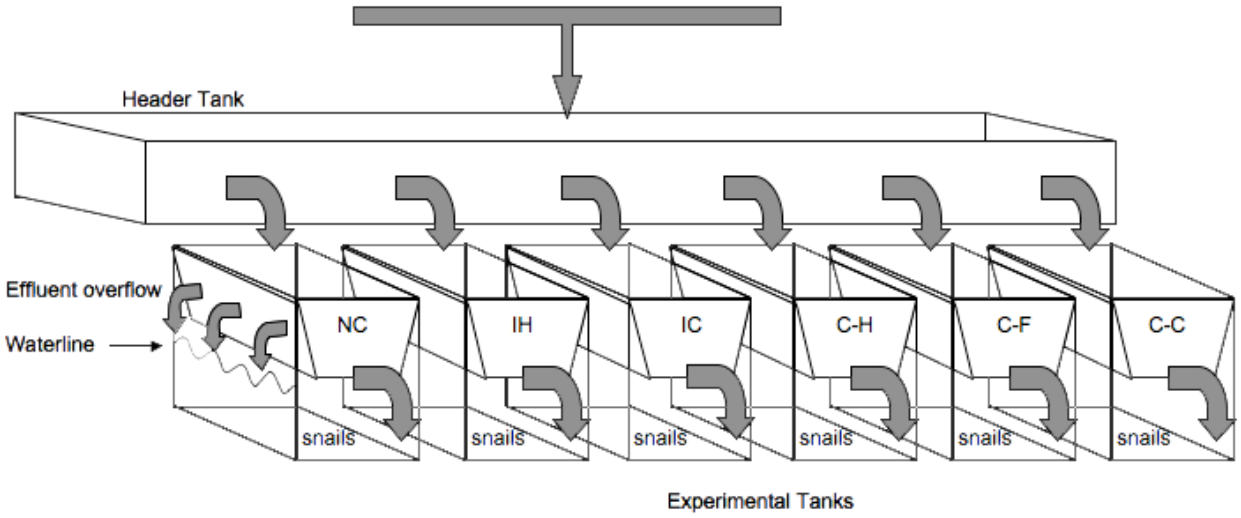


Fig. 1. Schematic of predation cue induction experiment. Experimental aquaria were fitted with tubs containing the cue source (NC – no cue, IH – injured heterospecific snails, IC – injured conspecific snails, C-H – crabs fed heterospecific snails, C-F – crabs fed fish, C-C – crabs fed conspecific snails). Overflow from the tubs delivered seawater and effluent to the experimental snails in each aquarium. Seawater drained through holes cut just below the level of the tub (gray arrows indicate direction of seawater flow).

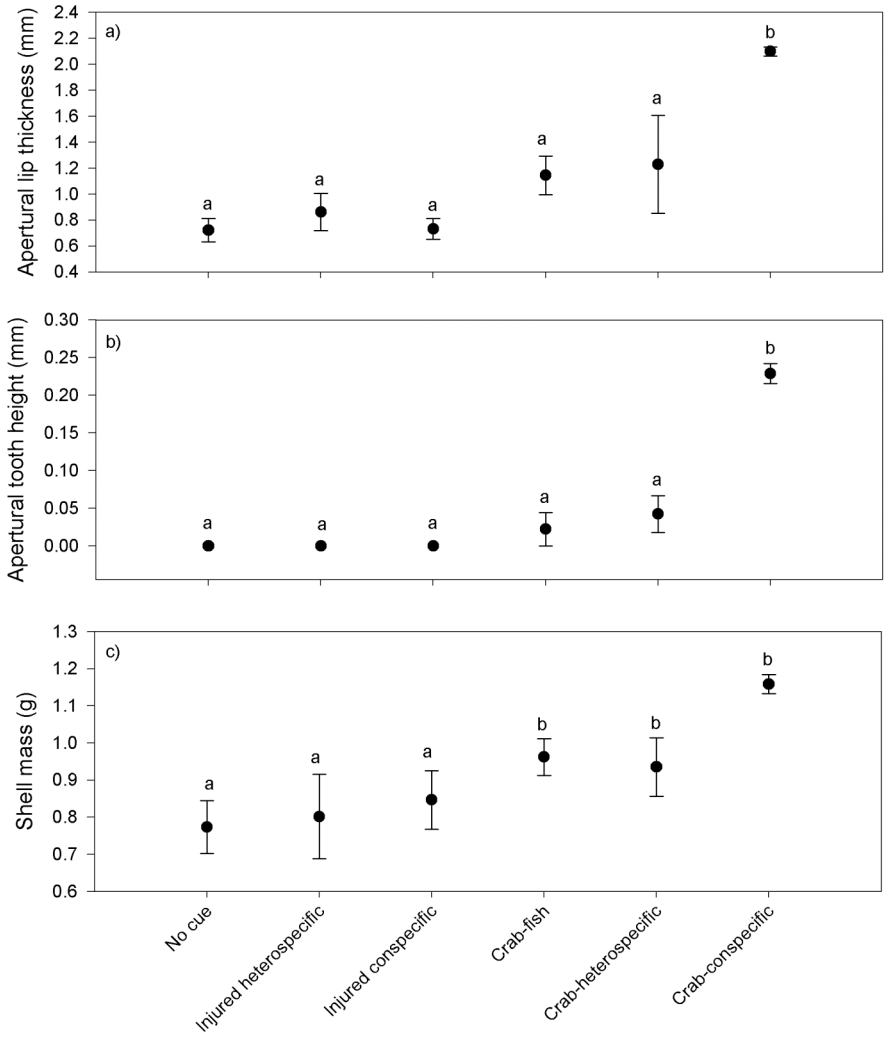


Fig. 2. Morphological responses of *Nucella lamellosa* (means ± 1 SE) to the presence of risk cues. Treatments were: Control with no cues added (no cue), injured heterospecifics, injured conspecifics, crabs fed fish (crab-fish), crabs fed heterospecific snails (crab-heterospecific), and crabs fed conspecific snails (crab-conspecific). Lowercase letters denote groups that are not significantly different ($P > 0.05$) based on a Fisher's LSD *post hoc* test.

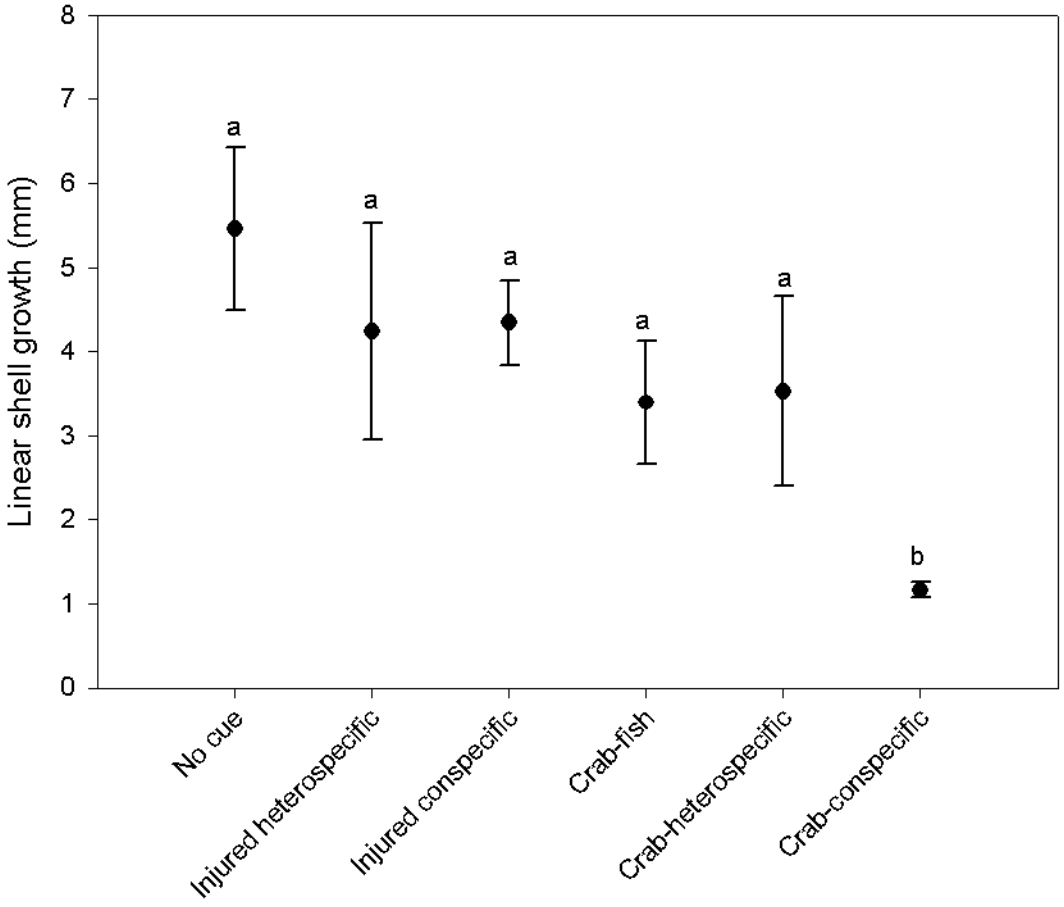


Fig. 3. Linear shell growth of *Nucella lamellosa* (means ± SE) in the presence of risk cues. Treatments were: Control with no cues added (no cue), injured heterospecifics, injured conspecifics, crabs fed fish (crab-fish), crabs fed heterospecific snails (crab-heterospecific), and crabs fed conspecific snails (crab-conspecific). Lowercase letters denote groups that are not significantly different ($P > 0.05$) based on a Fisher's LSD *post hoc* test.

References

Appleton RD, Palmer AR (1988) Water-borne stimuli released by predatory crabs and damaged prey induce more predator-resistant shells in a marine gastropod. Proceedings of the National Academy of Sciences of the United States of America 85:4387-4391

Avery R, Etter RJ (2006) Microstructural differences in the reinforcement of a gastropod shell against predation. Marine Ecology-Progress Series 323:159-170

Bamber SD, Naylor E (1997) Sites of release of putative sex pheromone and sexual behaviour in female *Carcinus maenas* (Crustacea: Decapoda). Estuarine Coastal and Shelf Science 44:195-202

Benard MF (2006) Survival trade-offs between two predator-induced phenotypes in Pacific treefrogs (*Pseudacris regilla*). Ecology 87:340-346

Bourdeau PE (2009) Prioritized phenotypic responses to combined predators in a marine snail. Ecology

Bronmark C, Pettersson LB (1994) Chemical cues from piscivores induce a change in morphology in crucian carp. Oikos 70:396-402

Brown GE, Dreier VM (2002) Predator inspection behaviour and attack cone avoidance in a characin fish: the effects of predator diet and prey experience. Animal Behaviour 63:1175-1181

Chivers DP, Smith RJF (1998) Chemical alarm signalling in aquatic predator-prey systems: A review and prospectus. Ecoscience 5:338-352

Chivers DP, Wisenden BD, Smith RJF (1996) Damselfly larvae learn to recognize predators from chemical cues in the predator's diet. Animal Behaviour 52:315-320

Colgan DJ, Ponder WF, Beacham E, Macaranas J (2007) Molecular phylogenetics of Caenogastropoda (Gastropoda : Mollusca). Molecular Phylogenetics and Evolution 42:717-737

Dalesman S, Rundle SD, Bilton DT, Cotton PA (2007) Phylogenetic relatedness and ecological interactions determine antipredator behavior. Ecology 88:2462-2467

Freeman AS (2007) Specificity of induced defenses in *Mytilus edulis* and asymmetrical predator deterrence. Marine Ecology-Progress Series 334:145-153

Griffiths CL, Richardson CA (2006) Chemically induced predator avoidance behaviour in the burrowing bivalve *Macoma balthica*. Journal of Experimental Marine Biology and Ecology 331:91-98

Hagen NT, Andersen A, Stabell OB (2002) Alarm responses of the green sea urchin, *Strongylocentrotus droebachiensis*, induced by chemically labelled durophagous predators and simulated acts of predation. Marine Biology 140:365-374

Hardege JD et al. (2002) Novel behavioural assay and partial purification of a female-derived sex pheromone in *Carcinus maenas*. Marine Ecology-Progress Series 244:179-189

Jacobsen HP, Stabell OB (1999) Predator-induced alarm responses in the common periwinkle, *Littorina littorea*: dependence on season, light conditions, and chemical labelling of predators. Marine Biology 134:551-557

Jacobsen HP, Stabell OB (2004) Antipredator behaviour mediated by chemical cues: the role of conspecific alarm signalling and predator labelling in the avoidance response of a marine gastropod. Oikos 104:43-50

Kats LB, Dill LM (1998) The scent of death: Chemosensory assessment of predation risk by prey animals. Ecoscience 5:361-394

Kristensen EA, Closs GP (2004) Anti-predator response of naive and experienced common bully to chemical alarm cues. Journal of Fish Biology 64:643-652

Laforsch C, Beccara L, Tollrian R (2006) Inducible defenses: The relevance of chemical alarm cues in *Daphnia*. Limnology and Oceanography 51:1466-1472

Lima SL, Dill LM (1990) Behavioral decisions made under the risk of predation - a review and prospectus. Canadian Journal of Zoology-Revue Canadienne De Zoologie 68:619-640

Lively CM (1986) Canalization versus developmental conversion in a spatially-variable environment. American Naturalist 128:561-572

McCollum SA, VanBuskirk J (1996) Costs and benefits of a predator-induced polyphenism in the gray treefrog *Hyla chrysoscelis*. Evolution 50:583-593

Miner BG, Sultan SE, Morgan SG, Padilla DK, Relyea RA (2005) Ecological consequences of phenotypic plasticity. Trends in Ecology & Evolution 20:685-692

Moran NA (1992) The evolutionary maintenance of alternative phenotypes. American Naturalist 139:971-989

Palmer AR (1982) Growth in marine gastropods - a non-destructive technique for independently measuring shell and body-weight. Malacologia 23:63-73

Relyea RA (2001) Morphological and behavioral plasticity of larval anurans in response to different predators. Ecology 82:523-540

Rittschof D, Cohen JH (2004) Crustacean peptide and peptide-like pheromones and kairomones. Peptides 25:1503-1516

Schoeppner NM, Relyea RA (2005) Damage, digestion, and defence: the roles of alarm cues and kairomones for inducing prey defences. Ecology Letters 8:505-512

Slusarczyk M (1999) Predator-induced diapause in *Daphnia magna* may require two chemical cues. Oecologia 119:159-165

Smee DL, Weissburg MJ (2006) Hard clams (*Mercenaria mercenaria*) evaluate predation risk using chemical signals from predators and injured conspecifics. Journal of Chemical Ecology 32:605-619

Smith ME, Belk MC (2001) Risk assessment in western mosquitofish (*Gambusia affinis*): do multiple cues have additive effects? Behavioral Ecology and Sociobiology 51:101-107

Stabell OB, Lwin MS (1997) Predator-induced phenotypic changes in crucian carp are caused by chemical signals from conspecifics. Environmental Biology of Fishes 49:145-149

Stabell OB, Ogbebo F, Primicerio R (2003) Inducible defences in *Daphnia* depend on latent alarm signals from conspecific prey activated in predators. Chemical Senses 28:141-153

Taylor HH, Taylor EW (1991) The dorsoventral muscles of *Carcinus maenas* - evidence for hydrostatic pressure control in a crab. Physiological Zoology 64:1110-1129

Teplitsky C, Plenet S, Joly P (2004) Hierarchical responses of tadpoles to multiple predators. Ecology 85:2888-2894

Tollrian R, Harvell CD (eds) (1998) The Ecology and Evolution of Inducible Defenses. Princeton University Press, Princeton, NJ

Trussell GC, Ewanchuk PJ, Bertness MD (2002) Field evidence of trait-mediated indirect interactions in a rocky intertidal food web. Ecology Letters 5:241-245

Trussell GC, Nicklin MO (2002) Cue sensitivity, inducible defense, and trade-offs in a marine snail. Ecology 83:1635-1647

Turner AM, Bernot RJ, Boes CM (2000) Chemical cues modify species interactions: the ecological consequences of predator avoidance by freshwater snails. Oikos 88:148-158

Van Buskirk J (2001) Specific induced responses to different predator species in anuran larvae. Journal of Evolutionary Biology 14:482-489

Vermeij GJ (1987) Evolution and Escalation: An Ecological History of Life. Princeton Univesrity Press, Princeton, NJ

Verschoor AM, Vos M, van der Stap I (2004) Inducible defences prevent strong population fluctuations in bi- and tritrophic food chains. Ecology Letters 7:1143-1148

Yamada SB, Boulding EG (1996) The role of highly mobile crab predators in the intertidal zonation of their gastropod prey. Journal of Experimental Marine Biology and Ecology 204:59-83

Chapter 3 - Specificity of cues that induce shell defenses in marine snails: Is a crab just a crab?

Introduction

Phenotypic plasticity for defensive traits is a potential adaptive mechanism for dealing with spatial and temporal heterogeneity in predation risk (Tollrian and Harvell 1998). Inducible defenses can alter species interactions, population dynamics and ecosystem functions and thus have important consequences at many levels of ecological organization (Miner et al. 2005). Defensive traits that are inducible are favored when there is spatial or temporal variation in predation risk (Moran 1992, Scheiner 1998), there is some opportunity cost to having a constitutive defense, reliable cues indicating a threat are available (Moran 1992, Getty 1996) and prey have the ability to respond with an effective defense within a relatively short time frame relative to environmental change (Padilla and Adolph 1996, Gabriel et al. 2005). Inducible defenses are widespread in nature, and can involve changes in behavior, morphology and life-history (Tollrian and Harvell 1998). In aquatic systems, prey organisms often detect and respond to waterborne chemical cues released by predators (Dodson et al. 1994, Bronmark

and Hansson 2000). These cues, if highly correlated with predation risk, can provide reliable information about the threat-level in the environment.

Little is known about the degree of specificity of inducible defenses to environmental cues. Previous studies have examined the specificity of prey responses to different concentrations of the same cue (Harvell 1998, Hawkins et al. 2007), to cues from damaged and consumed prey (Schoeppner and Relyea 2005, Laforsch et al. 2006), and to cues from multiple predatory species (Relyea 2003, Teplitsky et al. 2004, Bourdeau 2009a). However, because inducible defenses are often assumed to be predator-specific responses, studies do not often consider whether prey may respond to other species, including non-predatory species (but see Iyengar & Harvell, 2002; Langerhans & DeWitt, 2002).

In marine systems, many molluscs have been shown to produce thicker, heavier shells in response to chemical cues from predatory crabs (Appleton and Palmer 1988, Palmer 1990, Trussell 1996, Leonard et al. 1999, Caro and Castilla 2004, Dalziel and Boulding 2005, Bourdeau 2009a). These traits increase shell strength and thus enhance resistance to predation by durophagous (eating hard or hard-shelled organisms) crabs (Currey and Taylor 1974). Crab-induced shell-thickening is taxonomically widespread among molluscs and has been suggested to be a predator-induced defense specific to molluscivorous species (Tollrian and Harvell 1998). Previous studies of molluscan inducible defenses have only examined this response to chemical cues from predatory crabs, including species introduced to new shores (Freeman and Byers 2006, Edgell and Neufeld 2008).

However, a number of predatory and non-predatory crabs coexist in nature, and all might release cues detected by gastropods (Vermeij 1987). At present it is not known whether there is a specific cue associated with predatory species, or a generalized cue associated with all crabs that triggers defensive responses in molluscs. I tested whether the inducible shell defenses and associated changes in growth in *Nucella lamellosa* are specific to its most common predator, the rock crab *Cancer productus*, or if other predatory and non-predatory crabs can induce similar responses.

Methods

Study organisms and collection sites

Nucella lamellosa, is an intertidal snail that increases the size of its apertural teeth and the thickness and weight of its shell in response to the predatory crab *Cancer productus* (Appleton and Palmer 1988, Edgell and Neufeld 2008, Bourdeau 2009a). It lives sympatrically with a number of predatory and non-predatory species of crabs in the eastern north Pacific from Alaska to California (Kozloff 1987, Jensen 1995, Collins et al. 1996). As such, these species comprise an ideal system to test the specificity of the shell-thickening response.

All of the crabs used in this study are common on the rocky shores of protected bays in the eastern north Pacific, but differ in their diets and degree of durophagy (Kozloff 1987, Jensen 1995). The red rock crab, *Cancer productus,*

and the pygmy rock crab, *C. oregonensis,* are primarily durophagous. These crabs possess relatively large, strong chelae capable of producing powerful crushing forces (Yamada and Boulding 1998, Taylor et al. 2000). *C. oregonensis* (max carapace width = 53 mm) is a relatively sedentary species found mostly in shelters (e.g., crevices, dead barnacle tests and under rocks and cobbles) in the low intertidal and subtidal zones. Its effect on intertidal gastropod prey is small compared with that of the more abundant, larger and highly mobile *C. productus* (158-200 mm), which actively access the intertidal zone at high tide and consume more prey per unit body weight than *C. oregonensis* (Robles et al. 1989, Yamada and Boulding 1996, 1998). The purple shore crab, *Hemigrapsus nudus* (34 mm), is a generalist omnivore. It has smaller, weaker claws than either *C. oregonensis* and *C. productus* and can consume only the smallest snails (Yamada and Boulding 1998). I also included two species of anomurans, neither of which is durophagous. The grainyhand hermit crab, *Pagurus granosimanus* (19 mm), is mainly a scavenger and detritivore; it can feed opportunistically on *Nucella* hatchlings, but does so rarely (Gosselin 1997). The porcelain crab, *Petrolisthes eriomerus* (19 mm) is primarily a suspension feeder and detritivore and does not use its chelae to feed (Jensen 1995).

I collected 180 juvenile (< 25 mm shell length) *N. lamellosa* from the Westside Preserve, a current swept shore on the west side of San Juan Island, WA, USA (48°30'26.76"N, 123°8'35.20"W). This site was selected because although all crab species used in the study are present, they are rare (Bourdeau,

personal observation). This ensured that experimental snails had little previous exposure to cues from crabs in the field. Red rock crabs, *C. productus,* were trap-collected from the pier at the University of Washington's Friday Harbor Laboratories (FHL). All other crabs used in the experiment were collected by hand from nearby shores.

Experimental design

All experiments were conducted at FHL. Snails were exposed to six treatments: a negative control (no crab), *Pagurus*, *Petrolisthes*, *Hemigrapsus*, *C. oregonensis* and *C. productus*. Two replicate aquaria (30.5W X 19.1D X 20.3H cm) were used for each treatment. Because the crab species used in this experiment greatly differ in size, I used different numbers of crabs of each species in an effort to keep total crab biomass similar among treatments (2 *C. productus*, 3 *C. oregonensis*, 4 *H. sanguineus*, 5 *Pe. eriomerus* and 6 *Pa. granosimanus*). Crabs were placed in a plastic tub (1892 ml) fastened to the underside of the aquarium lid. Gravity fed seawater flowed from a header tank into the tub through a feeding hatch in the lid. Overflow from the tub provided the aquarium with seawater. Fifteen snails, individually numbered with bee tags, were added to each aquarium. This design allowed relatively constant flow-through of crab chemical cues while preventing physical contact between crabs and snails. Snails were fed *ad libitum* barnacles (*Balanus glandula*), encrusted on small stones. Barnacle-depleted stones were removed and replaced with new

barnacle-covered stones as needed. Crabs were fed frozen fish; so experimental snails were not exposed to the scent of injured snails in this study.

Shell thickness, shell mass and soft tissue growth

Shell thickness was measured to the nearest 0.01 mm with digital calipers at the mid-point of the apertural lip and at the lip suture, and averaged. Snails were weighed prior to the beginning of the experiment and after 60 days of exposure to experimental treatments. A nondestructive method whereby snails are weighed in air and then submerged in water (Palmer 1982) was used to separate shell mass from soft tissue mass. Shell mass was calculated as 1.572*(submerged weight)+0.0162. Soft tissue mass was calculated by subtracting shell mass from the weight in air.

Statistical analysis

All data were $\log_{10}$-transformed to better meet the assumptions of normality and homoscedasticity. Shell mass was analyzed with analysis of covariance (ANCOVA) with crab treatment as a fixed factor and with aquarium as a random factor nested within crab treatment. Final soft tissue mass was used as the covariate to control for size effects. To examine the somatic growth cost of responding to crab scent I analyzed soft tissue mass (hereafter body mass) with ANCOVA with treatment as a fixed factor, aquarium as a random factor nested within treatment and final shell mass as the covariate. Apertural lip thickness did not meet the assumption of equal variances (Levene's, $P = 0.027$), but because

parametric tests are not particularly sensitive to violations of this assumption, especially when variances are not dramatically different from each other, I used a parametric test on this variable (ANCOVA with treatment as a fixed factor, aquarium as a random factor nested within treatment and final shell length as the covariate). Covariate-by-treatment interactions that were not significant ($P > 0.25$) were removed from the models (Hendrix et al. 1982). I used post-hoc comparisons of covariate-adjusted means with Fisher's protected least significant difference test (LSD). For models with significant covariate-by-treatment interactions I used the Wilcoxon modification of the Johnson-Neyman procedure to determine the range of covariates over which response variables were significantly different among treatments (Huitema 1980). All analyses were conducted with Statistica software (v 6.1), except the Wilcox procedure, which was performed with the program WILCOX (Quinn and Keough 2002).

Results

Snails produced heavier shells and reduced their somatic growth in response to each of the crab species tested (Table 1; Fig. 1). Post-hoc analyses of size-adjusted means for both traits revealed significant differences between each crab species' effluent treatment and the control. Size-adjusted shell mass and body mass did not differ among any of the crab effluent treatments (Fig. 1).

For apertural lip thickness, ANCOVA revealed a significant interaction

between treatment and the covariate, shell length (Table 2). Only the regression line for *C. productus* differed in slope from the control (Table 2; Fig. 2). The Wilcoxon Johnson-Neyman test indicated that snails exposed to *C. productus* were predicted to have significantly thicker apertural lips than snails in the no crab treatment when the covariate, final length, was between 27.49 and 30.94 mm (Table 2), which was similar to the mean shell length (29.51 ± 4.43) among treatments. Snails exposed to *C. productus* were also predicted to have thicker apertural lips over a similar range of shell lengths (~ 27-32 mm) than snails exposed to all other crab treatments except *C. oregonensis* (Table 2).

Discussion

Nucella lamellosa responded to chemical cues from predatory and non-predatory crab species by reducing growth and producing heavier shells. These responses appear to be generalized responses to crabs, independent of degree of durophagy or potential threat as a predator. Because of the growth costs of producing thicker shells in a predator-free environment, *N. lamellosa* was predicted to show a specific induced response limited to predatory crabs, and a weak response or no response to non-predatory species. Instead, the response by *N. lamellosa* to two non-predatory anomuran crabs and a generalist omnivore was just as strong as that elicited by the molluscivorous *Cancer* species. In contrast, snails only produced thickened apertural lips in response to their major

predator, *Cancer productus*, indicating that response specificity was different for different traits. These results suggest that the observed phenotypic plasticity in lip thickness is a predator-specific extension of a generalized response to crabs. These results are in contrast to those found by Edgell & Neufeld (2008), who found that the introduced crab, *Carcinus maenas,* a species that does not overlap with *N. lamellosa,* does not induce growth reduction or shell thickening.

For plasticity to be adaptive, theoretical models predict that costs associated with the response must be balanced or outweighed by the benefits of the response (Levins 1968, Lively 1986). In *N. lamellosa,* shell thickening is a passive consequence of reduced feeding and growth (Chapter 4), thus the energetic costs of adding shell material may be absent or negligible (Palmer 1992). The reduced growth in snails exhibiting anti-predator responses result from predator avoidance behavior (reduced feeding activity and retreating into refuges, Chapter 4), thus the induced defenses are likely to be associated with a net survival benefit in environments containing predatory crabs. However, these animals do experience a growth cost due to reduced feeding. By growing slower, snails attain smaller size and have reduced fecundity (Harding et al. 2007) and are less likely (or take more time) to attain a size refuge from many common intertidal predators, including crabs (Harding 2003). Given these opportunity costs, responding to cues from non-predatory crabs would seem maladaptive.

This seemingly counterintuitive response could be explained by the functional link between growth rate and shell thickening. In *N. lamellosa,* growth

rate is mediated by feeding behavior (Chapter 4). Behavior can track temporal change in predation risk more rapidly than morphology. Therefore, snails that respond inappropriately to an over-generalized cue could reverse their 'mistake', or compensate for periods of inactivity with increased feeding and growth during periods when risk cues were absent (Arendt 1997, Stachowicz and Hay 1999). Thus, while opportunity costs of responding to general crab cues might exist over short time scales, long-term costs may be relatively minor and will be outweighed by the survival benefits of responding cautiously so as not to make a behavioral miscue in risky habitats.

Another potential explanation for the observed results is that each of the crab species tested is an indicator of a risky environment. Because all of the crabs in this study co-occur over broad geographic scales and on the same shores, detection of one should indicate the presence of another. While the presence of specific cues from predatory crabs may reliably indicate the presence of risk, the absence of these cues may not reliably indicate the absence of risk in a particular habitat. It on the order of weeks to months for these snails to produce an effectively thicker shell (Appleton and Palmer 1988, Bourdeau 2009a). If shell thickening is not induced until a predatory crab is actually present and detected, there may not be enough time before an encounter with a predator for the production of an effective defense (Padilla and Adolph 1996). However, the probability of producing a correct phenotype-to-habitat match can be increased if cues other than those of predator can be used to reliably judge the

relative risk of the habitat.

Indirect cues indicating predation risk should only be favored when they are positively associated with environments that are risky. For example, *Daphnia* responds to chemical cues released by fish with diel vertical migration as a predator avoidance behavior; responses to chemical cues from both planktivorous and piscivorous fish are similar, but piscivores co-exist with planktivorous fish, so their presence may indirectly indicate the presence of planktivores (von Elert and Pohnert 2000). In contrast, the morphological response of a freshwater snail to non-molluscivorous fish has been argued to be maladaptive because the presence of these non-predators is negatively correlated with the presence of molluscivorous species (Langerhans and DeWitt 2002).

In this system, the presence of non-predatory crabs is likely to be positively correlated with the presence of predatory crabs because of their overlapping ranges and the influence of water motion on the abundance of crabs in general. Crabs are often much more abundant on wave-protected shores, as most crabs have difficulty foraging on shores with heavy surf (Boulding et al. 1999, Hampton and Griffiths 2007). The association between non-predatory and predatory crabs is in the direction that would make the presence of non-predatory species a reliable indirect predictor of predatory species for snails. A recent study using similar methodology found that *N. lamellosa* does not reduce growth or thicken its shell in the presence of *Carcinus maenas* (Edgell and Neufeld 2008). *C. maenas*

is a voracious molluscivore, but does not overlap with *N. lamellosa* in its native range and therefore would not be a reliable indicator of the presence of native predators.

In this study, cue specificity depended upon the defensive trait under consideration. Increased shell mass and reduced soft tissue mass were both observed in the presence of all crabs, but apertural lip thickeneing occurred only in response to *C. productus* effluent. While shell mass is a good estimate of relative resistance to crushing, it may not necessarily reflect overall resistance to crab predation (especially to those crabs that peel shells from the aperture). Thickened apertural lips not only reinforce resistance to crushing, but also prevent shell-peeling, a tactic-employed by some molluscivorous crabs, including *C. productus* (Vermeij 1987). Thus, thickened apertural lips likely represent a more extreme defensive response than simply heavier shells. This response may be a simple extension of the way snails respond to generalized crab cues (Kraft et al. 2006) or an adaptive exaggeration co-opted from a general response that may not have even originated in through selection by extant or co-occurring species (Gould and Vrba 1982).

That significantly thicker lips were produced only in the presence of *C. productus* is consistent with theory, which predicts that prey should exhibit more extreme phenotypic responses in the presence of more dangerous predators (Lima and Dill 1990). The highly mobile and large-clawed *C. productus* exerts greater crushing forces and is responsible for greater mortality on intertidal snails

than any of the other crabs used in this study and thus represents a greater risk (Yamada and Boulding 1996, 1998). Consequently, it appears snails adjusted their phenotypic response in accordance with the heightened risk. This result is consistent with the findings in chapter 2; *N. lamellosa* produces a more extreme set of defenses in the presence of cues in the crab's diet that indicate elevated risk. Taken together, these findings highlight the importance of considering multiple traits when testing hypotheses about cue reliability and specificity, as one would draw different conclusions based on different traits.

Figures

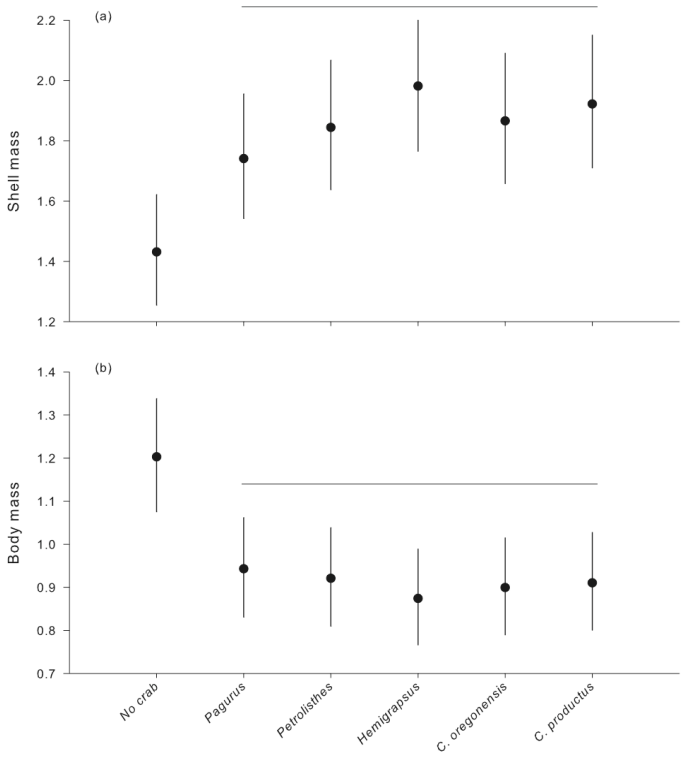


Figure 1. (a) Shell mass and (b) soft tissue mass of *Nucella lamellosa* raised in the six environments: negative control (No crab), grainyhand hermit crab (*Pagurus*), porcelain crab (*Petrolisthes*), purple shore crab (*Hemigrapsus*), pygmy rock crab (*C. oregonensis*) and positive control (red rock crab, *C. productus*). Values are back-transformed least squares means and 95% confidence intervals of $\log_{10}$-transformed data computed for covariates at their means. Horizontal lines indicate groups that were not significantly different from one another (LSD, $P > 0.05$) for both traits.

References

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

Arendt, J. D. 1997. Adaptive intrinsic growth rates: An integration across taxa. Quarterly Review of Biology **72**:149-177.

Boulding, E. G., M. Holst, and V. Pilon. 1999. Changes in selection on gastropod shell size and thickness with wave-exposure on Northeastern Pacific shores. Journal of Experimental Marine Biology and Ecology **232**:217-239.

Bourdeau, P. E. 2009. Prioritized phenotypic responses to combined predators in a marine snail. Ecology **in press**.

Bronmark, C. and L. A. Hansson. 2000. Chemical communication in aquatic systems: an introduction. Oikos **88**:103-109.

Caro, A. U. and J. C. Castilla. 2004. Predator-inducible defences and local intrapopulation variability of the intertidal mussel *Semimytilus algosus* in central Chile. Marine Ecology-Progress Series **276**:115-123.

Collins, T. M., K. Frazer, A. R. Palmer, G. J. Vermeij, and W. M. Brown. 1996. Evolutionary history of northern hemisphere *Nucella* (Gastropoda, Muricidae): Molecular, morphological, ecological, and paleontological evidence. Evolution **50**:2287-2304.

Currey, J. D. and J. D. Taylor. 1974. Mechanical-behavior of some molluscan hard tissues. Journal of Zoology **173**:395-406.

Dalziel, B. and E. G. Boulding. 2005. Water-borne cues from a shell-crushing predator induce a more massive shell in experimental populations of an intertidal snail. Journal of Experimental Marine Biology and Ecology **317**:25-35.

Dodson, S. I., T. A. Crowl, B. L. Peckarsky, L. B. Kats, A. P. Covich, and J. M. Culp. 1994. Nonvisual communication in freshwater benthos - an overview. Journal of the North American Benthological Society **13**:268-282.

Edgell, T. C. and C. J. Neufeld. 2008. Experimental evidence for latent developmental plasticity: intertidal whelks respond to a native but not an introduced predator. Biology Letters **4**:385-387.

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

Gabriel, W., B. Luttbeg, A. Sih, and R. Tollrian. 2005. Environmental tolerance, heterogeneity, and the evolution of reversible plastic responses. American Naturalist **166**:339-353.

Getty, T. 1996. The maintenance of phenotypic plasticity as a signal detection problem. American Naturalist **148**:378-385.

Gosselin, L. A. 1997. An ecological transition during juvenile life in a marine snail. Marine Ecology-Progress Series **157**:185-194.

Gould, S. J. and E. S. Vrba. 1982. Exaptation - a missing term in the science of form. Paleobiology **8**:4-15.

Hampton, S. L. and C. L. Griffiths. 2007. Why *Carcinus maenas* cannot get a grip on South Africa's wave-exposed coastline. African Journal of Marine Science **29**:123-126.

Harding, J. M. 2003. Predation by blue crabs, *Callinectes sapidus*, on rapa whelks, Rapana venosa: possible natural controls for an invasive species? Journal of Experimental Marine Biology and Ecology **297**:161-177.

Harding, J. M., R. Mann, and C. W. Kilduff. 2007. The effects of female size on fecundity in a large marine gastropod *Rapana venosa* (Muricidae). Journal of Shellfish Research **26**:33-42.

Harvell, C. D. 1998. Genetic variation and polymorphism in the inducible spines of a marine bryozoan. Evolution **52**:80-86.

Hawkins, L. A., A. E. Magurran, and J. D. Armstrong. 2007. Innate abilities to distinguish between predator species and cue concentration in Atlantic salmon. Animal Behaviour **73**:1051-1057.

Hendrix, L. J., M. W. Carter, and D. T. Scott. 1982. Covariance analyses with heterogeneity of slopes in fixed models. Biometrics **38**:641-650.

Huitema, B. E. 1980. The Analysis of Covariance and Alternatives. John Wiley and Sons, Inc., New York.

Jensen, G. C. 1995. Pacific Coast crabs and shrimps. Sea Challengers, Monterey, CA, USA.

Kozloff, E. N. 1987. Marine Invertebrates of the Pacific Northwest. University of Washington Press, Seattle, WA, USA.

Kraft, P. G., C. E. Franklin, and M. W. Blows. 2006. Predator-induced phenotypic plasticity in tadpoles: extension or innovation? Journal of Evolutionary Biology **19**:450-458.

Laforsch, C., L. Beccara, and R. Tollrian. 2006. Inducible defenses: The relevance of chemical alarm cues in *Daphnia*. Limnology and Oceanography **51**:1466-1472.

Langerhans, R. B. and T. J. DeWitt. 2002. Plasticity constrained: over-generalized induction cues cause maladaptive phenotypes. Evolutionary Ecology Research **4**:857-870.

Leonard, G. H., M. D. Bertness, and P. O. Yund. 1999. Crab predation, waterborne cues, and inducible defenses in the blue mussel, *Mytilus edulis*. Ecology **80**:1-14.

Levins, R. 1968. Evolution in changing environments. Princeton University Press, Princeton, NJ.

Lima, S. L. and L. M. Dill. 1990. Behavioral decisions made under the risk of predation - a review and prospectus. Canadian Journal of Zoology-Revue Canadienne De Zoologie **68**:619-640.

Lively, C. M. 1986. Canalization versus developmental conversion in a spatially-variable environment. American Naturalist **128**:561-572.

Miner, B. G., S. E. Sultan, S. G. Morgan, D. K. Padilla, and R. A. Relyea. 2005. Ecological consequences of phenotypic plasticity. Trends in Ecology & Evolution **20**:685-692.

Moran, N. A. 1992. The evolutionary maintenance of alternative phenotypes. American Naturalist **139**:971-989.

Padilla, D. K. and S. C. Adolph. 1996. Plastic inducible morphologies are not always adaptive: The importance of time delays in a stochastic environment. Evolutionary Ecology **10**:105-117.

Palmer, A. R. 1982. Growth in marine gastropods - a non-destructive technique for independently measuring shell and body-weight. Malacologia **23**:63-73.

Palmer, A. R. 1990. Effect of crab effluent and scent of damaged conspecifics on feeding, growth, and shell morphology of the Atlantic dogwhelk *Nucella lapillus* (L). Hydrobiologia **193**:155-182.

Palmer, A. R. 1992. Calcification in marine mollusks - how costly is it. Proceedings of the National Academy of Sciences of the United States of America **89**:1379-1382.

Quinn, G. P. and M. J. Keough. 2002. Experimental Design and Data Analysis. Cambridge University Press, New York.

Relyea, R. A. 2003. How prey respond to combined predators: A review and an empirical test. Ecology **84**:1827-1839.

Robles, C., D. A. Sweetnam, and D. Dittman. 1989. Diel variation of intertidal foraging by *Cancer productus* L in British Columbia. Journal of Natural History **23**:1041-1049.

Scheiner, S. M. 1998. The genetics of phenotypic plasticity. VII. Evolution in a spatially-structured environment. Journal of Evolutionary Biology **11**:303-320.

Schoeppner, N. M. and R. A. Relyea. 2005. Damage, digestion, and defence: the roles of alarm cues and kairomones for inducing prey defences. Ecology Letters **8**:505-512.

Stachowicz, J. J. and M. Hay. 1999. Reduced mobility is associated with compensatory feeding and increased diet breadth of marine crabs. Marine Ecology-Progress Series **188**:169-178.

Taylor, G. M., A. R. Palmer, and A. C. Barton. 2000. Variation in safety factors of claws within and among six species of *Cancer* crabs (Decapoda : Brachyura). Biological Journal of the Linnean Society **70**:37-62.

Teplitsky, C., S. Plenet, and P. Joly. 2004. Hierarchical responses of tadpoles to multiple predators. Ecology **85**:2888-2894.

Tollrian, R. and C. D. Harvell, editors. 1998. The Ecology and Evolution of Inducible Defenses. Princeton University Press, Princeton, NJ.

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

Vermeij, G. J. 1987. Evolution and Escalation: An Ecological History of Life. Princeton Univesrity Press, Princeton, NJ.

von Elert, E. and G. Pohnert. 2000. Predator specificity of kairomones in diel vertical migration of *Daphnia*: a chemical approach. Oikos **88**:119-128.

West-Eberhard, M. J. 2003. Developmental Plasticity and Evolution. Oxford University Press, New York.

Yamada, S. B. and E. G. Boulding. 1996. The role of highly mobile crab predators in the intertidal zonation of their gastropod prey. Journal of Experimental Marine Biology and Ecology **204**:59-83.

Yamada, S. B. and E. G. Boulding. 1998. Claw morphology, prey size selection and foraging efficiency in generalist and specialist shell-breaking crabs. Journal of Experimental Marine Biology and Ecology **220**:191-211.

Chapter 4 - Prioritized phenotypic responses to combined predators in a marine snail

Abstract

Although many species face numerous predators in nature, the combined impact of multiple predators on the inducible defenses of prey has rarely been studied. Prey may respond with an intermediate phenotype that balances the risk from several sources or may simply respond to the most dangerous predator. I examined the separate and combined effects of the presence of shell-breaking (crabs, *Cancer productus*) and shell-entry (seastars, *Pisaster ochraceous*) predators fed conspecific snails on the defensive shell morphology and antipredator behavior of a marine snail (*Nucella lamellosa*). When exposed to each feeding predator separately, snails responded with a combination of morphological defenses that reflect the attack mode of the predator and a generalized behavioral response. Snails responded to feeding crabs by increasing refuge use and producing a thick, rotund shell. Snails responded to feeding seastars with increased refuge use but produced elongate shells with high spires that allowed for greater retraction of the soft tissue. Seastar-induced phenotypes reduced susceptibility to seastars relative crab-induced phenotypes, but crab-induced phenotypes did not significantly reduce susceptibility to crabs, indicating an asymmetrical functional tradeoff. When feeding predators were

combined, snails produced a morphological phenotype similar to that expressed in the presence of the predator that imposed the highest mortality at the population level, suggesting that predator-induced morphology was prioritized according to predation risk. These results suggest that prioritizing conflicting defenses according to predator danger may be a common strategy for prey responding to combined predators, particularly in conjunction with generalized behavioral responses that reduce overall risk in multiple predator environments.

Introduction

To date, the majority of empirical and theoretical work on phenotypic plasticity has focused on single trait responses to single environmental factors (Pigliucci 2005). However, it is becoming increasingly clear that a shift toward studying how multiple traits vary in response to multiple factors is necessary for understanding the plastic responses of organisms to environmental change (DeWitt and Langerhans 2003, Pigliucci 2003). Some of the most frequently studied examples of phenotypic plasticity are predator-induced defenses (Tollrian and Harvell 1998, DeWitt and Scheiner 2003). The majority of studies of inducible defenses have focused on single traits and single predators, but in nature most organisms are faced with a diverse assemblage of predators with different attack modes (Lima and Dill 1990, Sih et al. 1998). This has resulted in a diversity of prey traits that are effective against different predator strategies, yet

we know relatively little about how prey change multiple traits in response to multiple-predator environments. Moreover, the few studies that have considered multiple traits demonstrate that focusing on a single trait is frequently insufficient to predict prey responses to combinations of predators (Relyea 2003). Thus, combinatorial experiments that quantify suites of defensive traits are essential for generating and testing predictive models of the evolution of inducible defenses in multi-predator systems (Lima 1992, Matsuda et al. 1996).

Defenses that are effective against one predator may not be effective against another, particularly if the predators use different attack modes (e.g., DeWitt et al. 2000). When different predators induce conflicting responses, the outcome of combined predator exposure will depend on the relative benefits of the opposing phenotypes as well as the energy limitations and developmental constraints imposed on the expression of conflicting responses. The result may either be an intermediate response with intermediate effectiveness against several predators, or a directional response to the disproportionately dangerous predator (Lima 1992, Matsuda et al. 1996). Support for these predictions have come from a limited number of single-trait studies focused on the behavioral responses of freshwater taxa (reviewed in Relyea 2003, but see (Hoverman and Relyea 2007). More studies are needed to determine whether we can generalize these patterns across a diversity of traits, taxa and systems.

Marine gastropods and their predators are an ideal system to study the effects of combined predators on prey defenses. A long evolutionary history with

their predators has produced a variety of inducible morphological and behavioral adaptations that reduce susceptibility to predation (Vermeij 1987). In nature, snails are often exposed to both shell-breaking and shell-entry predators, which can be encountered separately along an environmental gradient or simultaneously within a site. Because these predators differ in their attack modes, their presence could elicit conflicting responses. For example, shell-breaking predators may induce rotund shells which better disperse crushing forces, while shell entry predators induce elongate shells with high spires and narrow apertures which restrict shell entry (DeWitt et al. 2000). However, elongate shells can be easily crushed and might not provide effective armor, so a rotund, low-spired shell might be favored in the presence of shell crushers. In such a shell, however, deep withdrawal of the soft tissues is difficult (Vermeij 1987). Thus, a plastic response to one predator type would impose a tradeoff for protection against the alternative predator type.

I examined the morphological and behavioral responses of a marine snail, *Nucella lamellosa,* to the presence of predators with different attack modes: a shell-breaking crab, *Cancer productus,* and a shell-entering seastar, *Pisaster ochraceous*. Both predators can control *Nucella* populations through direct consumption (Paine 1974), or by inducing defensive behavioral or morphological traits (Appleton and Palmer 1988, Marko and Palmer 1991, Navarrete et al. 2000). At the population level, predator assemblages may vary from crab-dominated to seastar-dominated along a water motion gradient (Menge and

Lubchenco 1981), and within sites these predators often co-occur. Thus, in nature snails can encounter the two kinds of predators separately or simultaneously. Here I tested whether the two different predators induce conflicting responses and assessed the outcome of exposure to both predators simultaneously. I also measured crab and seastar predation on experimental snail populations to assess which predator represents the higher risk. My initial hypothesis was that if shell-breaking and shell-entry predators induce conflicting responses and differ in the risk they impose then prey should prioritize their defensive phenotype to the riskier predator.

Methods

Collection and Maintenance of Study Organisms

I collected juvenile *N. lamellosa* for an induction experiment from the intertidal zone between Cantilever Point and Collin's Cove, a wave-protected shore near the University of Washington's Friday Harbor Laboratories (FHL) on San Juan Island (48°33'N, 123°0l'W) in July 2006. Snails for predation trials were collected from two sites: False Bay, a wave-protected site on the west side of San Juan Island (48°30'N, 123°8'W) and the Westside Preserve, a moderately exposed site just north of False Bay (48°29'N, 123°4'W). Crabs were trap-collected from the FHL pier and seastars were collected by hand in the vicinity of FHL. Prior to

experimentation I marked snails by affixing a colored, numbered tag to the shell with cyanoacrylate adhesive. Red nail polish was also applied to the outside leading edge of the main body whorl. Snails and their predators were maintained separately in flow-through seawater tables so that snails were not exposed to predator cues. Snails were fed *ad libitum* barnacles (*Balanus glandula*), crabs were fed frozen fish and seastars were fed clams (*Nuttallia obscurata*). Predators were starved for at least 48 hours prior to experiments.

Comparing snail morphological responses to different predators

Prey responses to the separate and simultaneous presence of both predators were assessed in an induction experiment with four predator cue treatments: the absence of predators, the presence of feeding crabs, the presence of feeding seastars and the simultaneous presence of both feeding predators. Three replicates of each treatment were assigned randomly to 12 plastic covered aquaria (36.8 cm wide x 21.6 cm deep x 25.4 cm high). Each aquarium was provisioned with small stones encrusted with barnacles that served as both food and shelter for the snails. Eighteen individually marked juvenile snails (11.80-25.77 mm shell length) were haphazardly placed among the stones in each aquarium. Preliminary analysis indicated that there were no significant differences among treatments with respect to initial snail size and morphology. Stones were replaced when the barnacle supply was depleted. Plastic tubs (29.5 cm wide x 10.0 cm deep x 17.2 cm high) for holding predators were fastened to the underside of the cover of each aquarium for all treatments. Seawater flowed

directly into the tub through a feeding door in the aquarium top. Overflow from the tub provided seawater for the snails below. Seawater drained out of each aquarium through holes cut just below the level of the tub. This allowed the flow-through of chemical cues while preventing physical contact between the snails and the predator(s). In an effort to keep the total predator biomass and thus predator cue similar among predator treatments I standardized predator size; one large crab (mean carapace width = 144.77 mm) or large seastar (mean oral disc diameter = 71.33 mm) was placed in the single predator treatments and one small crab (mean carapace width = 114.2) and one small seastar (mean oral disc diameter = 55.44 mm) were placed together in the combined predator treatment. Predators in the combined predator treatment were physically separated in the tub to reduce interaction. A single snail was added to the predator tub every other day, for a total of 35 snails per predator over the course of the experiment. Snails presented to crabs were cracked to ensure consumption. Conspecific snails were chosen as a prey because the combination of predators and alarm cues has been shown to maximize defensive responses in snails (Jacobsen and Stabell 1999). All snails offered to predators during the course of the experiment were consumed, thus the amount of food consumed by each predator was similar. After 70 days, morphological responses of the snails were assessed.

Morphometric Measurements

I estimated the shell weight and body weight of each snail with a nondestructive

method (Palmer 1982). Snails were first suspended in seawater to estimate shell weight (immersed weight). Then, after allowing the shells to dry and gently pressing out extravisceral water, I weighed the snails in air (whole weight). I estimated body weight as the difference between the whole weight and the estimated shell weight. I measured shell length and shell width of each snail (Fig. 1A) to the nearest 0.01 mm with digital calipers and took two digital images (Canon PowerShot™ SD450 Digital Elph mounted to a lighted photo stand) of each individual before and after the experiment. For the first image snails were oriented with the axis of coiling perpendicular to the optical axis of the camera with the aperture facing up. I used these images to measure inner aperture area by tracing the inner circumference of the aperture and calculating its total area (mm^2). For the second image shells were embedded in sculpting clay, siphonal canal down, with the axis of coiling parallel to the optical axis of the camera with the apex facing up. I used this set of images to measure shell growth as the spiral distance from the edge of the initial nail polish mark to the leading edge of the last body whorl. All images were analyzed with Image-Pro Plus v 6.2. Shell aspect ratio was calculated as shell length (longest dimension along the coiling axis): shell width (widest dimension perpendicular to the coiling axis in the plane of the aperture). I estimated the ability of a snail to retract into its shell by measuring the unoccupied volume of the shell using a modification of the procedure in Palmer (1990). Snails that had been gently prodded into their shells were embedded aperture up in sculpting clay on a balance. After taring the

balance I pipetted distilled water into the aperture until it was flush with the lip of the aperture. I used the weight of water added as the unoccupied volume of the shell. To test the validity of this method for measuring withdrawal depth, I measured the unoccupied volumes of 15 wild-caught snails from the collection site and compared these values with the angular withdrawal distances taken relative to the maximum penetration distance into the shell (Edgell and Rochette 2008) of the same fifteen individuals.

Shell shape was analyzed with landmark-based geometric morphometrics (Bookstein 1989, Rohlf and Marcus 1993). This method retains the geometry of shape and facilitates visualization and statistical analysis of shape differences among groups and thus is a considerable improvement over linear distance measures, which provide only limited shape information. I digitized ten shell landmarks (Fig. 1B) on the first set of images with tpsDig software (Rohlf 2006). These landmarks were used in a generalized Procrustes analysis to remove the effects of specimen size, position and orientation in the digital images. The resultant aligned landmark configurations were used to generate shape variables as partial warp scores from a thin-plate spline analysis (TPS) and two uniform scores (tpsRelw v. 1.44; Rohlf, 2006). I then performed a relative warp analysis, which is analogous to a principal components analysis (PCA) of shape variation. Relative warps are principal components of the distribution of shapes and summarize the variation in local shape deformations among the specimens. To visualize shape differences among groups I projected the means of experimental

treatments onto the first two principal components, which accounted for 58.73% of the total shape variation.

Predator avoidance behavior

Over the course of the experiment I quantified snail avoidance behavior on 7 occasions approximately 10 d apart. Approximately 24 h after predators were fed I made 5 min scans of each experimental chamber, and recorded the number and position of each visible snail from a short (~ 1m) distance. I combined the number of snails hiding under stones (invisible snails) with the number of snails at or above the water line to determine the total number of snails taking refuge. I used the proportion of snails in avoidance in each aquarium as my behavioral response variable.

Statistical Analyses

Analyses were applied to shell characteristics (shell weight, aspect ratio, unoccupied volume) previously identified or hypothesized as having functional significance as antipredator traits (Vermeij 1987), and to multivariate geometric shell shape, anti-predator behavior and growth. I tested for heterogeneity of variance with Levene's test and transformed variables when significant heterogeneity was detected. Because size was affected by treatment, I used final body weight as a covariate to control for size effects on shell weight and aspect ratio. Because internal shell volume can be influenced by shell depth and shell thickness I used inner aperture as a covariate to control for the effects of shell

thickness on unoccupied shell volume. I ran 3 separate ANCOVAs, using the appropriate covariate and predator cue as fixed-effect factor, to compare treatments with respect to morphological variables. Because individual snails within a given aquaria may not be independent, replicates (aquaria) were treated as a random effect nested in the treatment effect. I used log-transformed values of all morphological variables except for aperture area, which was square root transformed to improve linearity. Shape parameters (i.e., the first 9 relative warps, which represent local deformations and account for 92.04% of the overall variation and the uniform components, which represent stretching and shearing) were analyzed with MANCOVA for variation attributable to predator treatments, with aquaria as a random effect nested in treatments and centroid size as a covariate to estimate and statistically adjust for multivariate allometry (i.e., the change in shape with growth). Post hoc comparisons among treatments were performed on the first two relative warps. Snail behavioral response did not differ among observation days so data were pooled across all observational periods for each replicate for statistical analysis. Proportions were arcsine square root transformed and analyzed with analysis of variance (ANOVA). Shell growth was analyzed with nested ANCOVA on spiral distance using initial shell length as a covariate. Body growth was analyzed with nested ANCOVA on final body weights using initial body weight as a covariate. Statistical models using covariates were initially run with the covariate by treatment interaction term included; however, $P > 0.25$ for all interactions, so the interaction terms were removed to simplify the

models (Hendrix et al. 1982). Multiple comparisons among groups were made with Tukey's HSD test. All statistical analyses were done with Statistica (v. 6.1, StatSoft).

Comparing relative risk of predators

I used a short-term predation experiment to determine the risk imposed on snail populations by crabs and seastars and to evaluate the relative vulnerability of elongate morphs and rotund morphs to each predator. I offered 20 individually marked, field-caught snails to each of four crabs (mean carapace width = 131.85 mm) and four seastars (mean oral disc diameter = 70.60 mm). The 20 snails were grouped into two classes of 10: elongate with thin shells (mean aspect ratio = 1.84 ± 0.01, mean lip thickness = 2.39 ± 0.25 mm) and rotund with thick shells (mean aspect ratio = 1.55 ± 0.01, mean lip thickness = 3.21 ± 0.08 mm). Snails in each class were size matched (24.95-41.43 mm) so that they differed primarily in the shape induced by each predator (Appendix 1) and so that predators were capable of handling the size range presented. All 20 snails were presented simultaneously to each predator. Predation trials took place in flow-through seawater tables (120 cm long x 45 cm wide x 20 cm deep) filled to a depth of ~15 cm and devoid of any structure. This simple environment snails restricted behavioral defenses (e.g., hiding under stones) other than fleeing. Crabs were given 24 hours to forage, seastars took considerably longer to capture and consume prey and so were given 120 hrs to feed. I first estimated the total

number of snails (of both phenotypes) that were captured and consumed by each predator over a 24 hr period. Differences between predator treatments in snail mortality over 24 hours were analyzed with a Mann-Whitney *U* test. Because survivorship of different prey types was not independent in my design, I did not directly analyze survival differences between prey types. Instead, I calculated survivorship of thin, elongate snails relative to thick, rotund snails as: (number of rotund snails eaten)/(number of elongate snails eaten + number of rotund snails eaten). Because prey phenotypes were offered to predators at equal densities, relative survivorship calculated in this manner is equivalent to Manly's electivity index (Manly et al. 1972), which assumes a value of 0.5 when there is equal preference or susceptibility for each phenotype: values greater than 0.5 indicate that elongate snails were more susceptible to predation and values less than 0.5 indicate that snails with rotund shells were more susceptible. A Mann-Whitney *U* test was used to compare electivity indices between predator treatments.

Results

Comparing morphological and behavioral responses to different predators

I found highly significant differences in the three measured morphological variables among experimental treatments (Appendix 2). However all traits did not respond similarly to all treatments. Compared to the no predator treatment, snails

produced heavier shells in all the predator treatments (crab-snail, seastar-snail, or seastar/crab-snail; ANCOVA, $F_{3,8} = 39.435$, $P < 0.0001$). Post-hoc tests showed significant pairwise differences between all predator treatments except crab-snail and seastar/crab-snail (Fig. 2A). Shell aspect ratio also differed significantly between treatments (ANCOVA, $F_{3,8} = 34.32$, $P < 0.0001$). Snails exposed to seastars fed conspecifics produced longer, narrower shells relative to snails in the no predator treatment, while snails exposed to feeding crabs produced shorter, wider shells. Pairwise post-hoc tests were significant, except between crab-snail and seastar/crab-snail (Fig. 2B). Thus, aspect ratio was similar among snails exposed to feeding crabs and snails exposed to crabs and seastars simultaneously. The volume of unoccupied shell was lager in snails exposed to feeding seastars relative to all other treatments (ANCOVA, $F_{3,8} = 8.844$, $P = 0.0025$). Unoccupied volume was highly correlated with angular retraction depth ($r^2 = 0.90$, $P < 0.001$, $N = 15$). Post-hoc tests revealed no significant difference in unoccupied shell between snails in the no predator treatment and those reared with feeding crabs or exposed to both predators simultaneously (Fig. 2C). Predator treatment had a strong effect on predator avoidance behavior (ANOVA, $F_{3,8} = 63.294$, $P < 0.0001$). Snails increased refuge use in predator treatments relative to snails in the no predator treatment, but this response was the same regardless of predator treatment (Fig. 2d).

Analysis of geometric morphometrics also revealed shape differences among treatments (MANCOVA $F_{3,8} = 80.084$, $P < 0.0001$; Appendix 3). Shell

shape varied with size ($F_{3,8}$ = 1596.396, P < 0.0001), indicating multivariate geometric allometry within treatments, however none of the treatment by covariate interactions approached significance (P > 0.20). Relative warp axis 1 (RW1), the major axis of shape variation, was associated with the predator effect and described the geometric analog of aspect ratio (Fig. 3). Feeding crabs and feeding seastars had opposite effects on RW1; relative to the no predator treatment, snails in the crab-snail treatment had wider shells with shorter spires (Tukey's HSD, P = 0.0379), while snails in the seastar-snail treatment had narrower shells with taller spires (Tukey's HSD, P = 0.0002). The combined predator treatment was not significantly different from the crab-snail treatment (Tukey's HSD, P = 0.0847). Shape variation along relative warp axis 2 (RW2), which explained less variance than RW1, described a widening of the aperture (Fig. 3). The seastar-snail treatment did not differ significantly from the no predator treatment (Tukey's HSD, P = 0.0877), while crab-induced snails and snails exposed to both predators produced similarly wider outer apertures than snails in the no predator treatment (Tukey's HSD, both P < 0.010). Thin-plate spline visualizations show that the crab-snail and seastar-snail treatment induced qualitatively different multivariate responses in snails and that the combined predator treatment induced a qualitatively similar multivariate response to that induced by the crab-snail treatment (Fig. 3).

Experimental treatment also had a strong effect on snail shell growth (ANCOVA, $F_{3,8}$ = 48.577, P < 0.0001) and body growth ($F_{3,8}$ = 89.658, P <

0.0001); Appendix 4). Snails grew fastest in the absence of predators, but decreased growth in the presence of feeding predators. Crabs fed conspecifics caused greater reduction in snail growth than feeding seastars. The presence of simultaneous predators reduced body growth to the same degree as the feeding crab treatment (Fig. 4A), but reduced shell growth more than either predator alone (Fig. 4B).

Predation risk and susceptibility of prey phenotype

In predation trials *Cancer* was a more dangerous predator for *N. lamellosa* than *Pisaster*: Crabs consumed an average of 13.5 snails in 24 hours while seastars consumed an average of only 1 snail per 24 hours (Mann-Whitney *U* test, n=4, $P < 0.05$). Manly's electivity index for elongate morphs was 0.65 ± 0.11 (± SE) for crabs and 0.05 ± 0.05 for seastars. Thus, snails with elongate, thin-walled shells were no more susceptible to crab predation than snails with rotund thick-walled shells ($t_3 = 1.606$, $P = 0.2066$), but snails with rotund, thick-walled shells were more susceptible to seastar predation than elongate snails ($t_3 = -8.933$, $P = 0.0030$). The relative susceptibility of morphs changed according to predator foraging mode ($t_8 = 4.433$, $P = 0.0044$).

Discussion

The outcome of exposure to the simultaneous presence of different predator risk

cues depended on which traits were considered. Morphological variables suggest that prey can discriminate between functionally different predators feeding on conspecific snails and produce phenotypes in accordance with the attack mode of the predator releasing risk cues. Snails exposed to feeding crabs produced thicker, squatter shells, which is a common defense against shell-breaking predators, while snails reared with feeding seastars could retract farther into more elongate, higher spired shells which made them less susceptible to this predator. Increased resistance to one predator increased susceptibility to the other, indicating a functional tradeoff. When exposed to both predators simultaneously, snails produced a phenotype similar to that expressed in the presence of crabs. This directional response suggests that snails distinguished between different predator cues in combination and optimized their phenotype to the most dangerous predator. These results contrast with those found in recent studies of freshwater organisms, where prey exposed to combinations of predators that induce opposing responses produced intermediate phenotypes (Relyea 2003, but see Teplitsky et al. 2004). Thus, prey can respond to the most risky predator in a combination even when each predator induces opposing phenotypes. In addition to morphological defenses that reflect predator-specific foraging modes, a generalized antipredator behavior, in the form of refuge use, was expressed to a similar degree in the separate presence of each predator. When both predators were combined, snails maintained the same level of refuge use, suggesting that prey might complement directional predator-specific

defenses with general defenses in multiple predator environments.

Thick, heavy shells are perhaps the most wide-spread defense in marine snails (Vermeij 1993) and the induction of these shells in the presence of shell-breaking predators are consistent with other examples in the literature (Palmer 1990, Trussell 1996). Numerous documentations of the attack phase of durophagous predation have shown that heavier shells are more resistant than thin, light ones (Bertness and Cunningham 1981, West et al. 1991). In addition to shell thickness, relatively rotund shells may spread crushing forces more evenly over a given amount of shell material, increasing crushing resistance (Anderson et al. 2004). Predator-induced shape change of this type is common in freshwater snails (DeWitt et al. 1998), but has not been previously documented in marine snails. In *N. lamellosa* and other marine snails, altering shell shape in combination with shell thickness may produce a more effective crush-resistant shell (e.g., trait complementation sensu DeWitt et al. 1999).

The observed production of elongate shells with increased retractability in the presence of seastars is consistent with reduced shell entry, making this a likely inducible defense against shell entry predators. Studies of the north Atlantic *Nucella lapillus* show that high-spired, small apertured shells are effective defenses against predators that attack prey by reaching in to the shell without damaging it (Vermeij 1987). Seastars are known to induce defensive morphological responses in other molluscs, most notably mussels, which produce larger adductor muscles in the presence of these predators (Freeman

2007), but this is the first evidence of induced morphological defense in a marine snail in response to water-borne stimuli by a predatory seastar and damaged prey.

While the production of morphological phenotypes appears to reflect the attack mode of the predator, snails could simply be responding to species-specific cues unrelated to the mode of attack employed by the predator. However, previous studies suggest that predator-specific responses reflecting the attack mode of the predator may be a common feature of inducible defenses in aquatic molluscs. For example, defensive responses in marine mussels appear to be adapted to the attack mode of the predators that induce them. In the presence of crabs, mussels develop significantly heavier, more crush-resistant shells; but develop heavier adductor muscles, which make shells more difficult to pry apart, in the presence of seastars (Reimer and Tedengren 1996, Smith and Jennings 2000). Additionally, my findings closely parallel those from a study of the freshwater snail *Physa heterostropha,* which produces an elongate shell in the presence of a shell-entering crayfish and a more rotund shell in the presence of a shell-breaking fish (DeWitt 1998).

Prey can express unique responses to different predators without distinguishing between them when different amounts of a general predation risk cue (e.g., alarm cues from damaged or consumed conspecifics) are produced and prey have an additive response to cue dosage (Van Buskirk and Arioli 2002). This experiment does not allow me to distinguish predator-specific effects from

conspecific alarm cue effects; however, several lines of evidence suggest that an additive response to alarm cue does not explain the results of my study. First, previous work has shown that induced morphological changes only occur when alarm cues are presented in conjunction with predator stimuli (Bourdeau, unpublished data). In this study, separate predators ate the same amount of prey each week and the prey were always consumed in between feedings. Because predator size and presumably total predator biomass was similar in each treatment, cue strength should have been similar across treatments, assuming the amount of cue produced by a predator scales to its size (Edgell and Neufeld 2008). Second, the phenotypes of snails reared in the crab-snail treatment were extremely similar those in the seastar/crab-snail treatment even though the latter treatment contained twice the number of predators and consumed snails as the single predator treatment. If the influence of predator density and/or the amount of food is additive, combined predators should have induced more extreme general defenses than either of the predators alone. None of the trait changes were congruent with this prediction.

Finally, while the observed changes in growth and shell thickness could reflect a graded response to a general alarm cue, my finding that shell shape changed in opposite directions in response to the different conspecific-fed predators along the principle component responsible for most of the shape change, is not consistent with this hypothesis. In other marine snails growth rates can affect shell elongation: slow growth is associated with the development

of thicker, more elongate shells and rapid growth is associated with the production of thinner, more globose shells (Kemp and Bertness 1984), If the shell shape change I observed here was governed solely by growth rate reduction in response to a general alarm cue, then the predicted effect would be that the slowest growing snails (i.e. crab induced) would develop the most elongate shells, and the fastest growing snails (i.e. those in the no predator treatment) would develop the most globose shells. That is, one would expect a graded pattern of shell elongation that tracks the pattern of growth rates. Instead, snails exposed to seastars produced the most elongate shells, despite growing intermediately between snails in the no predator treatment and the crab-snail treatment. This developmental shift is not consistent with a graded response to a general alarm cue and implies that snails perceive each predator-snail combination differently. Taken together, these results suggest that snails can distinguish between qualitatively different cues produced by crabs and seastars fed conspecific snails.

The prediction that prey should respond to the more risky predator in a combination when different predators select for similar phenotypes of different magnitudes is well supported by studies examining the behavioral responses of prey to combined predators (reviewed by Relyea 2003). However, to date there are few documented examples of prey responding to the most risky predator when predators favor opposite phenotypes (but see Teplitsky et al. 2004). When different predators favor opposite phenotypes, responding to only one of the two

predators inevitably puts the prey at a higher risk of being eaten by the other predator (i.e., risk enhancement, sensu Sih et al. 1998). Under this scenario, the optimal strategy would be to produce an intermediate phenotype, a compromise that presumably balances the overall risk of predation. However this strategy is only effective if each predator imposes similar or equal risk and if the intermediate phenotype offers an intermediate amount of protection. If the balance of risk is sufficiently subequal between predators, or an intermediate phenotype is developmentally constrained, then prey should optimally adjust their phenotype to the most dangerous predator, even if each predator induces opposing phenotypes. Here and in other studies (Teplitsky et al. 2004), the more dangerous predator is the one that imposes the highest mortality at the population level. In the lab predation trials, *Cancer* was the more dangerous predator, capturing and consuming many more snails in experimental populations than *Pisaster* over a 24-hour period. Such predation rates on field-caught snails in the laboratory may not necessarily reflect the risk imposed by different predators in the field. Indeed a number of factors that were not included in this study can affect the relative risk of predators, including habitat structure, predator density and the availability of other prey. Additionally, because prey can change their phenotype in the presence of predators, the risk imposed by a predator will depend on the prey's previous experience with the predator, its phenotype and how these influence vulnerability to that predator. While the present study does not include data on the relative risk of crabs and seastars under natural

conditions, the simple lab trials performed here corroborate previous observations that *Cancer* imposes higher mortality than *Pisaster* on natural *N. lamellosa* populations (Mauzey 1966, Spight 1974), suggesting crabs are the more dangerous predator for *N. lamellosa*.

Directional morphological responses to combined predators may reflect limits on morphological plasticity (DeWitt et al. 1998). In gastropods, the geometric constraints of shells limit them to being either rotund or elongate, but not both. Thus an intermediate phenotype that is neither relatively rotund nor relatively elongate may be particularly ineffective at defending against either predator. Secondly, unlike behavior which can often track rapid temporal change in the environment, morphology cannot be adjusted to short-term changes in predation risk (West-Eberhard 2003b). Thus, it may be more advantageous for prey to prioritize their response to the most dangerous predator in a pair rather than to continuously attempt to adjust their phenotype to changing predator regimes, particularly if lag times are long relative to the time scale of variability in predator presence (Padilla and Adolph 1996). As a result, asymmetries in predation risk probability and the relative fitness of each phenotype are likely driving the evolution of directional phenotypes in this system (Gabriel et al. 2005).

Field caught snails with high-spired, elongate shells survived predation by *Pisaster* in the lab better than snails with squat, heavy shells. Although I did not quantify the failure rate of seastars on elongate snails, their enhanced resistance is likely due to the coupling of the increased retractability associated with the

elongate, high-spired shell, which makes it more difficult for seastars to locate prey (Watanabe 1983), with the reduced transportation cost of a lighter shell, which may facilitate escape after contact with a predator (Daniel 1984). Consistent with this idea is the observation that snails in the predation trials initially withdrew into their shell in the presence of seastars and then fled only after contact with the predator (personal observation). Crabs were more effective than seastars at consuming snails but did not generate overall performance trade-offs. This suggests that phenotypic responses for seastars might evolve without trading-off the effectiveness against crab predation. However opposing phenotypes tended to increase resistance to the predator that induces them, suggesting that the form of selection and presence of tradeoffs may be frequency dependent in natural populations (Aigner 2004). These results also suggest that snails could experience enhanced mortality risk when they face multiple predators in nature. Risk enhancement (Sih et al. 1998) should occur when prey defenses induced by one predator conflict with defenses induced by the other predator. However, a reduction in risk is often observed because prey employ compensatory defenses that reduce the overall risk of predation despite the opposing defenses induced by different predators (Sih et al. 1998). Here, both feeding crabs and feeding seastars induced increased refuge use. Predator avoidance behavior is an effective defense because it affects predation at the pre-encounter phase by reducing the attack rate of the predator (Ramos-Jiliberto et al. 2007). This strategy should therefore be effective against predators

independent of foraging mode. Thus, snails that prioritize responses to crabs may not necessarily incur greater predation by seastars in the field. Future studies should determine how the predators select for alternative prey phenotypes and the survival abilities of different phenotypes for each predator in the field.

The results of this study have important implications for understanding the evolution of predator-induced plasticity in multiple predator environments. Because prey responses to combined predators depend on which traits are measured, examining the full suite of antipredator traits a prey can employ will provide a better predictive framework for understanding defense strategies against multiple predators (Relyea 2004, Miner et al. 2005). Here prey responded to the more risky of two predators, even when predator-specific risk cues induced opposite phenotypes and intermediate responses might have been predicted. I propose that the expression of generalized behavioral defenses that affect the pre-encounter phase of predation could dampen risk enhancement imposed by directional specialized responses to more dangerous predators in combined predator environments. The next step is to determine the relative contribution of generalized and specialized defensive traits to resistance against multiple predators.

Figures

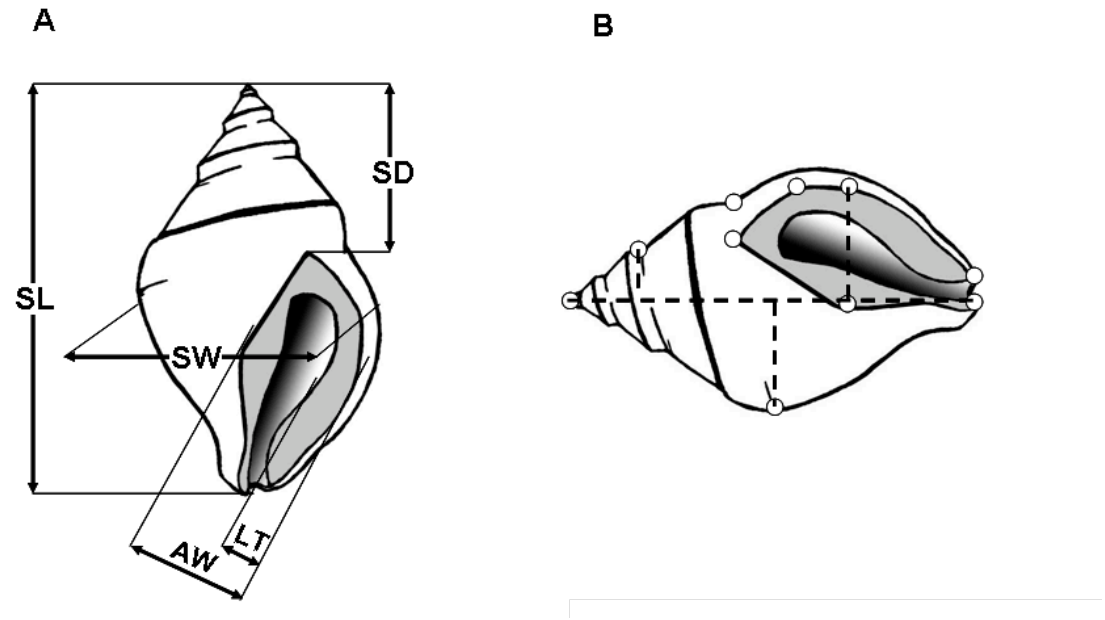


Fig. 1. Diagram showing (A) the linear measurements used in this study: shell length (SL) and shell width (SW); and (B) the position of 10 landmarks used in the geometric morphometric analysis.

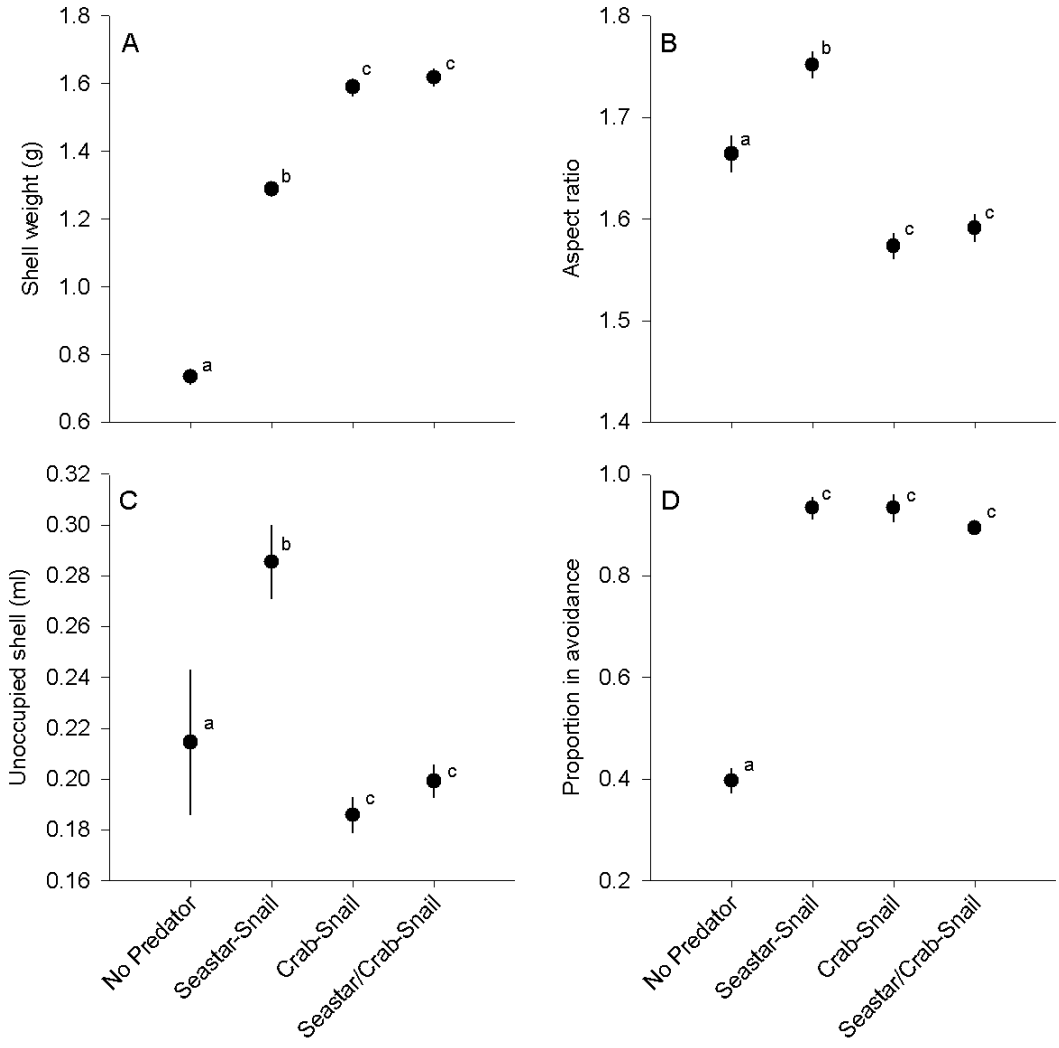


Fig. 2. Morphological responses and avoidance behavior of *N. lamellosa* to no predator (No Predator), feeding seastar (Seastar-Snail), feeding crab (Crab-Snails), and both predator (Seastar/Crab-Snail) treatments. Morphological values (A, B and C) are back-transformed least squared means and standard errors of $\log_{10}$-transformed data computed for covariates at their means (means ± SE); some error bars are obscured by the symbols. Behavioral values (D) are mean proportion of individuals showing an avoidance response. Groups that share lower case letters are not significantly different (Tukey's HSD, $P > 0.05$).

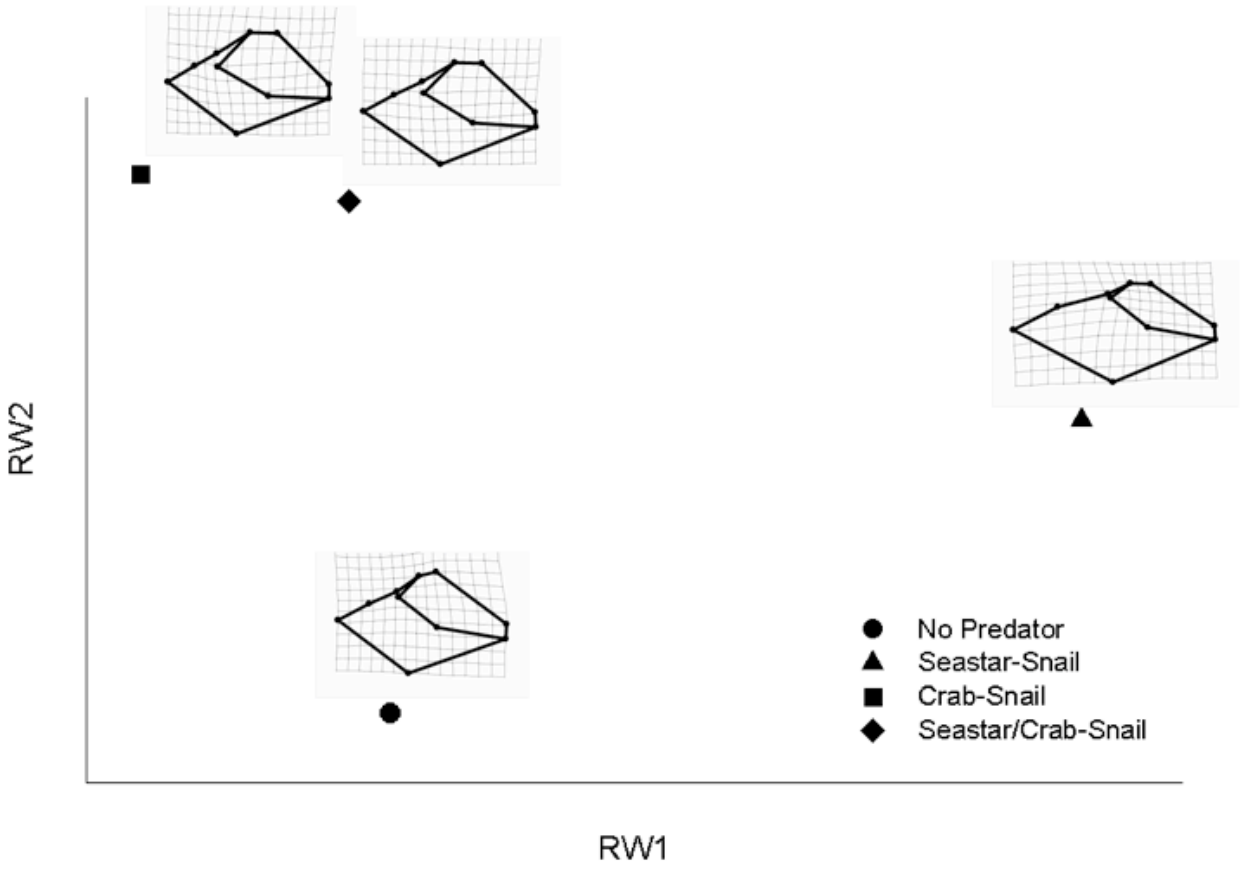


Fig. 3. Relative warp plot for *N. lamellosa*. The first two relative warps (RWs) are shown (with axis aspect preserved) accounting for 58.73% of the total non-uniform shape variation. Thin-plate spline deformation grids, 3X the observed range, are shown for each value, which are least-squares treatment means projected onto the first two RWs.

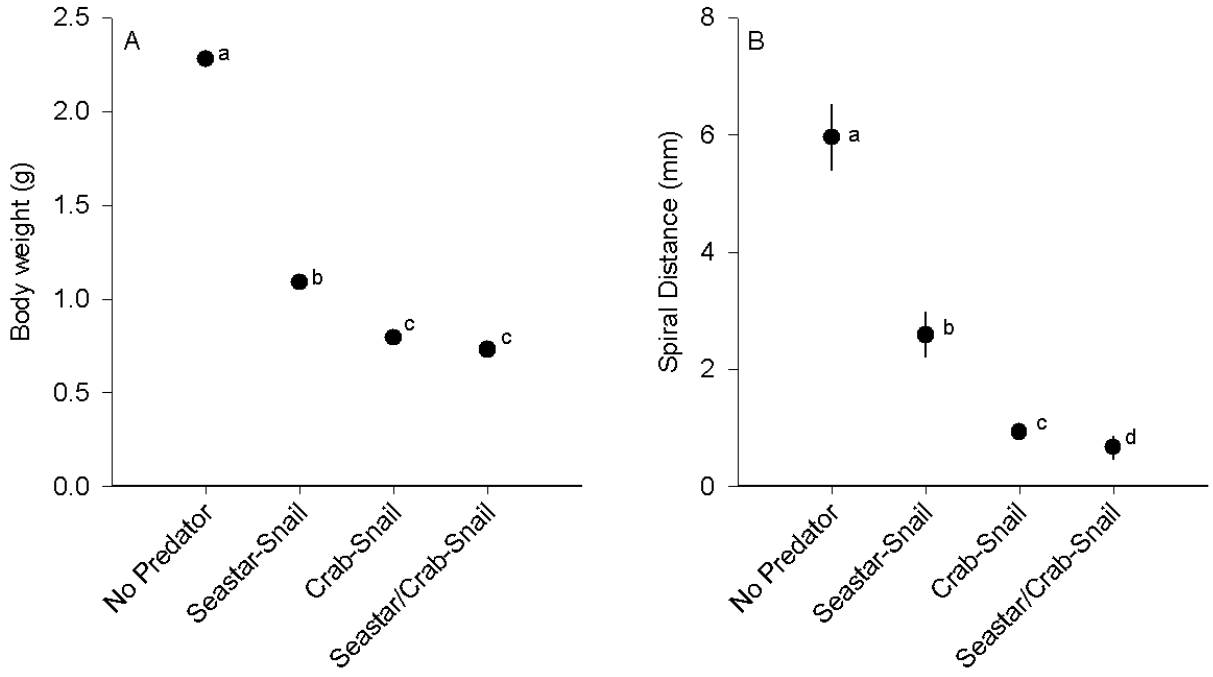


Fig. 4. Variation in (A) body weight and (B) spiral shell growth of *N. lamellosa* in response to different predator treatments. Values are back-transformed least squared means and standard errors of log10-transformed data computed for covariates at their means; some error bars are obscured by the symbols. Groups that share lower case letters are not significantly different (Tukey's HSD, $P > 0.05$).

References

Aigner, P. A. 2004. Floral specialization without trade-offs: Optimal corolla flare in contrasting pollination environments. Ecology **85**:2560-2569.

Anderson, K. E., J. B. Tharrington, P. A. Curtis, and F. T. Jones. 2004. Shell characteristics of eggs from historic strains of single comb white leghorm chickens and the relationship of egg shape to shell strength. International Journal of Poultry Science **3**:17-19.

Appleton, R. D. and A. R. Palmer. 1988. Water-Borne Stimuli Released by Predatory Crabs and Damaged Prey Induce More Predator-Resistant Shells in a Marine Gastropod. Proceedings of the National Academy of Sciences of the United States of America **85**:4387-4391.

Bertness, M. D. and C. Cunningham. 1981. Crab shell-crushing predation and gastropod architectural defense. Journal of Experimental Marine Biology and Ecology **50**:213-230.

Bookstein, F. L. 1989. Principal Warps - Thin-Plate Splines and the Decomposition of Deformations. Ieee Transactions on Pattern Analysis and Machine Intelligence **11**:567-585.

Daniel, T. L. 1984. Unsteady aspects of aquatic locomotion. American Zoologist **24**:121-134.

DeWitt, T. J. 1998. Costs and limits of phenotypic plasticity: Tests with predator-induced morphology and life history in a freshwater snail. Journal of Evolutionary Biology **11**:465-480.

DeWitt, T. J. and R. B. Langerhans. 2003. Multiple prey traits, multiple predators: keys to understanding complex community dynamics. Journal of Sea Research **49**:143-155.

DeWitt, T. J., B. W. Robinson, and D. S. Wilson. 2000. Functional diversity among predators of a freshwater snail imposes an adaptive trade-off for shell morphology. Evolutionary Ecology Research **2**:129-148.

DeWitt, T. J. and S. M. Scheiner, editors. 2003. Phenotypic Plasticity: Functional and Conceptual Approaches. Oxford University Press, New York, NY.

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

Edgell, T. C. and C. J. Neufeld. 2008. Experimental evidence for latent developmental plasticity: intertidal whelks respond to a native but not an introduced predator. Biology Letters **4**:385-387.

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

Freeman, A. S. 2007. Specificity of induced defenses in *Mytilus edulis* and asymmetrical predator deterrence. Marine Ecology-Progress Series **334**:145-153.

Gabriel, W., B. Luttbeg, A. Sih, and R. Tollrian. 2005. Environmental tolerance, heterogeneity, and the evolution of reversible plastic responses. American Naturalist **166**:339-353.

Hendrix, L. J., M. W. Carter, and D. T. Scott. 1982. Covariance analyses with heterogeneity of slopes in fixed models. Biometrics **38**:641-650.

Hoverman, J. T. and R. A. Relyea. 2007. The rules of engagement: how to defend against combinations of predators. Oecologia **154**:551-560.

Jacobsen, H. P. and O. B. Stabell. 1999. Predator-induced alarm responses in the common periwinkle, *Littorina littorea*: dependence on season, light conditions, and chemical labelling of predators. Marine Biology **134**:551-557.

Lima, S. L. 1992. Life in a multi predator environment: some considerations for antipredator vigilance. Annales Zoologici Fennici **29**:217-226.

Lima, S. L. and L. M. Dill. 1990. Behavioral decisions made under the risk of predation - a review and prospectus. Canadian Journal of Zoology-Revue Canadienne De Zoologie **68**:619-640.

Manly, B. F. J., P. Miller, and L. M. Cook. 1972. Analysis of a Selective Predation Experiment. American Naturalist **106**:719-&.

Marko, P. B. and A. R. Palmer. 1991. Responses of a Rocky Shore Gastropod to the Effluents of Predatory and Nonpredatory Crabs - Avoidance and Attraction. Biological Bulletin **181**:363-370.

Matsuda, H., M. Hori, and P. A. Abrams. 1996. Effects of predator-specific defense on community complexity in two-trophic-level communities. Evolutionary Ecology **10**:13-28.

Mauzey, K. P. 1966. Feeding Behavior and Reproductive Cycles in Pisaster Ochraceus. Biological Bulletin **131**:127-&.

Menge, B. A. and J. Lubchenco. 1981. Community Organization in Temperate and Tropical Rocky Inter-Tidal Habitats - Prey Refuges in Relation to Consumer Pressure-Gradients. Ecological Monographs **51**:429-450.

Miner, B. G., S. E. Sultan, S. G. Morgan, D. K. Padilla, and R. A. Relyea. 2005. Ecological consequences of phenotypic plasticity. Trends in Ecology & Evolution **20**:685-692.

Navarrete, S. A., B. A. Menge, and B. A. Daley. 2000. Species interactions in intertidal food webs: Prey or predation regulation of intermediate predators? Ecology **81**:2264-2277.

Padilla, D. K. and S. C. Adolph. 1996. Plastic inducible morphologies are not always adaptive: The importance of time delays in a stochastic environment. Evolutionary Ecology **10**:105-117.

Paine, R. T. 1974. Intertidal Community Structure - Experimental Studies on Relationship between a Dominant Competitor and Its Principal Predator. Oecologia **15**:93-120.

Palmer, A. R. 1982. Growth in Marine Gastropods - a Non-Destructive Technique for Independently Measuring Shell and Body-Weight. Malacologia **23**:63-73.

Palmer, A. R. 1990. Effect of crab effluent and scent of damaged conspecifics on feeding, growth, and shell morphology of the Atlantic dogwhelk *Nucella lapillus* (L). Hydrobiologia **193**:155-182.

Pigliucci, M. 2003. Phenotypic integration: studying the ecology and evolution of complex phenotypes. Ecology Letters **6**:265-272.

Pigliucci, M. 2005. Evolution of phenotypic plasticity: where are we going now? Trends in Ecology & Evolution **20**:481-486.

Ramos-Jiliberto, R., E. Frodden, and A. Aranguiz-Acuna. 2007. Pre-encounter versus post-encounter inducible defenses in predator-prey model systems. Ecological Modelling **200**:99-108.

Reimer, O. and M. Tedengren. 1996. Phenotypical improvement of morphological defences in the mussel *Mytilus edulis* induced by exposure to the predator Asterias rubens. Oikos **75**:383-390.

Relyea, R. A. 2003. How prey respond to combined predators: A review and an empirical test. Ecology **84**:1827-1839.

Relyea, R. A. 2004. Integrating phenotypic plasticity when death is on the line: Insights from predator-prey systems. Pages 176-193 *in* M. Pigliucci and K. Preston, editors. Phenotypic Integration: Studying the Ecology and Evolution of Complex Phenotypes. Oxford University Press, New York, NY.

Rohlf, F. J. 2006. tpsDig. Version 2.10.

Rohlf, F. J. and L. F. Marcus. 1993. A Revolution in Morphometrics. Trends in Ecology & Evolution **8**:129-132.

Sih, A., G. Englund, and D. Wooster. 1998. Emergent impacts of multiple predators on prey. Trends in Ecology & Evolution **13**:350-355.

Smith, L. D. and J. A. Jennings. 2000. Induced defensive responses by the bivalve *Mytilus edulis* to predators with different attack modes. Marine Biology **136**:461-469.

Spight, T. M. 1974. Sizes of Populations of a Marine Snail. Ecology **55**:712-729.

Teplitsky, C., S. Plenet, and P. Joly. 2004. Hierarchical responses of tadpoles to multiple predators. Ecology **85**:2888-2894.

Tollrian, R. and C. D. Harvell, editors. 1998. The Ecology and Evolution of Inducible Defenses. Princeton University Press, Princeton, NJ.

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

Van Buskirk, J. and M. Arioli. 2002. Dosage response of an induced defense: How sensitive are tadpoles to predation risk? Ecology **83**:1580-1585.

Vermeij, G. J. 1987. Evolution and Escalation: An Ecological History of Life. Princeton Univesrity Press, Princeton, NJ.

Vermeij, G. J. 1993. A Natural History of Shells. Princeton University Press, Princeton, NJ.

Watanabe, J. M. 1983. Anti-predator defenses of 3 kelp forest gastropods - contrasting adaptations of closely-related prey species. Journal of Experimental Marine Biology and Ecology **71**:257-270.

West, K., A. Cohen, and M. Baron. 1991. Morphology and behavior of crabs and gastropods from Lake Tanganyika, Africa - implicatons for lacustrine predator-prey coevolution. Evolution **45**:589-607.

West-Eberhard, M. J. 2003. Developmental Plasticity and Evolution. Oxford University Press, New York, NY.

Chapter 5 - An inducible morphological defence is a passive by-product of behaviour in a marine snail.

Abstract

Many organisms have evolved inducible defences in response to spatial and temporal variability in predation risk. These defences are assumed to incur large costs to prey; however, few studies have investigated the mechanisms and associated costs underlying these adaptive responses. I examined the proximate cause of predator-induced shell thickening in marine snails and tested whether induced thickening leads to an increase in structural strength. Results indicate that although predators (crabs) induce thicker shells, the response is a passive by-product of reduced feeding and soft tissue growth rather than an active physiological response to predation risk, as previously reported. Physical tests indicate that although the shells of predator-induced snails are significantly stronger, the increase in performance is no different than that of snails with limited access to food. Increased shell strength is attributable to an increase in the energetically inexpensive microstructural layer rather than to material properties changes in the shell. This mechanism suggests that predator-induced shell defences may be neither energetically nor developmentally costly. Positive correlations between antipredator behaviour and morphological defences may explain the commonly observed association between growth reduction and inducible defences in other systems and could have implications for the

evolutionary potential of these plastic traits.

Introduction

Phenotypic plasticity has become a major theme in studies of ecology and evolution (Miner et al. 2005, Pigliucci 2005). The underlying mechanics of plastic responses have, however, received less attention, despite the fact that a mechanistic explanation can enhance our understanding of the adaptive value and evolution of these traits (Windig et al. 2003). Specifically, determining whether plasticity can evolve more easily in certain traits requires identifying the costs and constraints on the expression of these traits. This is particularly true for correlated traits, where adaptively plastic phenotypes could be passive by-products of correlated plastic responses, rather than direct responses to specific environmental cues. Such genetic and phenotypic correlations are common, but their potential role in the evolution of plastic traits has rarely been investigated.

Some of the most well-studied and ecologically important forms of phenotypic plasticity are predator-induced defences (Tollrian and Harvell 1998), where prey species show adaptive plastic morphological shifts that increase resistance to predation in response to temporal or spatial heterogeneity in predation risk. These morphological changes are often accompanied by a reduction in feeding and growth (Harvell 1992, Trussell 2000b, Van Buskirk and

Schmidt 2000), inferring an energetic or developmental tradeoff between allocation of resources to defencive structures and growth (Palmer 1981, Trussell and Nicklin 2002). However, the conclusion of costs or tradeoffs requires careful scrutiny with regard to the exact causal chain between the predator cue and the phenotypic responses in the prey (Grunbaum 1997, Brookes and Rochette 2007). For example, previously unexplained growth costs associated with predator-induced defences in larval anurans have been found to be due in part to environmentally-induced changes in their feeding morphology (Relyea and Auld 2005). Such plastic changes in foraging traits, particularly feeding behaviour, may be important mechanistic links between the inducing environmental cue and the formation of the plastic phenotype.

Marine snails are excellent model prey organisms for examining the mechanisms and costs of inducible defences. A long evolutionary history with shell-breaking predators has increased the resistance of gastropod shells to crushing (Vermeij 1987) both as constitutive and inducible defences (Appleton and Palmer 1988, Trussell and Smith 2000). Numerous examples of shell thickening in marine snails in response to water-borne chemicals indicative of crab predation document a correlated reduction in feeding and somatic tissue growth (Appleton and Palmer 1988, Palmer 1990, Trussell 1996, Dalziel and Boulding 2005). In some cases, crab-induced shell thickening has been shown to be an actively modified morphological response to risk stimuli at the presumed expense of reduced soft tissue growth (Palmer 1990, Trussell and Nicklin 2002,

Brookes and Rochette 2007). However, whether this mechanism is widespread and whether reduced growth reflects a decrease in feeding activity or a direct tradeoff due to the cost of producing a more predator-resistant shell remain open questions.

To test for tradeoffs and underlying mechanisms that drive predator induced shell changes, I examined somatic growth, shell growth and shell morphology in the marine snail *Nucella lamellosa* by: 1) experimental manipulation of food availability, and 2) exposure to predation risk from the predatory crab *Cancer productus*. This design allowed me to distinguish between two alternative hypotheses: 1) shell thickening is a direct response to predator risk cues resulting from increased shell deposition, or 2) shell deposition rate remains constant and shell thickening is a by-product of reduced feeding activity and growth rate. If shell thickening is a direct response to risk cues, snails exposed to the scent of crabs should produce thicker shells than food-limited snails. Alternatively, if shell-thickening is a by-product of reduced feeding and growth, a reduction in somatic growth will restrict linear shell growth resulting in increased shell deposition perpendicular to the axis of linear shell growth, causing the shell to thicken with no additional energetic investment in the production of the defence. If this second mechanism is operating, then both predators and limited access to food should induce a similar shell-thickening response. I also measured shell strength to assess whether shell thickening induced by predators or food availability produced differences in resistance to crab predation.

Methods

Experimental set-up

On San Juan Island, WA, USA, juvenile snails (14-24 mm shell length) were collected from two current-swept sites where crabs are rare (Bourdeau, unpublished data) and test snails would have limited previous exposure to crabs: Westside Preserve (48°30'26.76"N, 123° 8'35.20"W) and San Juan County Park (48°32'29.92"N, 123° 9'35.99"W). Prior to experimentation, snails were individually numbered (bee tags attached with cyanoacrylate glue), fed barnacles in flow-through seawater tables for 48 hrs, and then measured and weighed. Ten snails were randomly allocated to plastic aquaria (30.5W X 19.1D X 20.3H cm) that served as experimental units. Aquaria were randomly assigned to one of three treatments corresponding to feeding frequency, in a non-orthogonal experimental design: 1) snails fed four out of six days (67%), 2) two of six days (33%) and 3) one of six days (16.7%). In a fourth treatment, snails were fed on a 67% feeding schedule while exposed to the non-lethal presence of the predatory crab, *Cancer productus*. Hereafter the treatments will be referred to as 'high food', 'moderate' food', 'low-food' and 'crab', respectively. Each treatment was replicated four times. Snails did not differ in initial values of morphometric variables among treatments (ANOVA, all *P*'s > 0.15). Snails were fed barnacles, *Balanus glandula*, attached to small stones. Stones were replaced as the

barnacle supply was depleted. Aquaria were connected to a header tank receiving coarsely filtered seawater. This gravity-fed flow through system ensured similar flows among treatments and a constant supply of chemical cues to experimental snails. Water flowed in through an opening in the aquaria top and into a plastic tub (1892 ml) fastened to the underside of the aquarium lid. The plastic tub housed the crab in the predator treatment. Overflow from the tubs supplied the aquaria with seawater. Seawater flowed out of circular openings 5 cm from the top of the aquaria. Crabs were fed pre-cracked *N. lamellosa* to ensure consumption. I used similar sized crabs in the replicates of the non-lethal predator treatment (mean CW =128.59). Crabs were replaced periodically. The experiment ran for 84 d.

Feeding

I quantified the number of barnacles eaten at the end of each feeding period to assess the effect of treatment on feeding rate. At the end of each feeding period I counted the number of barnacles missing opercular plates and measured the opercular diameter of each 'empty' test and removed empty tests to ensure they were counted only once.

Growth and morphology

At the end of the experiment I quantified the shell length, shell mass, shell cross sectional area (lip thickness*shell width) and body mass of each

experimental snail. Shell mass, which serves as a good estimate of overall shell thickness (Palmer 1982), was obtained with a non-destructive technique in which snails were weighed submerged in seawater and shell mass was estimated using the equation: 1.572*(submerged weight)+0.0162 (Palmer 1982). Snail soft tissue mass was calculated as the difference between the shell mass and the total wet mass in air. Shell length and shape covariates, shell width and lip thickness, were measured to the nearest 0.01 mm with digital calipers.

Performance

I estimated shell strength by measuring the force required to fracture shells after I took morphometric measurements. I quantified shell strength as the compressive force required to fracture shells in an Instron Universal Testing Machine (Instron Corporation, MA). Shells were placed aperture down so that force was applied perpendicular to the axis of coiling. Applying force to this plane provides a representative measure of resistance to forces exerted during a crushing attempt (Zipser and Vermeij 1978), and allowed all shells to be tested similarly.

Since differences among treatments in shell strength corrected for shell cross-sectional area could be indicative of either the material properties or microstructural differences of the shell, I also tested for differences in shell microstructure among high food, low food and crab-exposure treatments. The shell fragments of 20 snails from each treatment were examined after being

crushed, under a light microscope. Total shell thickness and microstructure layer thickness were measured to the nearest 0.01 um from light microscope images (Image Pro Plus). This technique provides similar estimates of shell layer thickness as scanning electron microscopy (Avery and Etter 2006). To ensure microstructural layers were quantified from new shell growth that occurred during the experiment, measurements were taken from the main body whorl just dorsal to the leading edge of the aperture. Total shell thickness and crossed lamellar layer (inner shell layer) thickness were quantified by taking measurements perpendicular to the inner edge of the shell. The thickness of the homogenous layer (outer shell layer) was calculated as the difference between the total thickness and the crossed lamellar layer thickness (Avery and Etter 2006).

Statistical analyses

I analyzed variation in shell mass using nested ANCOVA with treatment as a fixed factor, replicate aquaria as a random factor nested within treatment, and final soft tissue mass as the covariate. Soft tissue growth and linear shell growth were determined by analyzing the final measurement of each variable with the initial measurement a covariate. Analysis of body mass with shell mass as a covariate was used to determine whether there were tradeoffs between soft tissue growth and shell thickening.

I analyzed the effect of shell morphology on shell strength with multiple ANCOVAs using force to fracture as the dependent variable and shell mass and shell cross sectional area (lip thickness*shell width) as covariates. Increases in both shell mass and cross-sectional area represent increased production of shell material and should produce similar increases in shell strength (i.e. slopes should be similar) if shell material properties are similar among treatments. However, if snails can increase shell strength by altering shell material properties, shell strength should increase at different rates among treatments with respect to the amount of shell produced (i.e., the slopes should differ). Differences in microstructure layers among treatments were analyzed with a nested ANCOVA using final shell length as a covariate.

Adherence to the assumptions of normality and homoscedasticity were tested using Shapiro-Wilk's W and Cochran's Test respectively. All data were $\log_{10}$ +1 transformed to better meet these assumptions, with the exception of shell cross-sectional area, which was square root transformed. Because I was interested in how crab-exposed snails differed from those with high food availability and those with low food availability, I used a reverse Dunnett's test to compare each food availability treatment to the crab treatment. Statistica v6.1 was used for all analyses.

Results

Feeding

Access to food strongly influenced feeding rates (ANOVA, $F_{3,12} = 34.759$, $P < 0.001$). Snails in the high food treatment ate more barnacles than those in the moderate and low food treatments. Exposure to crab scent had a strong negative effect on the number of barnacles consumed. Snails exposed to crab effluent ate 51.9% fewer barnacles than those in the high food treatment (Dunnett's, $P < 0.001$), but consumed the same number of barnacles as snails in the low food treatment (Dunnett's, $P = 0.09$) (Fig.1).

Growth and morphology

Barnacle consumption determined snail growth. Snails produced longer shells ($R^2 = 0.82$, $P < 0.001$) and gained more body mass ($R^2 = 0.80$, $P < 0.001$) as they consumed more barnacles (Fig. 2a & b). Among treatments, both linear shell growth and soft tissue gain were significantly different (ANCOVA: linear growth, $F_{3,12} = 32.61$, $P < 0.001$; soft tissue gain, $F_{3,12} = 14.07$, $P < 0.001$). Snails exposed to crab effluent exhibited 88% less linear shell growth and 84% less body mass than 'high food' snails (Dunnett's: linear growth, $P < 0.001$; body growth, $P < 0.001$), but did not differ from 'low food' snails (Dunnett's: linear growth, $P = 0.723$; body growth, $P = 0.990$; Fig. 3a & b).

I found significant treatment x covariate interactions for both shell mass and shell accretion (ANCOVA, both P's < 0.05), indicating that the linear relationships between these response variables and their respective covariates (shell mass

and linear shell growth) differed among treatments. Because the slopes were different I used the Wilcoxon modification of the Johnson-Neyman procedure to determine the range of covariates over which treatments differed in the response variable of interest (Huitema 1980). For snails with a body mass greater than 0.3452 g, as was the case for the majority of the snails at the end of the experiment, crab-exposed snails had heavier shells than snails in the high food treatment. However, there was no difference in shell mass between crab-exposed snails and those in the low food treatment over the entire range of body sizes (Fig 4a). Interestingly, crab-exposed snails were predicted to deposit less shell material than 'high food' snails when linear shell growth exceeded 3mm. However, there was no difference between crab-exposed snails and 'low food' snails across the observed range of linear shell growth (Fig 4b).

Performance

Treatment had a significant effect on shell strength (ANCOVA, $F_{3,12}$ = 3.96, P = 0.030). For a given body size, crab-exposed snails produced shells that were 34% stronger than snails in the high food treatment (Dunnett's, P = 0.033), however, these shells were not any stronger than shells produced by snails in the low food treatment (Dunnett's, P = 0.626). Increases in both shell mass and shell cross-sectional area produced similar increases in shell strength among treatments, indicating that shell strength was increasing at similar rates among treatments, relative to the amount of shell material produced

(covariate*treatment interactions P's > 0.10). For a given shell mass, crab-exposed snails produced shells that were 36% stronger than shells produced by snails in the high food treatment (Dunnett's, $P = 0.002$), but did not produce shells any stronger than snails under low food conditions (Dunnett's, $P = 0.592$; Fig. 5a). Similarly, when corrected for shell cross sectional area, crab-exposed snails produced 15% stronger shells than 'high food' snails (Dunnett's, $P = 0.029$), but produced shells no stronger than 'low food' snails (Dunnett's, $P = 0.478$; Fig. 5b).

Treatment significantly affected overall shell thickness (ANCOVA, $F_{2,9} = 19.15$, $P < 0.001$). Shells from 'high food' snails had considerably thinner shells than those from crab-exposed snails (Dunnett's, $P < 0.001$), but 'low food' snails produced shells that were just as thick as crab-exposed snails (Dunnett's, $P = 0.631$). Thickness of the crossed lamellar layer did not differ among treatments ($F_{2,9} = 1.64$, $P = 0.244$), so the difference in shell thickness among treatments was due to an increase in the homogenous shell layer (ANCOVA, $F_{2,9} = 19.43$, $P < 0.001$). When controlled for differences in shell length, the homogenous layer of snails in the high food treatment was considerably thinner than the homogenous layer of shells from crab-exposed snails (Dunnett's, $P < 0.001$); however, the homogenous shell layer in snails in low food treatments was no different than that in the shells of crab-exposed snails (Dunnett's, $P = 0.462$).

Discussion

Predator-induced shell thickening in *N. lamellosa* is a passive consequence of reduced feeding rather than an active increase in calcification in response to predator risk. Snails exposed to predator risk cues ate fewer barnacles, grew less and produced thicker, more crab-resistant shells than frequently-fed snails, but showed no difference in any of these traits when compared to food-limited snails. In addition, the shells of crab-exposed snails were no stronger than the shells of food-limited snails. Because shell strength differed among treatments even when the amount of shell material was statistically controlled, another factor is also contributing to shell strength. It is unlikely that material properties of the shell are responsible because there were no differences among treatments in the properties of the shell material as they relate to shell strength. If such differences existed, one would expect significant differences in the slopes of force to fracture by size or cross-sectional area among treatments, which were not detected here. Instead, an increase in the thickness of the homogenous shell layer appears to be driving the observed pattern of shell strength. Because new shell in the crab-exposed and food-limited snails was added perpendicular to the axis of coiling rather than at the leading edge of the main body whorl, the resulting shells of these snails were thicker and thus better defended against crushing forces. Although the homogenous layer is mechanically weaker than the crossed-lamellar layer (Currey and Taylor 1974), its low organic content and passive

production make it an inexpensive material for shell-thickening (Palmer 1992, Avery and Etter 2006).

To my knowledge this is the first example of a predator-induced morphological defence that is a passive by-product of a behavioural response to predators. This result counters previous work on two other marine snails (Palmer 1990, Brookes and Rochette 2007), where there is evidence of active increase in the rate of calcification in response to predator cues. Thus, further experiments are needed to assess which mechanism is more common among other species of gastropods. My results, coupled with a recent study of freshwater snails (Rundle et al. 2004), suggest that adaptive inducible defences arising through environmental influences other than predation pressure via phenotypic modulation (West-Eberhard 2003a) may be a more common occurrence than previously appreciated.

The results of this study have profound implications for the ecology and evolution of inducible defences. The common finding that feeding and growth reductions accompany predator-induced morphological responses suggests that behaviour may play an important role in the development of morphological defences. Reductions in feeding alone, rather than energetic or developmental tradeoffs, may be responsible for many observed inducible morphologies, and many predator-induced morphological traits thought to be direct responses to particular environmental cues may instead be by-products of developmentally correlated behavioural responses (West-Eberhard 2003a). As a result, behaviour

may play an important role in shaping plastic phenotypes, as seen in the marked morphological changes in vertebrates due to correlated changes in behaviour (Wimberger 1991).

Another intriguing possibility is that this response is resource-mediated in the wild. Food availability can be limited in quiet-water habitats where crabs are abundant (Menge et al. 1994), so snails could be co-opting a pre-existing response (i.e. a thicker shell with slower growth in food limited habitats) to respond adaptively to the risk of predation (Christy 1995, Emerson and Boyd 1999). Regardless of the cue inducing this response, a reduction or change in feeding activity could produce morphological changes that further enhance resistance to predation, providing a positive feedback that would enhance the adaptive value of induced defences. This 'plasticity cascade' could provide a mechanism for the recent documentation of positive correlations among behavioural and morphological defences in other species (DeWitt et al. 1999).

My results also call into question the common conclusion that producing thicker shells in response to predation risk is and energetically (Palmer 1983, 1992) and developmentally expensive (Palmer 1981). First, the increased thickness in crab-exposed and food-limited snails was due to an increase in the less energetically expensive microstructure layer. Secondly, the reduction in growth resulting from both crab exposure and reduced food availability resulted in similar shell production relative to control snails, indicating that there are no additional metabolic or production costs to making a thicker shell in the presence

of predators over and above the cost of producing shell in a predator-free-environment. Therefore, decreased body size in the presence of predators likely reflects a decrease in feeding rather than a direct tradeoff with producing a thicker, more predator-resistant shell (Palmer 1990, Trussell and Nicklin 2002). These results suggest that rates of skeletal growth may not limit the rate of body growth, but rather that the rate of skeletal growth is passive and its direction (i.e. linear growth versus thickening) is driven by body growth (Vermeij 2002). Thus, in addition to being energetically inexpensive, thicker shells may not represent an important developmental cost as previously suggested (Palmer 1981). While reduced growth likely carries a fecundity cost, the positive fitness effect on survival may outweigh the cost of reduced growth. Further experimentation is needed to assess the relative importance of these factors and their net effect on fitness.

Here an induced morphological defence is not actively amplified in response to predator cues, but instead is a passive by-product of growth and thus environmentally-correlated with the feeding behaviour of the prey. Because phenotypic correlations that are genetically-based can strongly affect the evolutionary trajectory of the correlated traits (Lande and Arnold 1983), it seems likely that environmentally-mediated phenotypic correlations will also influence the evolution of plastic traits.

Figures

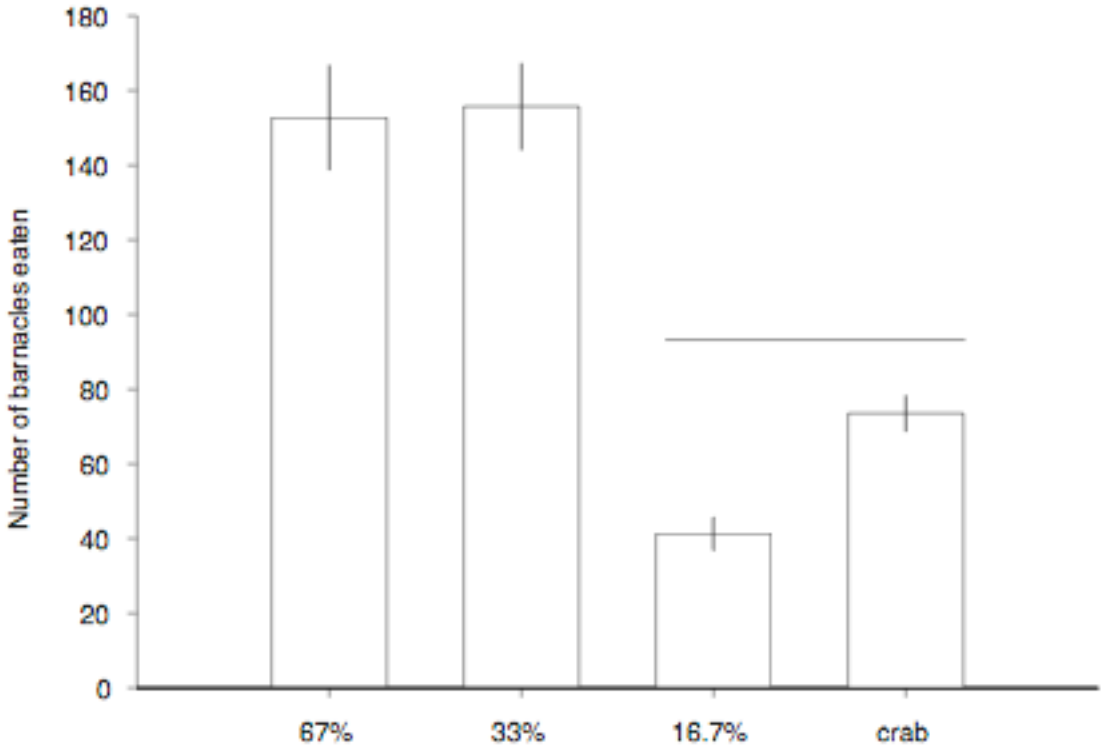


Figure 1. Number of barnacles eaten by *Nucella lamellosa* provided with high (67%), moderate (33%) and low (16.7%) food availability and high food availability while exposed to predator risk cues (crab). Values are back-transformed tank means and 95% confidence intervals of $\log_{10}$-transformed data. Horizontal lines indicate groups that are not significantly different.

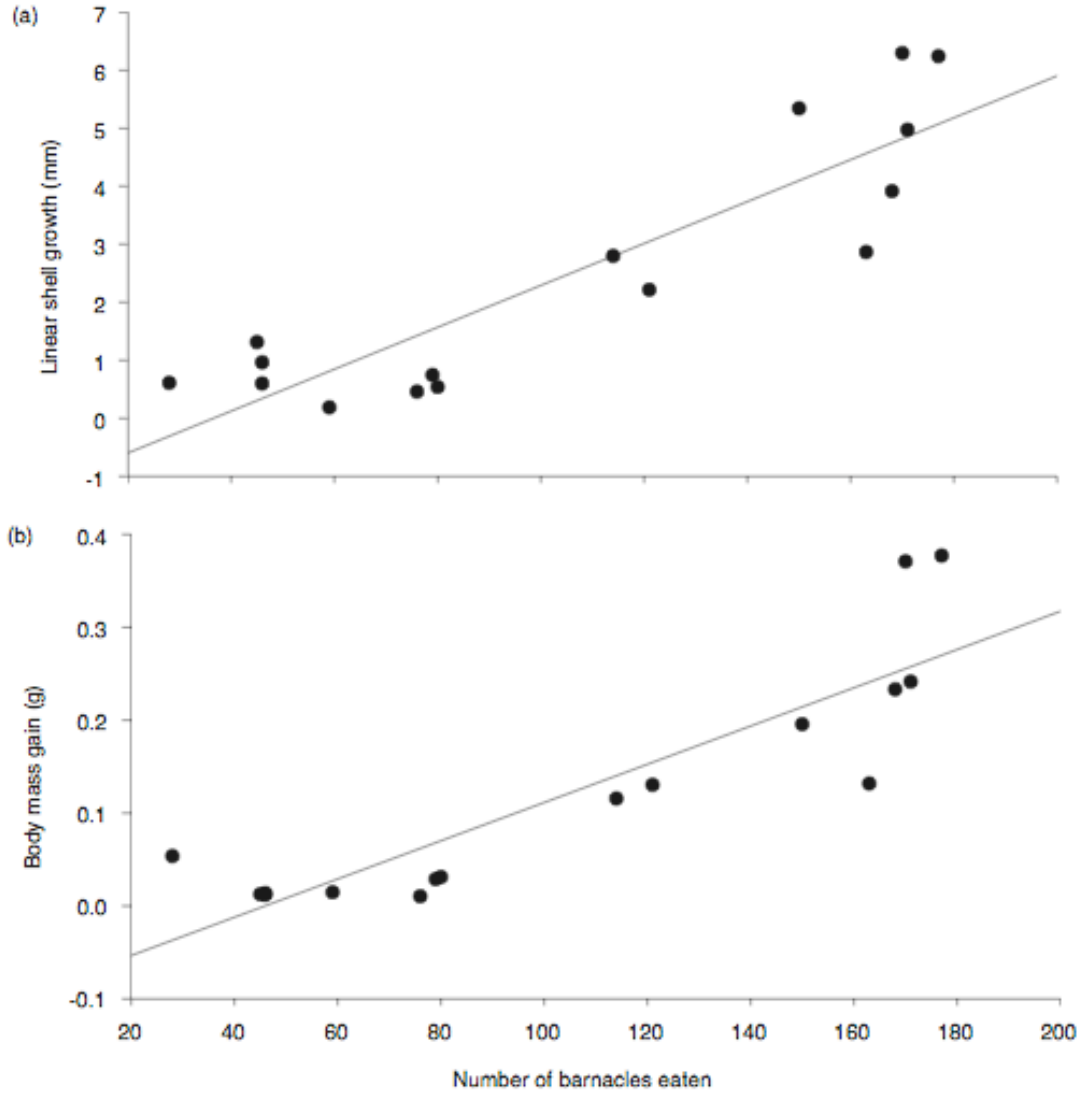


Figure 2. Relation between (a) linear shell growth and amount eaten and (b) body mass gain and amount eaten of *Nucella lamellosa* across all experimental treatments. Values are back-transformed tank means of $\log_{10}$-transformed data.

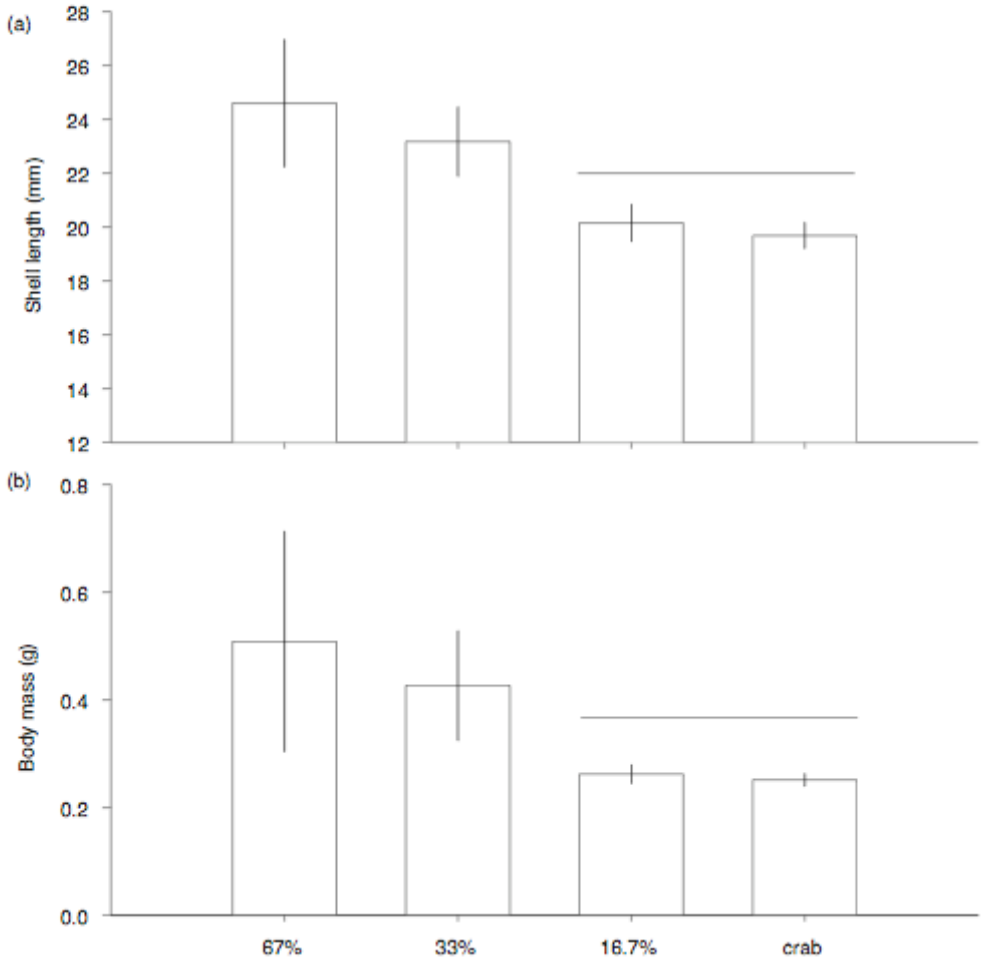


Figure 3. Variation in (a) final shell length and (b) body mass in *Nucella lamellosa* provided with high (67%), moderate (33%) and low (16.7%) food availability and high food availability while exposed to predator risk cues (crab). Values are back-transformed least squares means and 95% confidence intervals of $\log_{10}$-transformed data computed for covariates at their means. Horizontal lines indicate groups that are not significantly different.

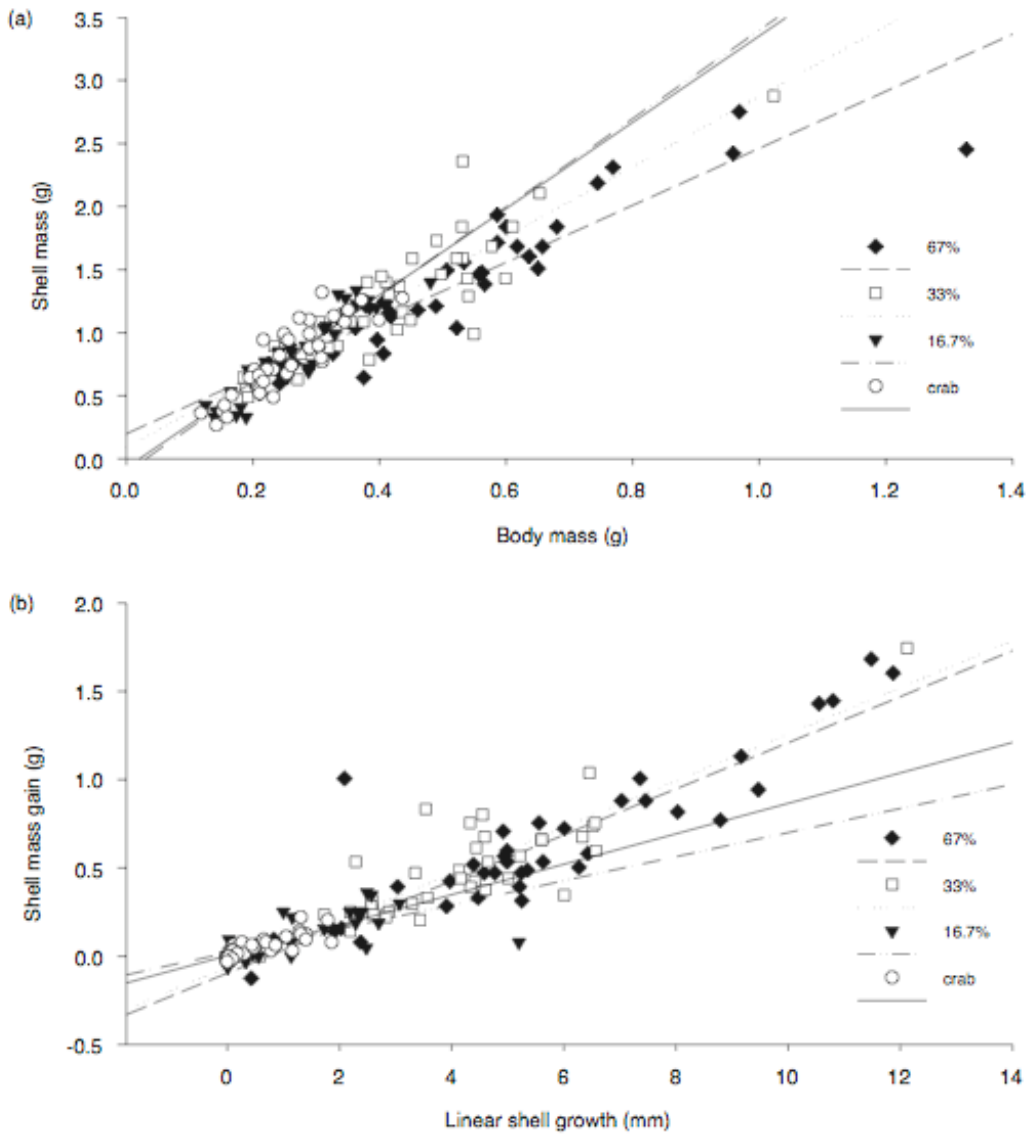


Figure 4. Relation between final body mass and final shell mass of *Nucella lamellosa* provided with high (67%), moderate (33%) and low (16.7%) food availability and high food availability while exposed to predator risk cues (crab). Values are back-transformed $\log_{10}$-transformed data.

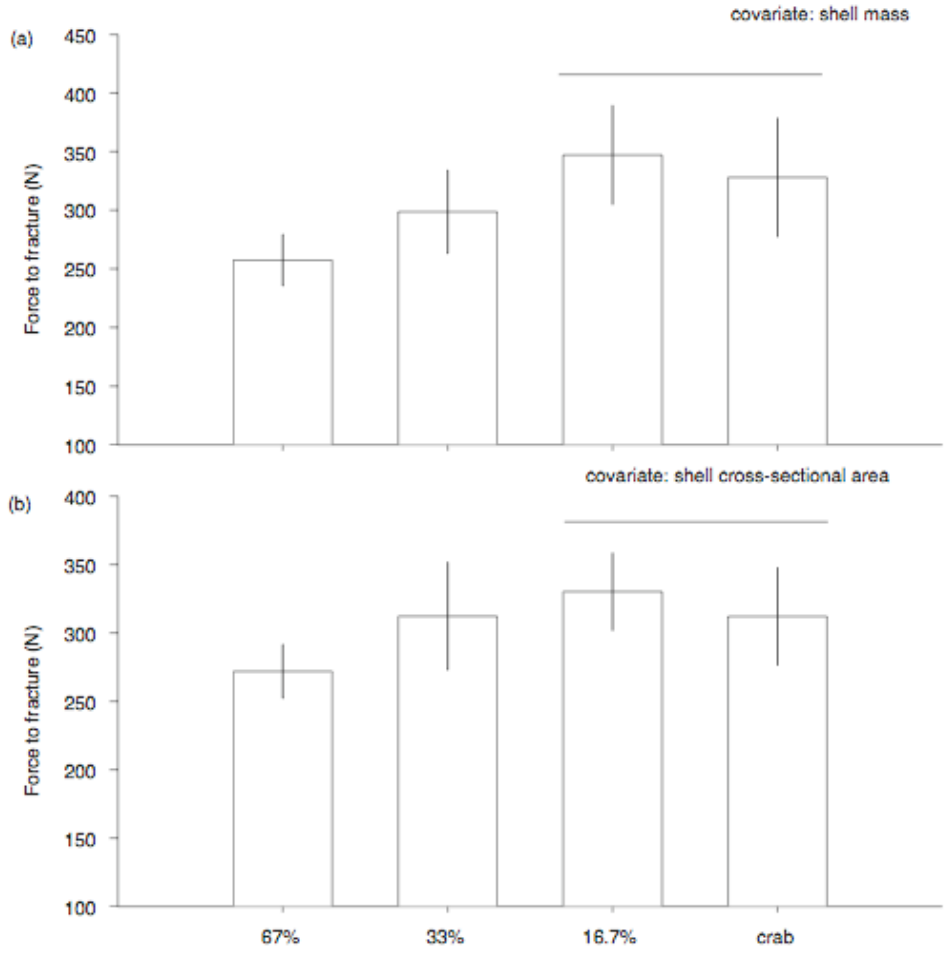


Figure 5. Variation in force to fracture of *Nucella lamellosa* provided with high (67%), moderate (33%) and low (16.7%) food availability and high food availability while exposed to predator risk cues (crab). Values are back-transformed least squares means and 95% confidence intervals of $\log_{10}$-transformed data computed for (a) shell mass and (b) shell cross-sectional area at their means. Horizontal lines indicate groups that are not significantly different.

References

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

Avery, R. and R. J. Etter. 2006. Microstructural differences in the reinforcement of a gastropod shell against predation. Marine Ecology-Progress Series **323**:159-170.

Brookes, J. I. and R. Rochette. 2007. Mechanism of a plastic phenotypic response: predator-induced shell thickening in the intertidal gastropod *Littorina obtusata*. Journal of Evolutionary Biology **20**:1015-1027.

Christy, J. H. 1995. Mimicry, mate choice, and the sensory trap hypothesis. American Naturalist **146**:171-181.

Currey, J. D. and J. D. Taylor. 1974. Mechanical-behavior of some molluscan hard tissues. Journal of Zoology **173**:395-406.

Dalziel, B. and E. G. Boulding. 2005. Water-borne cues from a shell-crushing predator induce a more massive shell in experimental populations of an intertidal snail. Journal of Experimental Marine Biology and Ecology **317**:25-35.

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

Emerson, S. B. and S. K. Boyd. 1999. Mating vocalizations of female frogs: Control and evolutionary mechanisms. Brain Behavior and Evolution **53**:187-197.

Grunbaum, D. 1997. Hydromechanical mechanisms of colony organization and cost of defense in an encrusting bryozoan, *Membranipora membranacea*. Limnology and Oceanography **42**:741-752.

Harvell, C. D. 1992. Inducible defenses and allocation shifts in a marine bryozoan. Ecology **73**:1567-1576.

Huitema, B. E. 1980. The Analysis of Covariance and Alternatives. John Wiley and Sons, Inc., New York.

Lande, R. and S. J. Arnold. 1983. The measurement of selection on correlated characters. Evolution **37**:1210-1226.

Menge, B. A., E. L. Berlow, C. A. Blanchette, S. A. Navarrete, and S. B. Yamada. 1994. The keystone species concept - variation in interaction strength in a rocky intertidal habitat. Ecological Monographs **64**:249-286.

Miner, B. G., S. E. Sultan, S. G. Morgan, D. K. Padilla, and R. A. Relyea. 2005. Ecological consequences of phenotypic plasticity. Trends in Ecology & Evolution **20**:685-692.

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

Palmer, A. R. 1982. Growth in marine gastropods - a non-destructive technique for independently measuring shell and body-weight. Malacologia **23**:63-73.

Palmer, A. R. 1983. Relative cost of producing skeletal organic matrix versus calcification - evidence from marine gastropods. Marine Biology **75**:287-292.

Palmer, A. R. 1990. Effect of crab effluent and scent of damaged conspecifics on feeding, growth, and shell morphology of the Atlantic dogwhelk *Nucella lapillus* (L). Hydrobiologia **193**:155-182.

Palmer, A. R. 1992. Calcification in marine mollusks - how costly is it. Proceedings of the National Academy of Sciences of the United States of America **89**:1379-1382.

Pigliucci, M. 2005. Evolution of phenotypic plasticity: where are we going now? Trends in Ecology & Evolution **20**:481-486.

Relyea, R. A. and J. R. Auld. 2005. Predator- and competitor-induced plasticity: How changes in foraging morphology affect phenotypic trade-offs. Ecology **86**:1723-1729.

Rundle, S. D., J. I. Spicer, R. A. Coleman, J. Vosper, and J. Soane. 2004. Environmental calcium modifies induced defences in snails. Proceedings of the Royal Society of London Series B-Biological Sciences **271**:S67-S70.

Tollrian, R. and C. D. Harvell, editors. 1998. The Ecology and Evolution of Inducible Defenses. Princeton University Press, Princeton, NJ.

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

Trussell, G. C. 2000. Predator-induced plasticity and morphological trade-offs in latitudinally separated populations of *Littorina obtusata*. Evolutionary Ecology Research **2**:803-822.

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

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

Van Buskirk, J. and B. R. Schmidt. 2000. Predator-induced phenotypic plasticity in larval newts: Trade-offs, selection, and variation in nature. Ecology **81**:3009-3028.

Vermeij, G. J. 1987. Evolution and Escalation: An Ecological History of Life. Princeton Univesrity Press, Princeton, NJ.

Vermeij, G. J. 2002. Characters in context: molluscan shells and the forces that mold them. Paleobiology **28**:41-54.

West-Eberhard, M. J. 2003. Developmental Plasticity and Evolution. Oxford University Press, New York.

Wimberger, P. H. 1991. Plasticity of jaw and skull morphology in the neotropical cichlids *Geophagus brasilensis* and *G. steindachneri*. Evolution **45**:1545-1563.

Windig, J. J., C. G. F. De Kovel, and G. De Jong. 2003. Genetics and Mechanics of Plasticity. Pages 31-49 *in* T. J. DeWitt and S. M. Scheiner, editors. Phenotypic Plasticity: Functional and Conceptual Approaches. Oxford University Press, New York, NY.

Zipser, E. and G. J. Vermeij. 1978. Crushing behavior of tropical and temperate crabs. Journal of Experimental Marine Biology and Ecology **31**:155-172.

Chapter 6 - Variation in inducible defenses in a marine snail from habitats with different predation regimes

Introduction

Predation is among the most important factors structuring natural populations and predation can affect the distribution and abundance of prey species (Paine 1966, Vermeij 1987). As a result, prey display a diversity of traits that defend against predators, including behavioral, morphological and chemical defenses, which may be a fixed property of the organism, or a phenotypically plastic response to the presence of predators (Lima and Dill 1990, Tollrian and Harvell 1998). In spatially and temporally variable predator environments, such inducible defenses reduce risk when predators are present, and confine the cost of defense to those individuals exposed to predators (Lively 1986).

Different populations, even in relatively close proximity, may experience very different predator environments and may exhibit different defensive phenotypes. Phenotypic variation among populations may be the result of local adaptation (Abjornsson et al. 2004), phenotypic plasticity in response to environmental heterogeneity (Trussell and Smith 2000), or variation in plastic responses among populations (Van Buskirk and McCollum 1999).

Marine snails offer an ideal system to test for variation in inducible defenses

and for studying the effects of predation risk on defensive traits. A long evolutionary history with shell breaking predators has produced snails with a variety of defensive shell structures such as increased shell thickness, sculpture and reduced apical spires (Vermeij 1987), either as fixed traits (Dalziel and Boulding 2005) or as inducible responses to chemical cues from predatory crabs (Appleton and Palmer 1988, Palmer 1990, Trussell 1996) . Shell-breaking crabs are important predators of intertidal zone snails (Boulding et al. 1999). Snails respond to the perceived risk of crab predation by reducing feeding activity and producing heavier shells, apertural teeth, and thicker apertural lips, all of which increase resistance to crab predation (Appleton and Palmer 1988, Palmer 1990, Marko and Palmer 1991, Trussell and Nicklin 2002). The presence of crabs varies across a water-motion gradient, from wave- and current-exposed habitats where crab predators are rare to protected habitats where crabs are locally abundant (Menge and Sutherland 1987). Snails also occur in different habitats along this gradient (Menge and Sutherland 1987).

The defenses of the snail *Nucella lamellosa,* a low shore predatory whelk, have been particularly well studied ((Appleton and Palmer 1988, Marko and Palmer 1991, Edgell and Neufeld 2008)Chapters 2-4). This species displays an array of shell and behavioral traits that can be induced by its major predator, the shell crushing crab, *Cancer productus.* Theory predicts that if the risk of predation is constant across space and time, prey should produce constitutive defenses (Lively 1986, Tollrian and Harvell 1998). However, if there is a cost to

defense (either a material or performance cost), then a plastic, inducible defense will be advantageous (Lively 1986, Tollrian and Harvell 1998), but only if the time to produce the defense is less than the temporal variation in predator risk experienced by the prey (Padilla and Adolph 1996). Snails that inhabit areas with abundant crabs might be particularly well defended against these predators both with fixed defensive traits and with plastic inducible defenses, while those in habitats where predators are rare, or episodically present, may benefit primarily from defenses that are phenotypically plastic. I examined the inducible and constitutive shell defenses of *Nucella lamellosa,* from habitats that vary in the abundance of predatory crabs.

Methods

Collection sites and experimental animals

This work was conducted on San Juan Island, WA, USA. Because the presence of crabs varies across a water motion gradient, I chose two current-swept sites (low crab abundance), County Park (PK; 48°32'29.92"N, 123°9'35.99"W) and Westside Preserve (WP; 48°30'26.76"N, 123° 8'35.20'W), and 2 quiet-water sites (high crab abundance), False Bay (FB; 48°28.87'N, 123°4.14'W) and a protected cove on the west side of Cattle Point (PT; 48°27.03' N, 122°57.70' W) (Fig. 1). Snails at current-swept sites are presumed to encounter crabs less often

because it is difficult for crabs to forage efficiently in wave- or current-swept conditions (Hampton and Griffiths 2007). At each site I verified the relative risk of predation by crabs by quantifying the incidence of repaired shell breaks on 50 snails, which are assumed to reflect sub-lethal encounters with crabs (Vermeij 1987). Snails were obtained by haphazardly tossing a 0.25 m^2 quadrat along a 10 meter transect in the low intertidal zone (*Hedophyllum* zone) of each site and collecting all the snails in each quadrat until 50 snails were obtained. I recorded the number of substantial breaks in the final shell whorl of each snail and then averaged the number of repaired breaks per shell at each site as a measure of relative predation risk at each site. Narrow, elongate shells with high spires characterized snails from current-swept sites (PK and WP), while snails from quiet-water sites (FB and PT) had rotund shells with low spires (Fig. 2).

Approximately 160 juvenile *Nucella lamellosa* (< 25 mm shell length) were collected from each site, 2 sites with abundant crabs and 2 sites without abundant crabs. Experimental snails from each population were kept in separate flow-through sea tables at the University of Washington's Friday Harbor Laboratories (FHL) and fed barnacles for 48 hours after collection from the field. Crabs (*Cancer productus*) were trap collected from the pier at FHL and held in sea tables separate from experimental snails. Crabs were fed frozen fish prior to the start of the experiment.

Laboratory induction experiment and morphometric measurements

Prior to the start of the experiment I individually numbered snails by affixing a numbered bee-tag to the spire of each shell with cyanoacrylate adhesive. After allowing the tags to dry, I measured shell dimensions (length, width, apertural lip thickness) with digital calipers (see Chapter 5). I weighed snails with a nondestructive method in which snails were weighed in air and then submerged in seawater and shell mass was estimated using the equation: 1.572*(submerged weight)+0.0162 (Palmer 1982). Soft tissue mass (hereafter body mass) was calculated by subtracting shell mass from the weight in air (total mass).

I randomly allocated plastic aquaria to one of two treatments: exposure and no exposure to effluent from crabs fed conspecific snails. Ten snails from each site were placed in one of four replicates for each treatment. Snails were fed four out of every six days their preferred prey (the barnacle *Balanus glandula*), attached to small stones, placed at the bottom of each aquarium. When the barnacles on a stone were nearly depleted, the empty stone was replaced with a new barnacle-covered stone. Seawater flowed into the tank through a trap door in the aquarium top and into a plastic tub fastened to the underside of the top. In the crab treatment, this tub housed a single crab that was fed a single conspecific snail three times a week. This treatment has been shown to induce the greatest morphological response in *N. lamellosa* in previous studies (Chapter 2). Overflow from the tub provided the aquarium with seawater. Holes cut into the aquarium, just below the level of the plastic tub, allowed seawater to drain from the aquarium. This apparatus facilitated the flow of chemical stimuli from

feeding crabs to the experimental snails below, while preventing physical contact with the predators. Snails were raised under these conditions for 84 days.

At the end of the experiment I quantified shell shape with landmark-based geometric morphometrics (Bookstein 1989, Rohlf and Marcus 1993), a method that retains the geometry of shape and facilitates visualization and statistical analysis of shape differences among groups. I took a digital image (Canon PowerShot™ SD450 Digital Elph mounted to a lighted photo stand) of each snail, oriented with the axis of coiling perpendicular to the optical axis of the camera. On each image I digitized ten shell landmarks on the with tpsDig software (Rohlf 2006). These landmarks were used in a generalized Procrustes analysis to remove the effects of specimen size, position and orientation in the digital images. The resultant aligned landmark configurations were used to generate shape variables as partial warp scores from a thin-plate spline analysis (TPS) and two uniform scores (tpsRelw v. 1.44; Rohlf, 2006). I then performed a relative warp analysis, which is analogous to a principal components analysis (PCA) of shape variation where relative warps are principal components of the distribution of shapes and summarize the variation in local shape deformations among the specimens. I also measured apertural lip thickness with digital calipers to the nearest 0.01 mm and obtained shell mass, which serves as a good estimate of overall shell thickness.

Shell breaking force

After obtaining morphometric measurements on all snails from all treatments, I estimated shell strength by measuring the compressive force required to fracture shells in an Instron Universal Testing Machine (Instron Corporation, MA). Shells were placed aperture down so that force was applied perpendicular to the axis of coiling. Applying force to this plane provides a good estimate of the resistance to forces exerted during a crab's crushing attempt, and allowed all shells to be tested similarly.

Statistical analyses

I tested adherence to the assumptions of normality and homoscedasticity with Shapiro-Wilk's W and Cochran's Test respectively. In an effort to meet these assumptions, all morphological data were $\log_{10}$ +1 transformed.

The effect of collection site on the incidence of sub-lethal encounters with crabs was analyzed with single factor ANOVA with mean number of repaired breaks per shell as the response variable and site as a fixed factor.

Because the responses of snails sharing an aquarium may not be independent, variation in shell shape was assessed with a nested ANCOVA using treatment as a fixed factor; replicate aquaria as a random factor nested within treatment, and centroid size as a covariate on the first principle component (RW1) generated from the geometric morphometric analysis.

At the end of the experiment, the size of snails differed among treatments, so ANCOVA could not be used to statistically control for size to assess difference

in shell traits (lip thickness, shell mass, shell strength) among treatments. Therefore, I regressed the value of each morphological trait against some metric of snail size within each replicate of each treatment and used the slope coefficient from each replicate as the response variable in a factorial ANOVA, with habitat and cue treatment as fixed factors. Lip thickness was regressed against shell length, shell mass was regressed against body mass, and shell strength was regressed against shell mass.

Tukey's Honestly Significant Difference (HSD) test was used for all post-hoc comparisons. Because shell strength could be affected by shell shape or shell thickness, I used a multiple regression to test the effects of shell shape (RW1) and shell thickness on shell strength. Statistica v6.1 was used for all analyses.

Results

Predation risk

Non-lethal encounters with crabs differed among habitat types (ANOVA, $F_{1,2}$ = 385.83, P = 0.0026). Overall the incidence of healed scars on shells was significantly greater in quiet water sites (0.765 ± 0.03) than in current-swept sites, (0.050 ± 0.03).

Laboratory induction experiment

For shell shape, positive scores on the first component (RW1) were associated with a wider body whorl and a shorter spire (Fig. 3). The shape of snails from habitats with abundant crabs was characterized by positive scores and was significantly different from snails from habitats without abundant crabs (ANCOVA, habitat effect, $F_{1,12} = 249.80$, $P < 0.001$; Table 1), which were characterized by negative scores (Fig. 3). Snails from habitats with crabs produced shorter, squatter shells than those from habitats without crabs. Shell shape was unaffected by the presence or absence of chemical cues from crabs (ANCOVA, cue effect, $F_{1,12} = 0.362$, $P = 0.552$;Table 1, Fig. 3) and there was not a significant treatment by interaction effect (ANCOVA, cue-by-habitat effect, $F_{1,12} = 11.93$, $P = 0.381$; Table 1).

For apertural lip thickness I found a highly significant cue-by-habitat type interaction, thus lip thickening responses to the presence of crabs differed between snails from different habitats (ANOVA, cue-by-habitat effect, $F_{1,12} = 24.61$, $P < 0.001$;Table 2). Crab cues induced apertural lip thickening in snails from habitats with abundant crabs but decreased lip thickening in snails from habitats without abundant crabs (Fig. 4).

There was also a highly significant cue-by-habitat interaction effect on shell mass gain (ANOVA, $F_{1,12} = 16.19$, $P = 0.002$; Table 3). Crab cues amplified shell mass gain per unit body mass but this response differed between snails from different habitats. Snails from habitats with abundant crabs had a large positive response while snails from habitats without abundant crabs had a very weak

positive response (Fig. 5).

Shell strengthening in response to crab cues also depended on source habitat as indicated a highly significant cue-by-habitat interaction effect on shell strength (ANOVA, $F_{1,12} = 6.84$, $P = 0.023$; Table 4). When exposed to crab cues snails from habitats with abundant crabs produced stronger shells per unit shell mass relative to controls, while those from habitats without crabs showed no response (Fig. 6).

Multiple regression showed that shell shape (RW1) and shell thickness, both contributed to shell strength. Shell thickness was a slightly stronger predictor for shell strength than shell shape ($\beta = 0.399$, $P < 0.001$, and $\beta = 0.302$, $P < 0.002$, respectively).

Discussion

Snails from different habitats that vary in predator abundance displayed different degrees of constitutive and inducible shell defenses. Snails from habitats with abundant crabs were less constitutively defended in the absence of crab cues, but displayed strong inducible responses to cues from predatory crabs. In contrast, snails from habitats without abundant crabs displayed slightly greater constitutive defenses in the absence of cues from predators, but responded weakly or not at all to cues from predatory crabs. Snails from habitats with

abundant crabs also displayed fixed developed of shorter, more rotund shells than snails from crab-fee habitats. For snails from crab-rich habitats, shell shape, together with induced increases in shell thickness, contributed to increases in shell strength in the presence of crabs that were greater than those snails from crab-free habitats.

That snails from crab-free habitats had higher levels of constitutive defense and lower levels of inducible defense and snails from crab-rich habitats had lower levels of constitutive defense and higher levels of inducible defense suggests that there may be a tradeoff between these two types of defense. As the production of both ever-present constitutive defense and inducible defenses may entail costs, it has been hypothesized that they should be traded off, such that investment in constitutive defense is predicted to be matched by low investment in induced defense, as seen here (Karban and Myers 1989). This tradeoff has been documented in a number of studies examining induced resistance to herbivores in terrestrial plants (Morris et al. 2006), but to my knowledge this is the first documentation of this pattern in an aquatic animal, a group, which in addition to terrestrial plants, commonly deploys inducible defenses (Tollrian and Harvell 1998).

Snails from crab-rich habitats can increase resistance to crab predation in different ways, with the fixed development of rotund shells and the inducible amplification of shell thickness and shell mass. Together these traits contribute to an increase in shell strength and thus resistance to crushing predators. A

rounder shell spreads crushing force more evenly over the shell surface, increasing the force required to break it (Anderson et al. 2004). Likewise, an increase in shell thickness when crabs are present increases the snails' resistance to crushing (Currey and Taylor 1974). Many studies have shown that prey often employ only one type of defense, presumably saving the cost of a second defense (DeWitt et al. 1999). For example, prey with weak morphological defense often show stronger antipredator behavior than morphologically well-defended prey (Rundle and Bronmark 2001, Cotton et al. 2004). Here snails employ both constitutive defense with inducible defense, presumably enhancing overall defense against shell-breaking crabs (trait cospecialization, sensu DeWitt et al. 1999).

Phenotypic differences in shell shape between snails from crab-rich and crab-free habitats in the no-cue controls suggest that some of the phenotypic variation, particularly shape, is inherited. Thus, while *N. lamellosa* from crab-rich habitats appears to have fixed shell shape development that increases resistance to crabs, the inducible amplification of shell thickness is likely to compliment local adaptation. It would appear that the performance of snails in crab-rich habitats benefits from the combined effect of natural selection and plasticity shaping locally adapted phenotypes. At present we do not know if these differences among populations are due to genetic differences, or early juvenile experience, or maternal effects. However, genetic differentiation is possible as these animals are direct developers, slow moving, and the sites are separated by

a diversity of habitat types making each relatively isolated. Studies on other gastropod species, similarly lacking a dispersal stage, suggest that plastic traits are often combined with fixed trait differences to improve adaptation to local conditions (Trussell 2000a, Dalziel and Boulding 2005).

Only snails from habitats with abundant crabs exhibited plasticity for each of the traits. This suggests that plasticity is evolving differently within local populations of the same species over relatively small spatial scales. Thinner, elongate shells increase vulnerability to predators because thinner shells are more readily crushed and crabs can easily break a tall spire to access a snail's soft tissue. However, when released from the selective pressure of crab predation the production of thinner, elongate shells may provide fitness benefits. For example, shell elongation creates a taller spire, which provides the shell with greater habitable volume (Stone 1999). Likewise, thinner shells can increase the volume of living space inside the shell (Kemp and Bertness 1984). Thus the production of thinner, elongate shells may allow for greater investment in reproductive and somatic tissues.

Alternatively, the fixed development of elongate shells may be constrained by some other selective force, like predators with contrasting attack strategies or water-motion. For example, *N. lamellosa* from another San Juan Island site, can alter shell shape in the presence of predators with different attack modes (Bourdeau 2009a). Snails from this habitat, which has abundant crabs and seastars, make short, rotund shells in the presence of shell-breaking crabs, and

elongate, tall-spired shells in the presence of shell-entry seastars. Elongate shells with tall spires reduce susceptibility to seastars, by providing more shell volume into which the snail can withdraw its soft tissues. Thus, environments with few crabs, but abundant seastars, such as current-swept shores, may select for taller, more elongate shells. If this selection pressure is strong enough, it may favor canalization for elongate shell shape and constrain the production of a crab-resistant phenotype through plastic development (DeWitt 1998). Such limits to evolve plasticity might even preclude adaptation to crab-rich habitats.

This study suggests that crab predation plays an important role in shaping intraspecific variation in shell morphology and inducible defenses in *Nucella lamellosa*. Higher risk habitats appear to favor snails with thicker, shorter, more rotund shells. This shell morphology may function to increase a snail's ability to exploit sheltered shores by reducing the snail's susceptibility to locally-abundant shell-breaking crabs. The presence of crabs in these habitats may also induce the development of thicker, stronger shells thereby allowing individuals inhabiting these environments to better resist more numerous crab attacks.

Tables

Table 1. Factorial ANCOVA for the effects of origin (habitats with and without abundant crabs on San Juan Island, WA, USA), treatment (control, crab cue), size (centroid size) and their interactive effects on *Nucella lamellosa* shell shape (RW1).

Source	df	MS	*F*	*P*
Centroid size	1	0.000802	1.9742	0.162239
Treatment	1	0.000191	0.3623	0.551632
Habitat	1	0.158291	249.8028	0.000000
Treatment X Habitat	1	0.000541	11.9322	0.380547
Aquarium(Treatment X Habitat)	12	0.000651	1.6036	0.097093
Error	139	0.000406		

Table 2. Factorial ANCOVA for the effects of origin (habitats with and without abundant crabs on San Juan Island, WA, USA), and treatment (control, crab cue), and their interactive effects on *Nucella lamellosa* apertural lip thickening.

Source	df	MS	*F*	*P*
Treatment	1	0.024007	0.41590	0.531126
Habitat	1	0.504874	8.74646	0.011977
Treatment X Habitat	1	1.420301	24.60535	0.000331
Error	12	0.057723		

Table 3. Factorial ANCOVA for the effects of origin (habitats with and without abundant crabs on San Juan Island, WA, USA), and treatment (control, crab cue), and their interactive effects on *Nucella lamellosa* shell mass gain.

Source	df	MS	*F*	*P*
Treatment	1	6.95910	45.2715	<0.0001
Habitat	1	1.24907	8.1257	0.01461
Treatment X Habitat	1	2.48828	16.1872	0.0017
Error	12	0.15372	0.15372	

Table 4. Factorial ANCOVA for the effects of origin (habitats with and without abundant crabs on San Juan Island, WA, USA), and treatment (control, crab cue), and their interactive effects on *Nucella lamellosa* shell strength change.

Source	df	MS	*F*	*P*
Treatment	1	0.35945	2.8616	0.1165
Habitat	1	0.00039	0.0031	0.9565
Treatment X Habitat	1	0.85936	6.8415	0.0226
Error	12	0.12561		

Figures

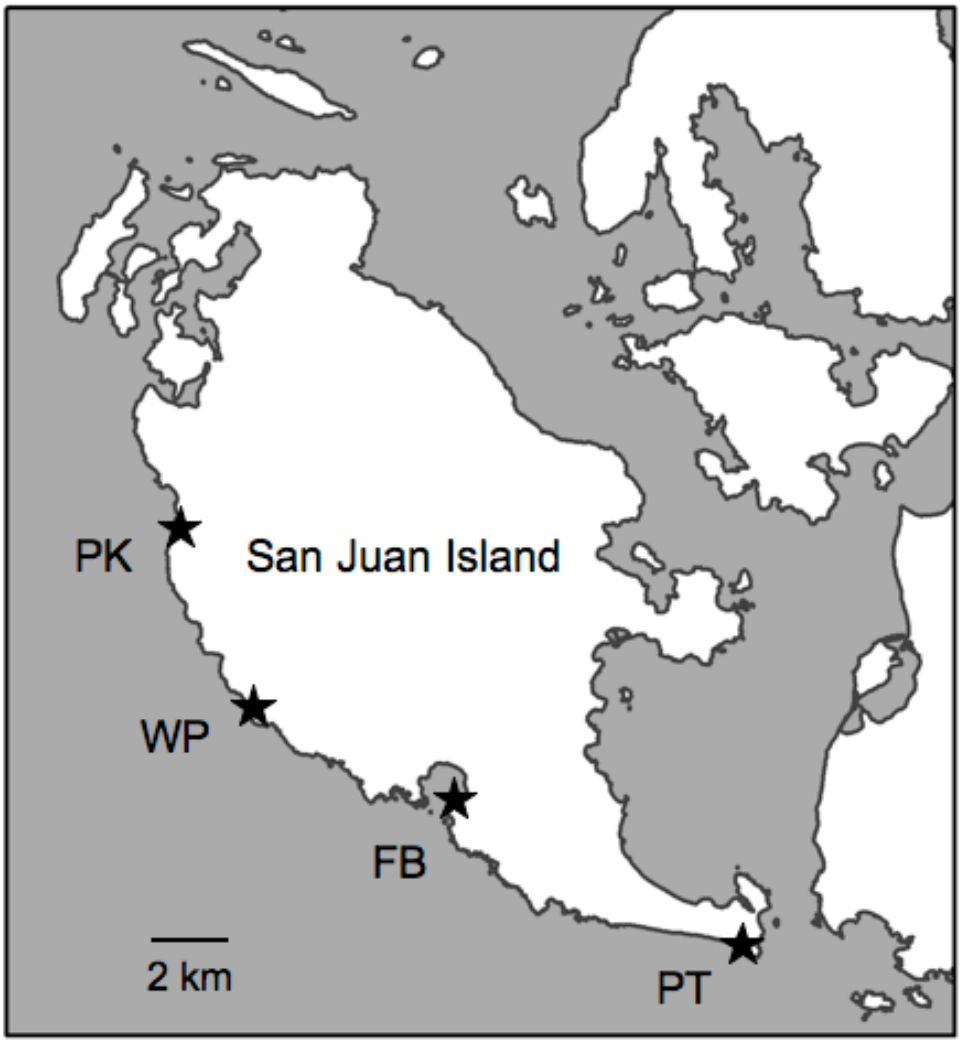


Fig. 1. Source habitats on San Juan Island, Washington, USA: PK – San Juan County Park; WP – Westside Preserve; FB – False Bay; PT – Cattle Point.

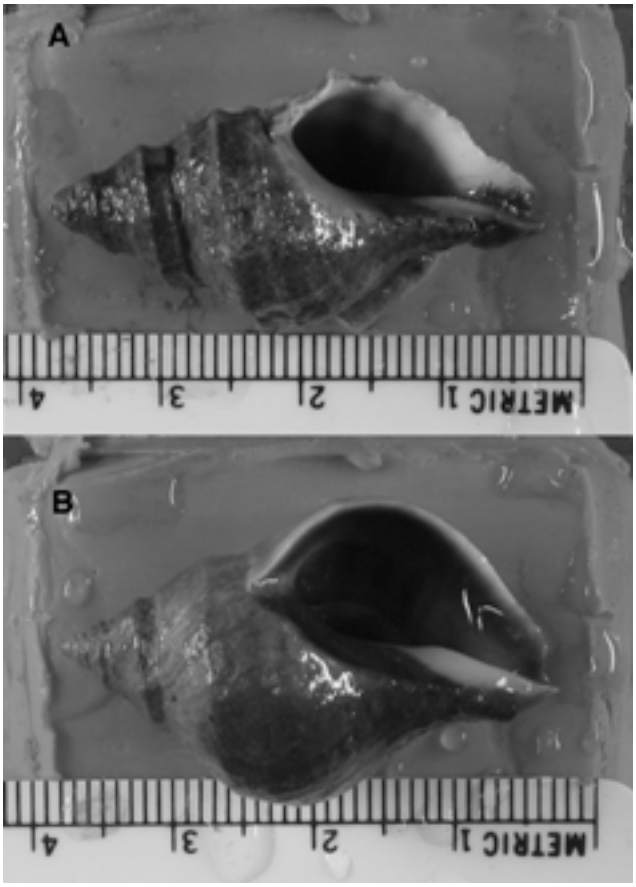


Fig. 2. Typical shell forms of *Nucella lamellosa* from sites without (A) and with (B) abundant crabs. Scale in mm.

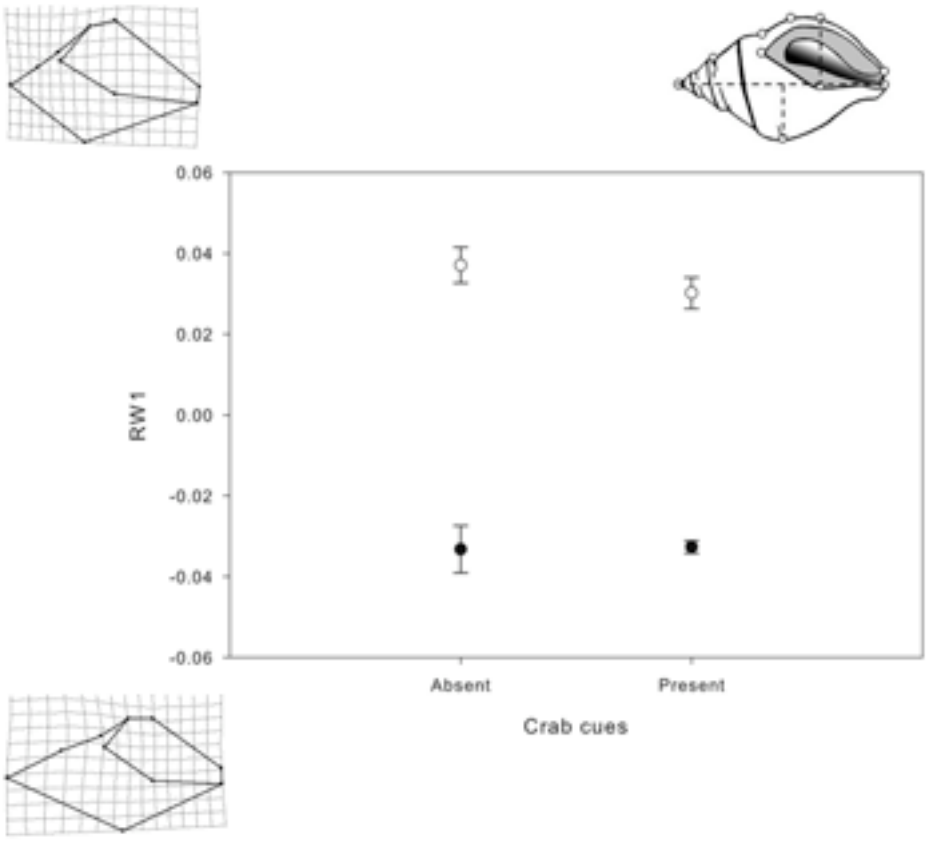


Fig. 3. Shell shape in response to treatment (with or without cues from predatory crabs) for *Nucella lamellosa* from habitats with (white circles) and without (shaded circles) abundant crabs. The first relative warp (RW1) and thin-plate spline deformation grids, 3X the observed range are shown on the vertical axis. Positive scores along RW1 are associated with squatter shells with shorter spires and negative scores with elongate shells with taller spires. Symbols are least-squares treatment means at covariate mean centroid size (669.37). Error bars represent ±1 SE.

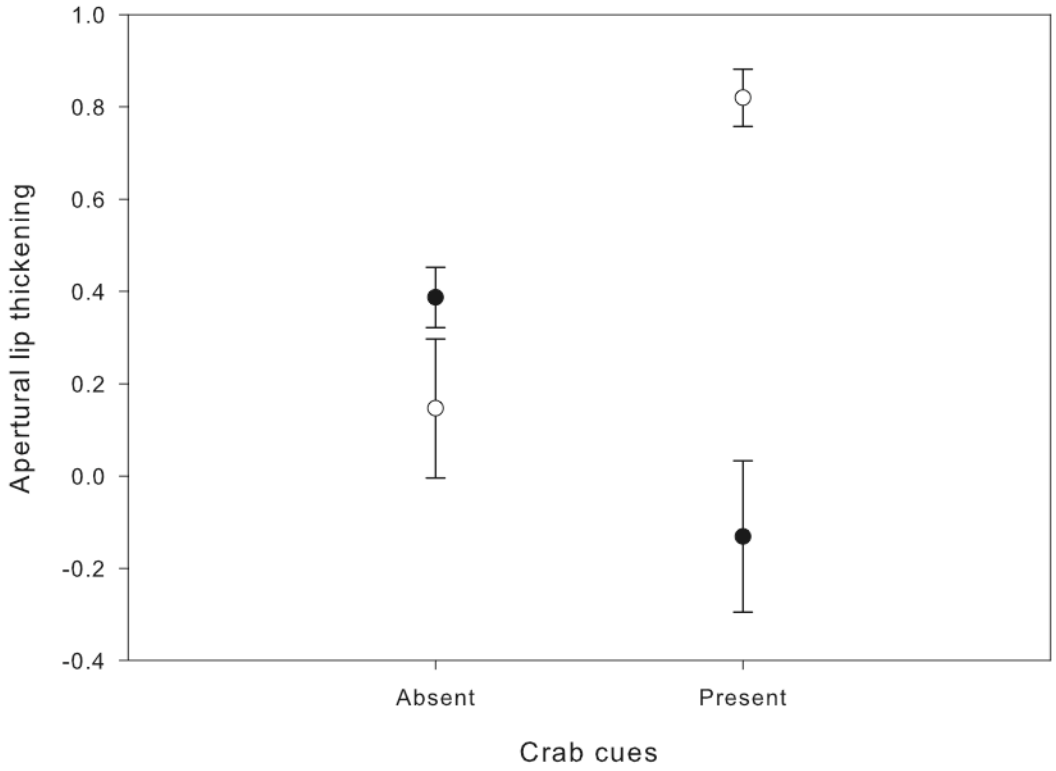


Fig. 4. Variation in apertural lip thickening in response to treatment (with or without cues from predatory crabs) for *Nucella lamellosa* from habitats with (white circles) and without (shaded circles) abundant crabs. Apertural lip thickening on the vertical axis represents the linear slope coefficient of log lip thickness (originally measured in mm) regressed against log shell length (originally measured in mm) estimated for each replicate aquarium. Symbols represent treatment means and error bars represent ±1 SE.

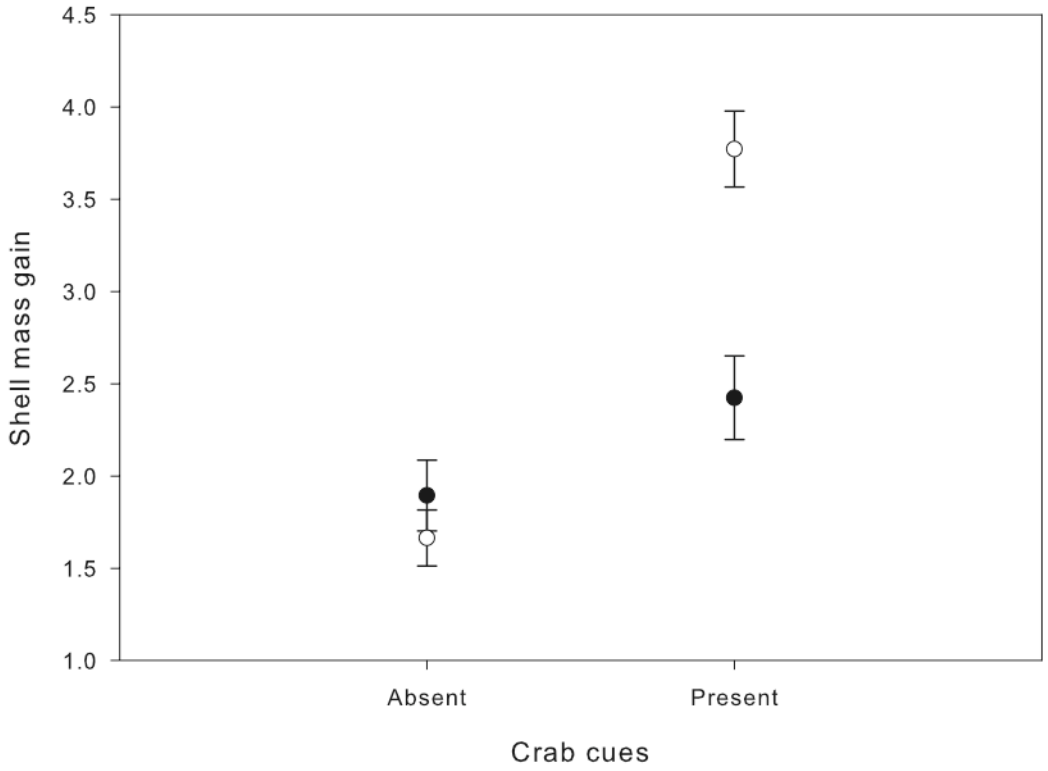


Fig. 5. Variation in shell mass gain in response to predation treatment for *Nucella lamellosa* from habitats with (white circles) and without (shaded circles) abundant crabs Shell mass gain on the vertical axis represents the linear slope coefficient of log final shell mass (originally measured in g) regressed against log final body mass (originally measured in g) estimated for each replicate aquarium. Symbols represent treatment means and error bars represent ±1 SE.

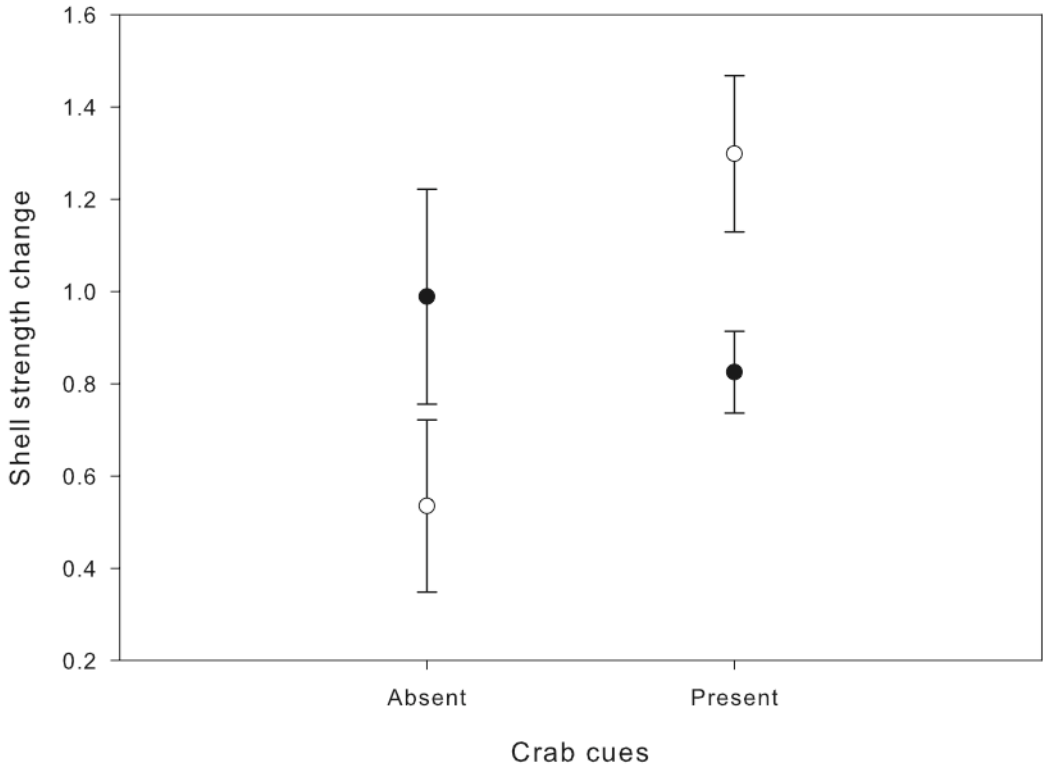


Fig. 6. Variation in shell strength change in response to treatment (with or without cues from predatory crabs) for *Nucella lamellosa* from habitats with (white circles) and without (shaded circles) abundant crabs. Shell strength gain on the vertical axis represents the linear slope coefficient of log force to fracture (originally measured in N) regressed against log final shell mass (originally measured in g) estimated for each replicate aquarium. Symbols represent treatment means and whiskers represent standard errors.

References

Abjornsson, K., L. A. Hansson, and C. Bronmark. 2004. Responses of prey from habitats with different predator regimes: Local adaptation and heritability. Ecology **85**:1859-1866.

Anderson, K. E., J. B. Tharrington, P. A. Curtis, and F. T. Jones. 2004. Shell characteristics of eggs from historic strains of single comb white leghorm chickens and the relationship of egg shape to shell strength. International Journal of Poultry Science **3**:17-19.

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

Bookstein, F. L. 1989. Principal Warps - Thin-Plate Splines and the Decomposition of Deformations. Ieee Transactions on Pattern Analysis and Machine Intelligence **11**:567-585.

Boulding, E. G., M. Holst, and V. Pilon. 1999. Changes in selection on gastropod shell size and thickness with wave-exposure on Northeastern Pacific shores. Journal of Experimental Marine Biology and Ecology **232**:217-239.

Bourdeau, P. E. 2009. Prioritized phenotypic responses to combined predators in a marine snail. Ecology **in press**.

Cotton, P. A., S. D. Rundle, and K. E. Smith. 2004. Trait compensation in marine gastropods: Shell shape, avoidance behavior, and susceptibility to predation. Ecology **85**:1581-1584.

Currey, J. D. and J. D. Taylor. 1974. Mechanical-behavior of some molluscan hard tissues. Journal of Zoology **173**:395-406.

Dalziel, B. and E. G. Boulding. 2005. Water-borne cues from a shell-crushing predator induce a more massive shell in experimental populations of an intertidal snail. Journal of Experimental Marine Biology and Ecology **317**:25-35.

DeWitt, T. J. 1998. Costs and limits of phenotypic plasticity: Tests with predator-induced morphology and life history in a freshwater snail. Journal of Evolutionary Biology **11**:465-480.

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

Edgell, T. C. and C. J. Neufeld. 2008. Experimental evidence for latent developmental plasticity: intertidal whelks respond to a native but not an introduced predator. Biology Letters **4**:385-387.

Hampton, S. L. and C. L. Griffiths. 2007. Why *Carcinus maenas* cannot get a grip on South Africa's wave-exposed coastline. African Journal of Marine Science **29**:123-126.

Karban, R. and J. H. Myers. 1989. Induced plant-responses to herbivory. Annual Review of Ecology and Systematics **20**:331-348.

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

Lima, S. L. and L. M. Dill. 1990. Behavioral decisions made under the risk of predation - a review and prospectus. Canadian Journal of Zoology-Revue Canadienne De Zoologie **68**:619-640.

Lively, C. M. 1986. Canalization versus developmental conversion in a spatially-variable environment. American Naturalist **128**:561-572.

Marko, P. B. and A. R. Palmer. 1991. Responses of a rocky shore gastropod to the effluents of predatory and nonpredatory crabs - avoidance and attraction. Biological Bulletin **181**:363-370.

Menge, B. A. and J. P. Sutherland. 1987. Community regulation: variation in disturbance, competition, and predation in relation to environmental-stress and recruitment. American Naturalist **130**:730-757.

Morris, W. F., M. B. Traw, and J. Bergelson. 2006. On testing for a tradeoff between constitutive and induced resistance. Oikos **112**:102-110.

Padilla, D. K. and S. C. Adolph. 1996. Plastic inducible morphologies are not always adaptive: The importance of time delays in a stochastic environment. Evolutionary Ecology **10**:105-117.

Paine, R. T. 1966. Food web complexity and species diversity. American Naturalist **100**:65-&.

Palmer, A. R. 1982. Growth in marine gastropods - a non-destructive technique for independently measuring shell and body-weight. Malacologia **23**:63-73.

Palmer, A. R. 1990. Effect of crab effluent and scent of damaged conspecifics on feeding, growth, and shell morphology of the Atlantic dogwhelk *Nucella lapillus* (L). Hydrobiologia **193**:155-182.

Rohlf, F. J. 2006. tpsDig. Version 2.10.

Rohlf, F. J. and L. F. Marcus. 1993. A Revolution in Morphometrics. Trends in Ecology & Evolution **8**:129-132.

Rundle, S. D. and C. Bronmark. 2001. Inter- and intraspecific trait compensation of defence mechanisms in freshwater snails. Proceedings of the Royal Society of London Series B-Biological Sciences **268**:1463-1468.

Stone, J. R. 1999. Using a mathematical model to test the null hypothesis of optimal shell construction by four marine gastropods. Marine Biology **134**:397-403.

Tollrian, R. and C. D. Harvell, editors. 1998. The Ecology and Evolution of Inducible Defenses. Princeton University Press, Princeton, NJ.

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

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

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

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

Van Buskirk, J. and S. A. McCollum. 1999. Plasticity and selection explain variation in tadpole phenotype between ponds with different predator composition. Oikos **85**:31-39.

Vermeij, G. J. 1987. Evolution and Escalation: An Ecological History of Life. Princeton Univesrity Press, Princeton, NJ.

Chapter 7 - Predator-induced plasticity and habitat partitioning in congeneric marine snails distributed along a vertical intertidal gradient.

Introduction

In marine systems, predation risk decreases with increasing vertical elevation over small spatial scales in the intertidal zone of rocky shores (Vermeij 1987). The number, diversity and composition of predators, as well as the intensity of predation all change along this gradient because most intertidal zone predators are unable to survive the longer duration of emersion at high tidal elevations due to desiccation and heat stress (Menge and Sutherland 1976, 1987). During low tides feeding generally ceases, and mobile predators are usually inactive or confined to the subtidal zone or isolated rock pools (Robles et al. 1989). The physiological or temporal limitation that periodic emergence imposes on predators means that upper-shore prey species are exposed to predation risk less frequently than their lower shore congeners.

For relatively slow-moving gastropods in the intertidal zone, shell architectural defense is the primary mode of protection against their highly mobile, shell-breaking crab predators (Vermeij 1987). Crab predation has selected for increased resistance of gastropod shells to crushing, both as a constitutive defense and as an inducible response to chemical cues released

from feeding crabs (Vermeij and Currey 1980, Palmer 1990, Trussell and Nicklin 2002, Dalziel and Boulding 2005, Bourdeau 2009a). This production of thicker shells is accompanied by a reduction in feeding and subsequent somatic growth (Palmer 1981, 1990, Trussell 2000b). In addition, shell accretion is dependent upon submergence. While shell features that confer resistance to crushing become progressively more evident in species inhabiting lower tidal elevations on most rocky shores (Vermeij 1987), it is not known whether predator-induced shell plasticity and associated growth tradeoffs exhibit a similar pattern. However, if the importance of shell defenses differs among habitats or accross time within a habitat, species from different habitats would be expected to differ in their degree of constitutive shell defenses and the ability to amplify those defenses (inducible defenses).

Whelks in the genus *Nucella* provide an appropriate system for studying defensive traits and their plasticity across a predation risk gradient. As mobile primary predators in the intertidal zone, these species face the conflicting demands of foraging and avoiding predation during periods of immersion. Three species, *N. canaliculata*, *N. lamellosa* and *N.ostrina* overlap considerably across their geographic ranges in the Northeastern Pacific (Collins et al. 1996, Sorte and Hofmann 2005). Within extant members of the genus *Nucella*, these three species form a monophyletic group along with *N. emarginata*, with *N. lamellosa* basal to the other three species (Collins et al. 1996). These three species tend to be segregated along a vertical gradient on moderately wave-exposed shores,

where they commonly co-occur. *N. ostrina* is found highest on the shore, *N. canaliculata* occurs at mid-shore and *N. lamellosa* is restricted to low tide heights (Bertness 1977, Palmer 1980). Thus, the three species are exposed to different periods of tidal immersion, which results in different exposure to predation risk and different amounts of time for foraging and shell accretion. I tested whether these three species differed in their response to predation risk in terms of activity, growth, and constitutive and inducible morphological defenses.

Materials and methods

Nucella *distribution and density*

To confirm the relative vertical distributions and to assess the density of these species along a vertical gradient, I sampled two moderately wave-exposed shores on the southwest coast of San Juan Island, WA, USA where all three species co-occur (Cattle Point - 48°27.03' N, 122°57.70' W, Eagle Cove - 48°28' N, 123°03' W). In May and June 2007, 6 m horizontal transects (three at Cattle Point, four at Eagle Cove) were established parallel to the shore at the low water mark along a stretch of rocky outcrop at each site. Along each horizontal transect I set up vertical transects spaced 2 m apart. Using a surveying level and measuring tape I established tide height markers at each 0.25 m tidal elevation along each vertical transect starting at -0.25 m below MLLW and ending at 2.0 m

above MLLW, for a total of 10 markers. I sampled at each marked tidal elevation by counting the number of whelks of each species within a 0.25 x 0.25 m quadrat.

Immersion time at different tidal heights

To estimate the relative risk of predation at different tidal heights, I calculated the proportion of time that each marked tidal height was immersed. Because crabs only forage when submerged (Robles et al. 1989), the proportion of time immersed is a good estimate of the maximum amount of time whelks at a given tidal height could be exposed to predators. I used NOAA tidal height observations for these sites from 1-June through 31-August, 2007 (tides at these sites are approximately the same as those at Port Townsend, WA, USA,48°06′59″N 122°46′31″W; http://tidesandcurrents.noaa.gov/), which provides predicted and observed tidal height readings at 6-minute intervals. I calculated the proportion of time that a given marked tidal height was immersed over the three-month period as the proportion of NOAA observations that were greater than or equal to that marked height.

Morphological responses of Nucella *to predation risk*

In June 2007, small juveniles of each species (shell length (mm) ± SE; *N. ostrina*, 13.02 ± 0.31, *N. canaliculata*, 15.45 ± 0.28, *N. lamellosa*, 19.42 ± 0.27) were collected at Cattle Point and Eagle Cove across the range of the vertical

distribution of each species at each site. I conducted a common garden experiment using the experimental apparatus described in chapter 2, where juveniles of each species were either exposed to chemical cues associated with risk to crab predation (the rock crab, *Cancer productus*, fed conspecific whelks for each test species) or a control, where they were not exposed to cues associated with risk. All whelks were raised in aquaria provided with flow-through seawater and small stones encrusted with their preferred food, barnacles (*Balanus glandula*). Each species-by-treatment combination was replicated in four aquaria with 10 whelks in each. The experiments began on 9-10 June and lasted for 76 days, until 24-25 August 2007.

Prior to the experiment whelks were individually marked with a colored, numbered tag (bee tag) affixed to the spire of their shell with cyanoacrylate adhesive. I measured the total shell length of each whelk to the nearest 0.01 mm with digital calipers. Initial shell weights and body weights were estimated following the non-destructive procedure of (Palmer 1982). At the completion of the experiment I re-measured each trait. During the experiment, activity levels were quantified for all replicates of each species in each treatment by taking visual scans of each aquarium. I used the proportion of whelks active at each scan as my response variable. A whelk was considered active if was mobile (foot extended and tentacles in motion) or if it was feeding (clinging to an individual barnacle). A whelk was considered inactive if it was immobile and attached to bare rock or the side or bottom of the aquarium.

Shell defense and magnitude of inducible response

Upon completion of the experiment, I quantified the compressive strength of shells by determining the force required to fracture shells with an Instron Dynamic Testing Machine (Instron Corporation, Canton, Massachusetts, Model 1350; 1000 N load cell). Whelks were placed aperture down on a stationary bottom platen and force was applied perpendicular to the axis of coiling by a top platen lowered onto the shell at a constant rate of 10 mm/min. Force was applied until the shell failed (i.e., fractured to the point of exposing the soft tissue of the whelk). I defined shell strength as the maximum force withstood by the shell immediately prior to shell failure. The compressive strength of shells is a good estimate of whelk vulnerability to shell-crushing crabs. To test for differences in the magnitude of predator-induced phenotypic responses among species, I calculated a size-corrected relative response by subtracting the size-corrected mean shell strength of each species in control aquaria from the size-corrected aquaria means in the crab cue treatments (Morris et al. 2006).

Statistical analysis

For each species, I used a Kruskal-Wallis test to compare densities among tidal heights along its vertical range (*N. ostrina*, 0.5-2.0 m; *N. canaliculata*, 0.25-1.5 m; *N. lamellosa*, 0.0-0.75 m). For tidal heights where two species overlapped I used a Wilcoxon matched-pairs signed-ranks tests to compare the density of each

species (*N. ostrina* – *N. canaliculata*, 0.75-1.5 m; *N. canaliculata* – *N. lamellosa*, 0.25-0.75 m, *N. lamellosa* – *N. ostrina*, 0.75 m). Non-parametric tests were used for these analyses because the data violated assumptions of normality and homoscedasticity and transformations did not remedy the situation.

For behavioral data I used a factorial analysis of variance (ANOVA) with treatment and species as fixed factors. The statistical unit was the mean proportion of whelks active per replicate aquarium. Proportions were arcsine square-root-transformed to meet the assumptions of normality and homoscedasticity.

Growth and morphological data were analyzed with a two-factor nested ANCOVA with treatment and species as fixed factors. Replicate aquaria were treated as a random factor nested within each treatment-by-species combination. All growth and morphological data were $\log_{10}$-transformed to better meet the assumptions of normality and homoscedasticity. Analysis of linear shell growth and body mass growth used initial shell length and initial body mass as covariates, respectively. Initial body mass had no effect on body mass growth, therefore I removed it from the initial model and ran a two-factor nested ANOVA on body mass growth data. Analysis of final shell mass used shell length and body mass as covariates. Because I was interested in the potential consequences of increased shell production on tissue mass, I used shell mass as the covariate for body mass. The scaling relationships (slopes) between some traits varied among groups negating a test for among-group differences in

Y-intercepts for some traits. However, because ANCOVA first tests for among-group differences in scaling relationships by assessing slope heterogeneity, I used this method to directly examine species-specific responses to predators by comparing the slopes of the scaling relationships between different groups (Huitema 1980, Sokal and Rohlf 1995). Non-significant slope terms were removed from the initial ANCOVA models (Hendrix et al. 1982).

Exposure to chemical cues from crabs significantly reduced whelk growth, so the final sizes of some experimental groups did not overlap, requiring that size-adjusted means be extrapolated beyond the range of available data. To validate the use of ANCOVA for size-correction on experimental whelks, I collected additional reference samples of each species from the same sites from which the experimental whelks were collected. Each sample consisted of 30 whelks, 15 were raised in the presence chemical cues from crabs and 15 were raised in the absence of chemical cues from crabs. Reference whelks were reared for a similar duration (84 days) as the experimental whelks. Size (shell length) ranges for each species in the reference samples were chosen so that final sizes (after exposure to cues associated with predation risk) would broadly overlap with those of experimental whelks (see Appendix I for information about reference samples). At the end of the exposure period, shells from the reference samples were crushed in an Instron following the protocol described above. I then conducted ANCOVA on both experimental and reference whelks to test for among-group differences in scaling relationships between shell strength and

shell length. This analysis revealed equal slopes (Appendix I) among all experimental and reference groups, indicating no change in the relationship between shell strength and shell length with increasing size among any of the groups. Consequently, I used ANCOVA to correct for the effects of size (shell length) before comparing shell strength of experimental whelks at the end of the experiment.

The magnitude of the inducible response was measured as the difference between the covariate-adjusted mean response from control treatment tanks and the covariate adjusted tank means from the predation-cue treatments (Morris et al. 2006). The resultant values were analyzed with single-factor ANOVA with species as the factor, to test for species differences. Post-hoc comparisons were made with Tukey's honestly significant difference (HSD) test. Statistica v6.1 (Statsoft) was used for all statistical analyses.

Results

Whelk distribution and density

All three species of *Nucella* were found along transects on both shores, but differed in their vertical distributions (Fig. 1). At both sites, *N. ostrina* were rare or absent below the 0.75-m level. *N. ostrina* reached maximum densities between the 1.0 and 1.5-m level at Cattle Point (Kruskal-Wallis test: $H_{(6, N= 41)} = 27.05841$,

$P = 0.0001$) and then showed a sharp decline in abundance at the 1.75-m level (Fig. 1b). There was no difference in the density of *N. ostrina* between the 0.75 and 1.75-m levels for Eagle Cove ($P > 0.05$ for all comparisons), but its density was lower at the 0.5-m level (Kruskal-Wallis test: $H_{(6, N= 41)} = 27.05841$, $P = 0.0001$; Fig. 1a). *N. canaliculata* was absent above the 1.5-m level and below the 0.25 m level on both shores (Fig. 1). Its density was similar across this range on each shore (Kruskal-Wallis test: Cattle Point $H_{(5, N= 41)} = 2.75$, $P = 0.7384$; Eagle Cove $H_{(5, N= 45)} = 5.14$, $P = 0.3994$; Fig. 1). *N. lamellosa* was found lowest on the shore at both sites (from 0.0-0.75 m at Eagle Cove and 0.0-1.0 m at Cattle Point). At Eagle Cove, *N. lamellosa* reached a peak density at the 0.0 m level ($H_{(3, N= 41)} = 11.38$, $P = 0.0098$; Fig. 1a). The density of *N. lamellosa* was similar across tidal heights where it was found at Cattle Point (Kruskal-Wallis test: $H_{(3, N= 33)} = 4.84$, $P = 0.1835$; Fig. 1b).

Nucella ostrina and *N. canaliculata* were similarly abundant across their region of overalp on the shore at Cattle Point ($P > 0.20$ for all comparisons; Fig. 1b). At Eagle Cove, *N. ostrina* was significantly more abundant than *N. canaliculata* from the 1.25-1.5-m level ($P = 0.0117$). *N. canaliculata* was more abundant than *N. lamellosa* at the 0.5-m level (Eagle Cove, $P = 0.0077$; Cattle Point, $P = 0.0277$), but similar in abundance to *N. lamellosa* at the 0.25-m level ($P > 0.10$ at both sites). *N. lamellosa* was the only species found at the 0.0-m level at both sites (Fig. 1).

Predation risk at different tidal levels

At the 0.0 m level, organisms were submerged 91.4% of the time and 53.2% of the time at the 1.5 m level (Fig. 1). Between the 0.0 and 1.0 m level, immersion time decreased gradually from 87.1% to 68.1% and then more rapidly from 53.2% to 35.1% between 1.5 m and 2.0 m (Fig. 1). Although prey are vulnerable to highly mobile crab predators at all tidal heights, their risk of predation becomes progressively less as tidal height increases above the 1.5 m tidal level (Fig.1).

Activity, growth, and morphology

For all three species, individuals raised in the presence of cues associated with risk were less active than those reared in the absence of cues associated with predation risk (Table 1, Fig. 2) and grew significantly less than those raised in the absence of cues associated with predation risk (Table 2, Fig. 3a & b). The effect of cues associated with predation risk depended on whelk species (Table 2). Linear shell growth was similar among the three species in the presence of cues associated with predation risk, but the mid- and low-shore species grew faster than high-shore species in the absence of cues associated with predation risk (Fig. 3a). Thus, species differed in linear shell growth response (ANOVA, $F_{2,9}$ = 9.0103, P = 0.007103) with cues associated with predation risk, producing the greatest reduction in shell growth for the two lower-shore species (Fig. 3c). Differences between the two lower-shore species and the high-shore species were even more pronounced for body mass growth (Fig. 3b). *N. canaliculata* and

N. lamellosa both gained similar amounts of body mass, which were greater than *N. ostrina* in the absence of cues associated with predation risk. In the presence of cues associated with predation risk, *N. lamellosa* gained less body mass than *N. ostrina*, with *N. canaliculata*, intermediate between the two. Predator-induced reduction in body mass growth differed among species (ANOVA, $F_{2,9} = 37.7498$, $P = 0.000042$); *N. canaliculata* and *N. lamellosa* exhibited the greatest reduction in body mass growth (Fig. 3d).

In general, cues associated with predation risk affected the slope of the scaling relationships between morphological traits in *Nucella* (Table 3). Shell mass as a function of shell length decreased in the presence of cues associated with predation risk, relative to controls, for all species (Table 4, Fig. 4a-c). In contrast, slopes relating shell mass and body mass diverged between treatments only in the mid-shore and low-shore species (*N. canaliculata* and *N. lamellosa*, respectively) but not in the high shore species, *N. ostrina* (Table 4, Fig. 4d-i). For the mid-shore and high-shore species, cues associated with predation risk caused an increase in the amount of shell produced per unit body mass (Fig. 4e & f) and a concomitant decrease in the amount of body mass gained per unit shell mass (Fig. 4h & i).

I also observed species-specific differences in the slopes of morphological scaling relationships (Table 5, Fig. 4a-i). The low-shore species, *N. lamellosa*, was able to add more shell mass per unit shell length than either of the mid-shore or high shore species in both the presence and absence of cues associated with

predation risk (Table 5, Fig. 4a-c). However, for slopes relating shell mass and body mass, species-specific differences were most demonstrable in the presence of cues associated with predation risk. In the presence of cues associated with predation risk, both *N. canaliculata* and *N. lamellosa* produced more shell mass per unit body mass than *N. ostrina* (Table 5; Fig. 4d-f, open circles), but *N. lamellosa* did not add as much body mass per unit shell mass as *N. ostrina* (Table 5; Fig. 4g-i, open circles). These species-specfic differences were not observed in the absence of cues associated with predation risk (Table 5; Fig. 4a-I, closed circles).

Shell defense

Overall, whelks reared in the presence of cues associated with predation risk produced significantly stronger shells than whelks reared in the absence of cues associated with predation risk (Table 6, Fig. 5a). However, the effect of cues associated with predation risk differed according to species (Table 6, Fig. 5a). I found significant differences between species in the magnitude of their inducible response (Fig. 5b; $F_{2, 9} = 53.0868$, $P = 0.000010$). Post-hoc tests revealed significant pairwise differences among all three species (Tukey's HSD, all $P < 0.05$). *N. lamellosa* showed the greatest increase in shell strength in the presence of cues associated with predation risk, *N. ostrina* showed the lowest, and *N. canaliculata* was intermediate (Fig. 5b).

Discussion

Both the mid- and low-shore species, (*N. canaliculata* and *N. lamellosa,* respectively*)* grew faster in the absence of cues associated with predation risk than the high-shore species, *N. ostrina*. In addition, these two species gained less body mass and produced more shell mass per unit body mass in the presence of cues associated with predation risk than the high-shore species. The plastic re-scaling of shell production relative to somatic growth presumably enable *N. canaliculata* and *N. lamellosa* to produce stronger, more crab-resistant shells, in the presence of crabs than their high shore congener. *N. lamellosa*, the species distributed lowest on the shore and exposed to the risk of crab predation most frequently, produced the most shell material per unit shell length, the strongest shells and exhibited the greatest increase in shell defense.

Species-specific differences in plasticity, defense and growth reflect the growth/mortality tradeoffs that are associated with their vertical distribution. In gastropods, rapid growth may be favored because body size is positively associated with many aspects of fitness (Harding et al. 2007). However, rapid growth, which concomitantly produces thinner shells, may also increase the risk of mortality from predators (Arendt 1997, Gotthard 2000). This may be particularly true in *Nucella*, where the production of thicker shells reduces vulnerability to crab predation, but also comes at the cost of reduced growth,

either through architectural constraints imposed by thicker shells (Palmer 1981) or to lost feeding opportunity (Ch. 4). If producing a thicker shell limits body growth, or if reduced growth results in a thicker shell, increasing growth rate will reduce the amount of shell produced per unit body mass, which will lead to thinner, more vulnerable shells. Differences in scaling relationships between shell mass and body mass and shell strength between treatments suggest the presence of a growth/mortality tradeoff in this system.

Because smaller whelks are more vulnerable to crabs than larger whelks, *Nucella* must also balance a tradeoff between growing slowly and increasing shell strength and growing fast to decrease the amount of time spent being small. As with somatic growth/shell thickening tradeoffs, this growth/mortality tradeoff can be mediated by phenotypically plastic changes in foraging activity (Werner and Anholt 1993, Anholt and Werner 1995). Whelks reduce feeding activity and growth when predators are present and produce a thickened, more crab-resistant shell. But, because at a given shell thickness, shell strength increases with size (Chapter 6), and larger whelks are less vulnerable to crabs, whelks should grow quickly to reach large sizes as quickly as possible before thickening their shells when crabs are absent. Predator-induced reductions in activity and growth and concomitant increases in shell strength among species supports the hypothesis that plasticity in foraging activity mediates the growth-mortality tradeoff in *Nucella*. The resolution of this tradeoff is probably more important for the mid- and low-shore species, because they reach larger sizes and are more frequently

exposed to foraging crabs than the high-shore species.

Temporal variation in predation risk low on the shore will favor rapid growth in the absence of crabs and slow growth, and thicker shells, when crabs are present. When foraging time is constrained, theoretical models predict a reduced growth response to risk (Ludwig and Rowe 1990, Rowe and Ludwig 1991, Werner and Anholt 1993, Abrams and Rowe 1996). Because the foraging activity of whelks is limited to periods of immersion, time constraints are greater for *N. ostrina* relative to *N. canaliculata* and *N. lamellosa*. Consequently, *N. canaliculata* and *N. lamellosa* are expected to display greater changes in activity and growth relative to *N. ostrina*. While I did not detect species-specific differences in the alteration of activity that match this prediction (Fig. 2), greater growth reduction and increases in shell strength in *N. canaliculata* and *N. lamellosa* relative to *N. ostrina* are consistent with these expectations (Fig. 3a-d). Species-specific differences in growth and shell plasticity may therefore play a critical role in maintaining the distribution of *Nucella* species across the vertical gradient in predation risk (Skelly 1995, Stoks and McPeek 2003, Schiesari et al. 2006).

If natural selection can shape the trade-offs between shell production and growth reduction, one would expect differences in the scaling of these traits to exist among species differentially distributed along a predation gradient. Specifically, the slope of the relationship between body mass and shell mass in the risk cue treatments should be steeper for lower-shore species (i.e., species

exposed to predation risk more often) compared to high-shore species. The slopes of the lines describing the trade-off in body mass as a function of shell mass did differ among the low shore and high shore species, but in contrast to expectations, *N. lamellosa,* the low-shore species, had a less steep slope than *N. ostrina*, the high shore species, and a similar slope to the *N. canaliculata*, the mid-shore species (Fig 4g-i). This result is inconsistent with defense theory, which predicts that natural selection will reduce the magnitude of trade-offs associated with defense production (Tollrian and Harvell 1998). However, this result is consistent with two alternative hypotheses. First, production of a thicker shell may restrict body growth, and this architectural constraint may prevent natural selection from reducing the growth tradeoff associated with shell production (Palmer 1981). Secondly, shell thickening may be a passive by-product of reduced body growth resulting from crab-induced reduction in feeding activity. Consequently, low-shore species could be adaptively reducing feeding to the degree necessary to produce an effectively thicker shell (Ch. 4).

N. ostrina showed slower growth in the absence of cues associated with predation risk and less growth and defense plasticity between risk treatments than either of its lower-shore congeners (Fig. 3a-d). However, *N. ostrina* reaches smaller adult sizes than either *N. canaliculata* and *N. lamellosa*, thus juvenile N. ostrina used in this experiment may have had less scope for growth. Nevertheless, *N. ostrina* did not change allocation to shell among risk cue treatments, as observed in the other species. Lower defense levels and reduced

plasticity may reflect an evolutionary loss of plastic shell thickening/growth reduction as this lineage invaded a less permanently immersed habitat with less frequent exposure to predation, reducing the need for rapid growth, and stronger, more crab-resistant shells.

In *Nucella*, the slopes of the scaling relationships between shell defense and body mass are phenotypically plastic and positively related to exposure to predation along a vertical tidal gradient (Fig. 4d-f). Increases in shell mass production per unit size also paralleled increases in shell strength between treatments and among species (Fig. 4a-f). *N. lamellosa*, the species distributed lowest on the shore, increased shell production relative to body mass in the presence of cues associated with predation risk and had the steepest slope for the relationship between shell mass and shell length among species. It also produced the strongest shells and exhibited the greatest predator-induced increase in shell strength (Fig. 5b). Thus, plastic changes in scaling relationships appear to amplify the differences in shell strength among species (Fig. 5a), which are known to be adaptive (Palmer 1985). These data suggest that scaling relationships themselves may be adaptive. Furthermore, plastic slope increase in the presence of crabs is also in an adaptive direction, allowing low-shore species to increase shell strength in risky habitats.

These results highlight the importance of considering scaling relationships as phenotypically plastic patterns, and not just fixed developmental trajectories (Emlen and Nijhout 2000, McCoy 2007). Examining the plasticity of scaling

relationship slopes in closely related taxa can provide insight into why relationships have a particular steepness or shape. Future studies of developmental plasticity should consider not just the size-adjusted plastic traits but also the environmentally-contingent expression of trait relationships to body size.

At present, we do not know what factors drive the vertical distribution limits of these species, nor do we know the historical context in which these traits evolved. However, growth rate and morphological development are particularly informative traits in understanding how trade-offs affect species distributions across habitat gradients, because they reflect an integration of multiple behavioral and physiological processes (Pigliucci 2003, West-Eberhard 2003a). At the very least, results indicate that genetic variation for growth and defense plasticity in *Nucella* existed in the past and there is an adaptive match between growth, morphological plasticity and exposure to predation risk. Studying the relationships between species' growth/defense strategies and their habitat distributions should provide insight into the forces structuring communities and the trade-offs species resolve in habitats along predation gradients in intertidal systems (Connell 1961, Hahn and Denny 1989, Rilov et al. 2004).

Tables

Table 1. Results of factorial ANOVA on activity of three species of Nucella (*N. canaliculata*, *N. lamellosa* and *N. ostrina*) raised in the presence and absence of cues associated with the risk of predation.

Effect	SS	Df	MS	F	P
Cue (C)	5.39222	1	5.39222	89.9115	0.000000
Species (S)	0.08221	2	0.04110	0.6854	0.516575
C*S	0.17488	2	0.08744	1.4580	0.258914
Error	1.07951	18	0.05997		

Table 2. Analysis of growth (ANCOVA and ANOVA) of three species of *Nucella* (*N. canaliculata*, *N. lamellosa* and *N. ostrina*) raised in the presence or absence of cues associated with the risk of predation.

Effect	SS	Df	MS	F	P
Log shell length increase (*Y*) vs. initial shell length (*X*)					
Cue (C)	35.33369	1	35.33369	785.7696	0.000000
Species (S)	0.17182	2	0.08591	2.2977	0.122316
C*S	0.70925	2	0.35462	7.8956	0.003290
Aquarium(C*S)	0.83235	18	0.04624	2.7911	0.000262
Covariate	0.39918	1	0.39918	24.0945	0.000002
Error	3.11468	188	0.01657		
Log body mass gain					
Cue (C)	2.154131	1	2.154131	416.5811	0.000000
Species (S)	0.063118	2	0.031559	6.0697	0.009379
C*S	0.158805	2	0.079403	15.2715	0.000120
Aquarium(C*S)	0.095486	18	0.005305	2.3673	0.002079
Error	0.423512	189	0.002241		

Table 3. Results of nested analyses of covariance (ANCOVA) on morphological traits for three species of *Nucella* (*N. ostrina*, *N. canaliculata*, *N. lamellosa*) raised in the presence or absence of cues associated with the risk of predation.

Effect	SS	Df	MS	F	P
Log body mass (*Y*) vs. Log shell mass (*X*)					
Cue (C)	0.000627	1	0.000627	1.3743	0.242372
Species (S)	0.121473	2	0.060737	76.220	0.000000
Aquarium(C*S)	0.018559	18	0.001031	2.4608	0.001240
C*S	0.003437	2	0.001719	2.1710	0.132592
Covariate	0.290644	1	0.290644	693.6803	0.000000
C*Covariate	0.028859	1	0.028859	68.8778	0.000000
Error	0.086312	206	0.000419		
Log shell mass (*Y*) vs. Log shell length (*X*)					
Cue (C)	0.057907	1	0.057907	83.048	0.000000
Species (S)	0.069595	2	0.034797	49.903	0.000000
C*S	0.070684	2	0.035342	45.946	0.000000
Aquarium(C*S)	0.021158	18	0.001175	1.687	0.043748
Covariate	1.0717671	1	1.071671	1538.042	0.000000
C*Covariate	0.049093	1	0.049093	70.458	0.000000
S*Covariate	0.076565	2	0.038283	54.942	0.000000

Error	0.142142	204	0.000697		
Log shell mass (*Y*) vs. Log body mass (*X*)					
Cue (C)	0.010228	1	0.010228	6.1422	0.019879
Species (S)	0.031024	2	0.015512	9.2525	0.000935
C*S	0.003303	2	0.001652	0.9851	0.387007
Tank(C*S)	0.033491	18	0.001861	1.6607	0.049894
Covariate	0.432011	1	0.432011	285.1640	0.000000
Aquarium*Covariate	0.045199	18	0.002511	2.2413	0.003847
C*S*Covariate	0.013808	2	0.006904	4.2154	0.020065
Cue*Covariate	0.031678	1	0.031678	20.9100	0.000019
S*Covariate	0.014256	2	0.007128	4.3524	0.017835
Error	0.206148	184	0.001120		

Table 4. Differences in the allometeric growth rates of three species of *Nucella* (*N. ostrina*, *N. canaliculata*, *N. lamellosa*) raised in the presence or absence of cues associated with the risk of predation. Pairwise comparisons were performed between the different cue treatments for each species. Probabilities in bold are significant after sequential Bonferroni corrections.

	Log body mass vs. log shell mass		Log shell mass vs. log shell length		Log shell mass vs. log body mass	
	F	*P*	*F*	*P*	*F*	*P*
	(Df = 1,6)		(Df = 1,6)		(Df = 1,6)	
Species						
N. canaliculata	11.47	**0.001175**	31.88	**<0.000001**	27.39	**0.000002**
N. lamellosa	44.17	**<0.000001**	17.90	**0.000064**	65.81	**<0.000001**
N. ostrina	4.93	0.030453	22.70	**0.000013**	2.97	0.090152

Table 5. Analyses of allometric scaling relationships among three species of *Nucella* (*N. ostrina*, *N. canaliculata*, *N. lamellosa*). Pairwise comparisons between species in the presence and absence of cues associated with predation risk. Probabilities in bold are significant after sequential Bonferroni corrections. Degrees of freedom = 1, 6.

	Log body mass vs. log shell mass		Log shell mass vs. log shell length		Log shell mass vs. log body mass	
Comparison	*F*	*P*	*F*	*P*	*F*	*P*
Cues associated with predation risk present						
N. canaliculata – N. lamellosa	0.92	0.341718	49.85	**<0.000001**	2.86	0.095344
N. canaliculata – N. ostrina	1.00	0.320918	1.00	**0.002613**	11.72	**0.001216**
N. lamellosa – N. ostrina	8.11	**0.006059**	51.23	**<0.000001**	19.47	**0.000044**
Cues associated with predation risk absent						
N. canaliculata – N. lamellosa	0.41	0.522370	8.63	**0.004396**	0.30	0.584172
N. canaliculata – N. ostrina	1.91	0.206085	57.42	**<0.000001**	2.82	0.097160

N. lamellosa – N. ostrina	5.09	0.026998	12.41	**0.000742**	4.92	0.029609

Table 6. Results of nested analyses of covariance on shell strength for three species of *Nucella* (*N. ostrina*, *N. canaliculata*, *N. lamellosa*) raised in the presence or absence of cues associated with predation risk.

Effect	SS	Df	MS	*F*	*P*
Cue (C)	0.693044	1	0.693044	59.31032	0.000000
Species (S)	0.906244	2	0.453122	34.36780	0.000000
C*S	0.424494	2	0.212247	15.19517	0.000112
Aquarium(C*S)	0.253372	18	0.014076	1.26309	0.230424
Shell length	0.506026	1	0.506026	45.40666	0.000000
Error	1.047565	94	0.011144		

Figures

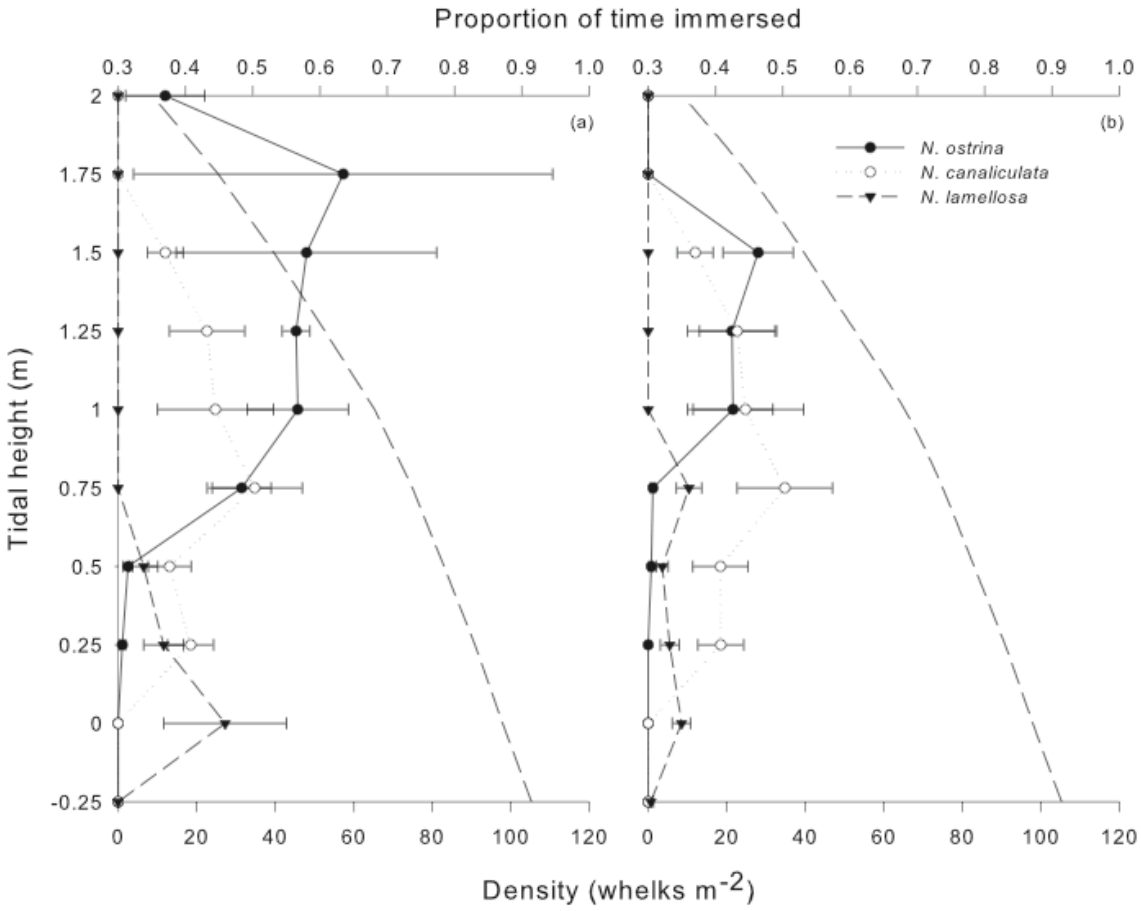


Figure 1. Intertidal distributions and mean densities of three species of *Nucella* (*N. ostrina, N. canaliculata, N. lamellosa*) in relation to tidal immersion (dashed curve) on two moderately wave-exposed, San Juan Island, Washington shores; (a) Eagle Cove and (b) Cattle Point. The number of quadrats per tidal level was between three and ten. Tidal height is taken from U.S. tables in which 0 m is defined as mean lower low water.

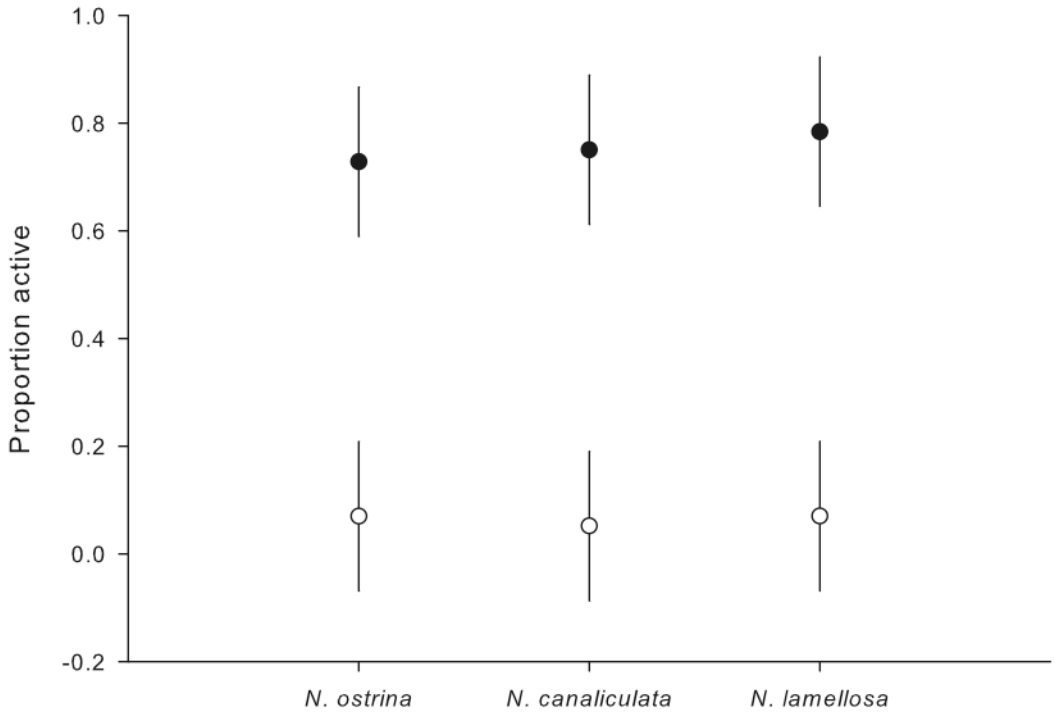


Figure 2. Activity (mean ± SE) of three species of *Nucella* (*N. ostrina, N. canaliculata, N. lamellosa*) in the presence (solid symbols) and absence (open symbols) of cues associated with predation risk.

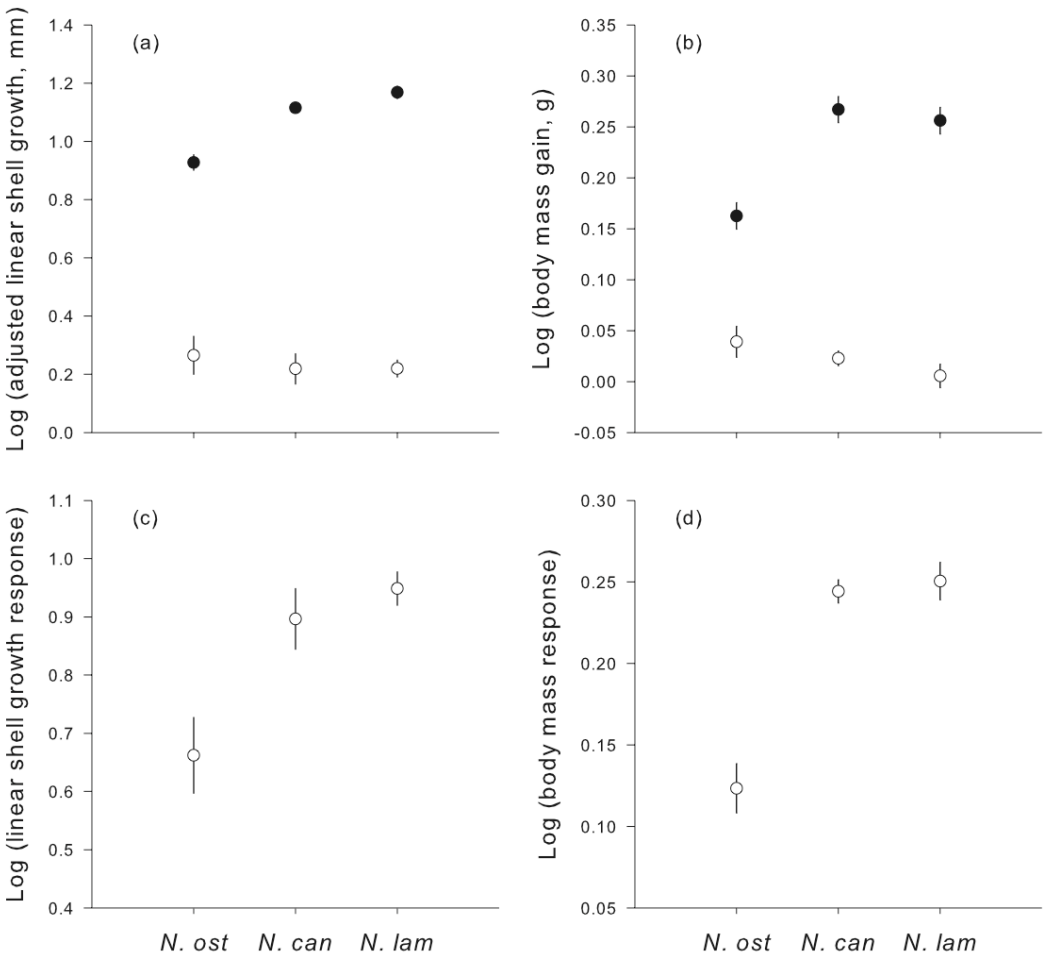


Figure 3. (a) Adjusted mean log linear shell growth (± SE) at a covariate mean log initial shell length of 1.226 (originally measured in mm), and (b) mean log body mass growth for whelks of three species of *Nucella* (*N. ostrina*, *N. canaliculata* and *N. lamellosa*) in the presence (open symbols) and absence (solid symbols) of cues associated with predation risk; and (c) mean induced response for shell growth and (d) mean induced response for body mass growth: post hoc tests for both growth responses, *N. ostrina* < *N. canaliculata* = *N. lamellosa.*

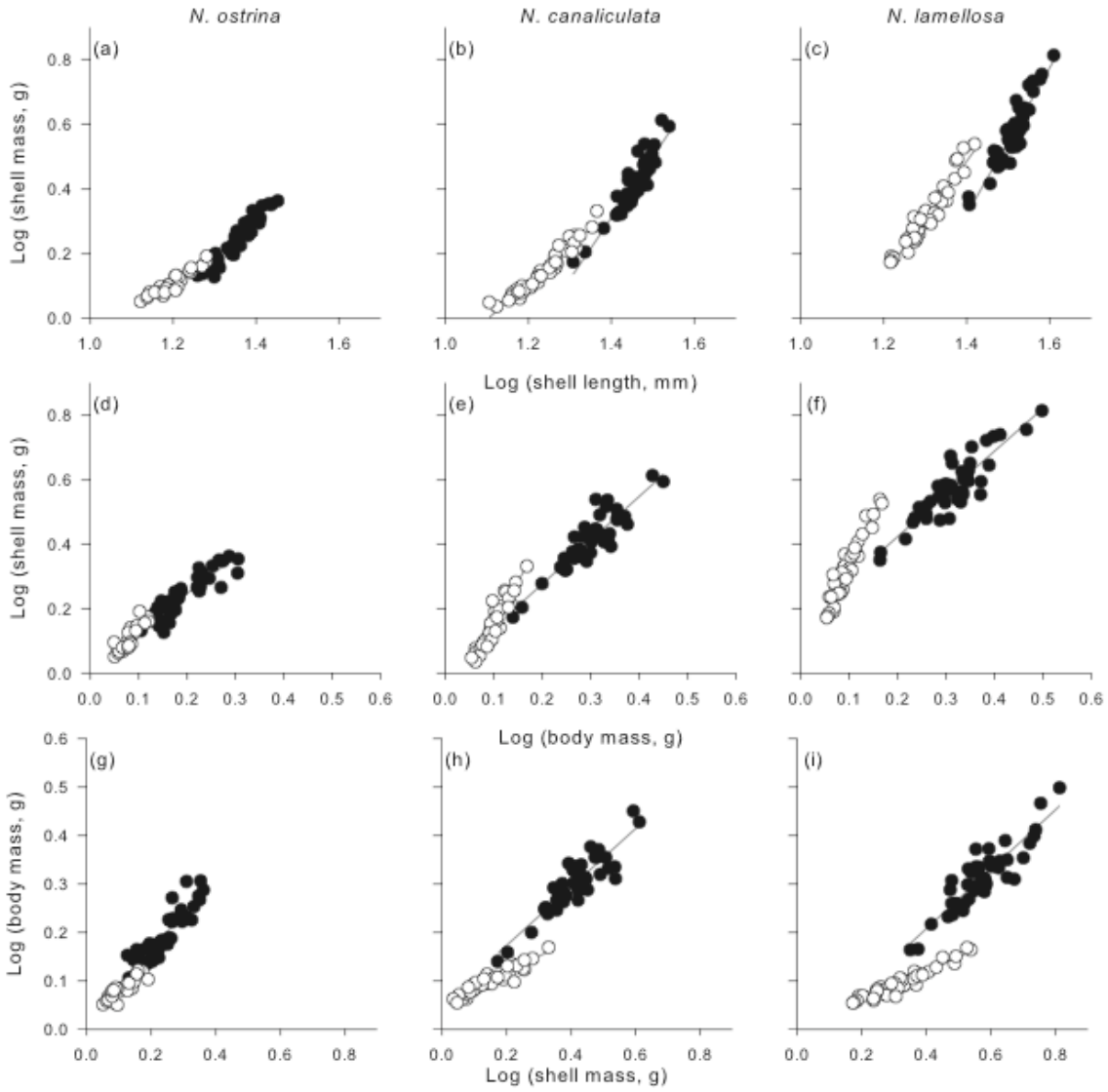


Figure 4. Scaling relationships of (a-c) log shell mass and log shell length, (d-f) log shell mass and log body mass, and (g-i) log body mass and log shell mass in three species of *Nucella* (*N. ostrina, N. canalicualta*, *N. lamellosa*) in the presence (open symbols) and absence (solid symbols) of cues associated with predation risk.

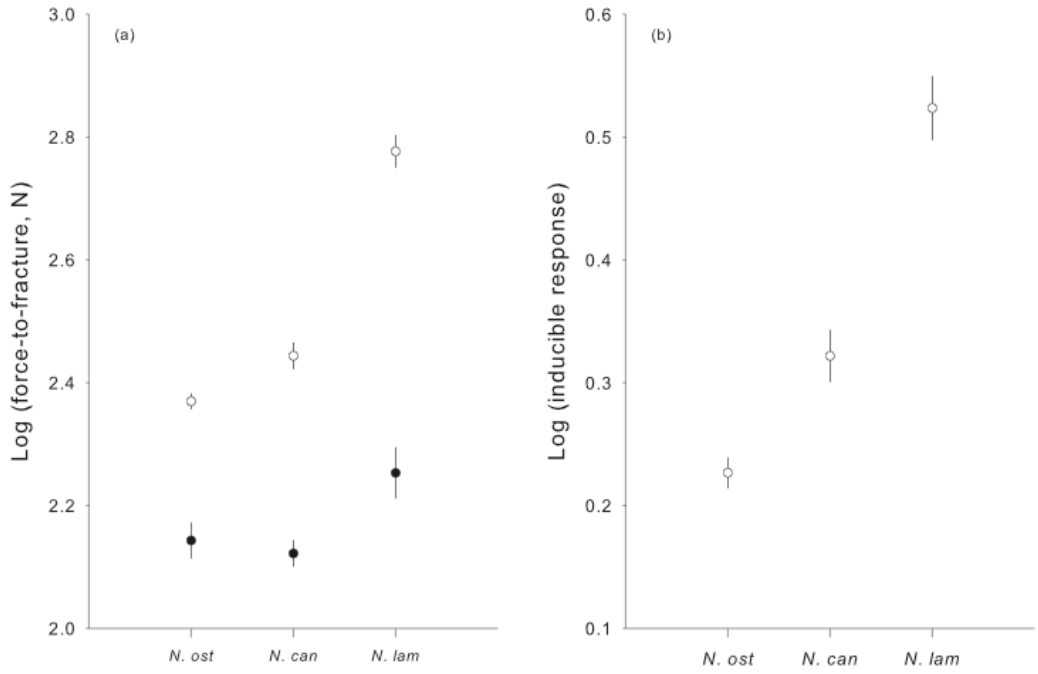


Figure 5. Interspecific comparisons of (mean ± SE) among three species of *Nucella* (*N. ostrina, N. canaliculata*, *N. lamellosa*) raised in the presence (open symbols) or absence (solid symbols) of cues associated with predation risk. (a) Size adjusted mean log force-to-fracture at a covariate mean log shell length of 1.36 (originally measured in mm); and (b) induced shell strength in the presence of crab cues; post hoc tests, *N. ostrina < N. canaliculata < N. lamellosa.*

References

Abrams, P. A. and L. Rowe. 1996. The effects of predation on the age and size of maturity of prey. Evolution **50**:1052-1061.

Anholt, B. R. and E. E. Werner. 1995. Interaction between food availability and predation mortality mediated by adaptive behavior. Ecology **76**:2230-2234.

Arendt, J. D. 1997. Adaptive intrinsic growth rates: An integration across taxa. Quarterly Review of Biology **72**:149-177.

Bertness, M. D. 1977. Behavioral and ecological aspects of shore-level size gradients in *Thais lamellosa* and *Thais emarginata*. Ecology **58**:86-97.

Bourdeau, P. E. 2009. Prioritized phenotypic responses to combined predators in a marine snail. Ecology **in press**.

Collins, T. M., K. Frazer, A. R. Palmer, G. J. Vermeij, and W. M. Brown. 1996. Evolutionary history of northern hemisphere *Nucella* (Gastropoda, Muricidae): Molecular, morphological, ecological, and paleontological evidence. Evolution **50**:2287-2304.

Connell, J. H. 1961. Influence of interspecific competition and other factors on distribution of barnacle *Chthamalus stellatus*. Ecology **42**:710-&.

Dalziel, B. and E. G. Boulding. 2005. Water-borne cues from a shell-crushing predator induce a more massive shell in experimental populations of an intertidal snail. Journal of Experimental Marine Biology and Ecology **317**:25-35.

De Block, M., M. A. McPeek, and R. Stoks. 2007. Winter compensatory growth under field conditions partly offsets low energy reserves before winter in a damselfly. Oikos **116**:1975-1982.

Emlen, D. J. and H. F. Nijhout. 2000. The development and evolution of exaggerated morphologies in insects. Annual Review of Entomology **45**:661-708.

Gotthard, K. 2000. Increased risk of predation as a cost of high growth rate: an experimental test in a butterfly. Journal of Animal Ecology **69**:896-902.

Hahn, T. and M. Denny. 1989. Tenacity-mediated selective predation by oystercatchers on intertidal limpets and its role in maintaining habitat partitioning by *Collisella scabra* and *Lottia digitalis*. Marine Ecology-Progress Series **53**:1-10.

Harding, J. M., R. Mann, and C. W. Kilduff. 2007. The effects of female size on fecundity in a large marine gastropod *Rapana venosa* (Muricidae). Journal of Shellfish Research **26**:33-42.

Hendrix, L. J., M. W. Carter, and D. T. Scott. 1982. Covariance analyses with heterogeneity of slopes in fixed models. Biometrics **38**:641-650.

Huitema, B. E. 1980. The Analysis of Covariance and Alternatives. John Wiley and Sons, Inc., New York.

Ludwig, D. and L. Rowe. 1990. Life-history strategies for energy gain and predator avoiance under time constraints. American Naturalist **135**:686-707.

McCoy, M. W. 2007. Conspecific density determines the magnitude and character of predator-induced phenotype. Oecologia **153**:871-878.

McPeek, M. A. 2004. The growth/predation risk trade-off: So what is the mechanism? American Naturalist **163**:E88-E111.

Menge, B. A. and J. P. Sutherland. 1976. Species diversity gradients: synthesis of roles of predation, competition, and temporal heterogeneity. American Naturalist **110**:351-369.

Menge, B. A. and J. P. Sutherland. 1987. Community regulation: variation in disturbance, competition, and predation in relation to environmental stress and recruitment. American Naturalist **130**:730-757.

Palmer, A. R. 1980. A comparative and experimental study of feeding and growth in thaidid. PhD Dissertation. University of Washington, Seattle, WA, USA.

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

Palmer, A. R. 1982. Growth in marine gastropods - a non-destructive technique for independently measuring shell and body-weight. Malacologia **23**:63-73.

Palmer, A. R. 1985. Adaptive value of shell variation in *Thais lamellosa* - effect of thick shells on vulnerability to and preference by crabs. Veliger **27**:349-356.

Palmer, A. R. 1990. Effect of crab effluent and scent of damaged conspecifics on feeding, growth, and shell morphology of the Atlantic dogwhelk *Nucella lapillus* (L). Hydrobiologia **193**:155-182.

Pigliucci, M. 2003. Phenotypic integration: studying the ecology and evolution of complex phenotypes. Ecology Letters **6**:265-272.

Rilov, G., Y. Benayahu, and A. Gasith. 2004. Life on the edge: do biomechanical and behavioral adaptations to wave-exposure correlate with habitat partitioning in predatory whelks? Marine Ecology-Progress Series **282**:193-204.

Robles, C., D. A. Sweetnam, and D. Dittman. 1989. Diel variation of intertidal foraging by *Cancer productus* L in British Columbia. Journal of Natural History **23**:1041-1049.

Rowe, L. and D. Ludwig. 1991. Size and timing of metamorphosis in complex life-cycles: time constraints and variation. Ecology **72**:413-427.

Schiesari, L., S. D. Peacor, and E. E. Werner. 2006. The growth-mortality tradeoff: evidence from anuran larvae and consequences for species distributions. Oecologia **149**:194-202.

Skelly, D. K. 1995. A behavioral trade-off and its consequences for the distribution of *Pseudacris* treefrog larvae. Ecology **76**:150-164.

Sokal, R. R. and F. J. Rohlf. 1995. Biometry: the principles and practice of statistics in biological research. 3rd edition. W.H. Freeman and Co., New York.

Sorte, C. J. B. and G. E. Hofmann. 2005. Thermotolerance and heat-shock protein expression in Northeastern Pacific *Nucella* species with different biogeographical ranges. Marine Biology **146**:985-993.

Stoks, R. and M. A. McPeek. 2003. Antipredator behavior and physiology determine Lestes species turnover along the pond-permanence gradient. Ecology **84**:3327-3338.

Tollrian, R. and C. D. Harvell, editors. 1998. The Ecology and Evolution of Inducible Defenses. Princeton University Press, Princeton, NJ.

Trussell, G. C. 2000. Predator-induced plasticity and morphological trade-offs in latitudinally separated populations of *Littorina obtusata*. Evolutionary Ecology Research **2**:803-822.

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

Vermeij, G. J. 1987. Evolution and Escalation: An Ecological History of Life. Princeton Univesrity Press, Princeton, NJ.

Vermeij, G. J. and J. D. Currey. 1980. Geographical variation in the strength of Thaidid snail shells. Biological Bulletin **158**:383-389.

Werner, E. E. and B. R. Anholt. 1993. Ecological consequences of the trade-off between growth and mortality rates mediated by foraging activity. American Naturalist **142**:242-272.

West-Eberhard, M. J. 2003. Developmental Plasticity and Evolution. Oxford University Press, New York.

Chapter 8 - Conclusions

In evolutionary ecology, phenotypic plasticity has become a central theme in studies that address how organisms relate to their environment and how populations evolve (Schlichting and Pigliucci 1998, DeWitt and Scheiner 2003, West-Eberhard 2003a). In this dissertation, I used snails in the genus *Nucella* to examine several outstanding questions about phentypic plasticity, in particular, inducible defenses. I used a multiple trait and multiple inducing agent approach to examine the specificity of cues that trigger phenotypic responses, the proximate mechanisms that produce phenotypes and intra- and interspecific variation in predator-induced behavior and morphology.

I found that cues from injured con- and hererospecific snails do not trigger a inducible defenses in *N. lamellosa.* However, in combination with cues from the major predatory crab, *Cancer productus*, cues from injured conspecifics act synergistically to signal predation risk and trigger inducible defenses in this species. Surprisingly, I found that a wide range of crabs, including non-predatory species, trigger morphological defenses in *N. lamellosa*. Although mis-identifying potential predators may result in higher than needed predator avoidance costs, if other species of crabs are indicators of a high predation risk environment, such a generalized response may be advantageous.

Despite the inability to distinguish among crabs, *N. lamellosa* could discriminate among predators with different attack modes (crabs and seastars) and adjust its morphological defenses accordingly. Snails produced both

predator-specific morphological responses (crabs – thickened and rotund shells, stars – elongate shells with tall spires) and generalized behavioral responses to these contrasting predators. Predator specific responses to crabs and seastars reflected a survival tradeoff; phenotypes that reduced susceptibility to one predator, increased susceptibility to the other. In the presence of both predators snails reconcile this tradeoff by responding to the more lethal of the two predators (crabs), but maintained general responses that would be effective against either predator. Combining general and specific defenses may be a way to reduce the overall risk to predation in multiple predator environments.

The results of this dissertation also suggest that crab-induced shell thickening is a passive by-product of reduced feeding and growth rather than an active physiological response to predation risk. Predation cues cause snails to reduce feeding activity, which in turn causes a reduction in the rate of body growth and linear shell translation. Calcification rate remains unchanged, however, and reduced shell elongation causes calcium carbonate to be deposited in the shell parallel to the axis of linear translation. This suggests that the response is neither energetically or developmentally costly, but that costs associated with the deployment of this defense are due to lost feeding opportunity. This response has important evolutionary implications because morphological development is tied to behavior, which could affect its evolutionary trajectory.

Both intra- and inter-specific differences in response to predatory crabs were found. In both cases, this variation reflected differences in environmental risk of predation by crabs. *N. lamellosa* from shores with abundant crabs exhibited greater inducible defenses than did those from shores with few crabs. Among three eastern North Pacific congeners of *Nucella*, the species with the greatest potential to experience crab predation exhibited the greatest responses.

These findings highlight the importance of considering multiple traits, multiple potential inducing cues, as well as the spatial and temporal variability in risk when considering inducible defenses. This work also raised a number of important questions. Most notably, what is the chemical nature of cues released by predators and injured snails (Chivers and Smith 1998)? Is predation regime responsible for patterns of morphological variation in prey in the field (Trussell and Smith 2000)? How do the plastic responses of prey in complex cue environments with multiple predators influence community structure and population dynamics in nature (Werner and Peacor 2003, Verschoor et al. 2004)? And, what are the ultimate causes of population-level differences in inducible responses(Grosberg and Cunningham 2001), and what is the role of history of exposure and maternal environment (Agrawal et al. 1999)?

References

Agrawal, A. A., C. Laforsch, and R. Tollrian. 1999. Transgenerational induction of defences in animals and plants. Nature **401**:60-63.

Chivers, D. P. and R. J. F. Smith. 1998. Chemical alarm signalling in aquatic predator-prey systems: A review and prospectus. Ecoscience **5**:338-352.

DeWitt, T. J. and S. M. Scheiner, editors. 2003. Phenotypic Plasticity: Functional and Conceptual Approaches. Oxford University Press, New York, NY.

Grosberg, R. K. and C. W. Cunningham. 2001. Genetic structure in the sea: from populations to communities. Pages 61-84 *in* M. D. Bertness, S. D. Gaines, and M. E. Hay, editors. Marine Community Ecology. Sinauer, Sunderland, MA.

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

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

Verschoor, A. M., M. Vos, and I. van der Stap. 2004. Inducible defences prevent strong population fluctuations in bi- and tritrophic food chains. Ecology Letters **7**:1143-1148.

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

West-Eberhard, M. J. 2003. Developmental Plasticity and Evolution. Oxford University Press, New York.

Bibliography

References for Chapter 1

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

Bronmark, C. and J. G. Miner. 1992. Predator-induced phenotypical change in body morphology in crucian carp. Science **258**:1348-1350.

Collins, T. M., K. Frazer, A. R. Palmer, G. J. Vermeij, and W. M. Brown. 1996. Evolutionary history of northern hemisphere *Nucella* (Gastropoda, Muricidae): Molecular, morphological, ecological, and paleontological evidence. Evolution **50**:2287-2304.

Crothers, J. H. 1984. Some observations on shell shape variation in Pacific Nucella. Biological Journal of the Linnean Society **21**:259-281.

Fordyce, J. A. 2006. The evolutionary consequences of ecological interactions mediated through phenotypic plasticity. Journal of Experimental Biology **209**:2377-2383.

Harvell, C. D. 1992. Inducible defenses and allocation shifts in a marine bryozoan. Ecology **73**:1567-1576.

Karban, R. and J. H. Myers. 1989. Induced plant-responses to herbivory. Annual Review of Ecology and Systematics **20**:331-348.

Kincaid, T. 1957. Local races and clines in the marine gastropod *Thais lamellosa* Gemlin. The Calliostoma Co., Seattle, WA, USA.

Kitching, J. A. 1976. Distribution and changes in shell form of *Thais* spp (Gastropoda) near Bamfield, BC. Journal of Experimental Marine Biology and Ecology **23**:109-126.

Leonard, G. H., M. D. Bertness, and P. O. Yund. 1999. Crab predation, waterborne cues, and inducible defenses in the blue mussel, *Mytilus edulis*. Ecology **80**:1-14.

Lima, S. L. and L. M. Dill. 1990. Behavioral decisions made under the risk of predation - a review and prospectus. Canadian Journal of Zoology-Revue Canadienne De Zoologie **68**:619-640.

Lively, C. M. 1986. Canalization versus developmental conversion in a spatially-variable environment. American Naturalist **128**:561-572.

Miner, B. G., S. E. Sultan, S. G. Morgan, D. K. Padilla, and R. A. Relyea. 2005. Ecological consequences of phenotypic plasticity. Trends in Ecology & Evolution **20**:685-692.

Moran, N. A. 1992. The evolutionary maintenance of alternative phenotypes. American Naturalist **139**:971-989.

Padilla, D. K. and S. C. Adolph. 1996. Plastic inducible morphologies are not always adaptive: The importance of time delays in a stochastic environment. Evolutionary Ecology **10**:105-117.

Palmer, A. R. 1985. Adaptive value of shell variation in *Thais lamellosa* - effect of thick shells on vulnerability to and preference by crabs. Veliger **27**:349-356.

Palmer, A. R. 1990. Effect of crab effluent and scent of damaged conspecifics on feeding, growth, and shell morphology of the Atlantic dogwhelk *Nucella lapillus* (L). Hydrobiologia **193**:155-182.

Palmer, A. R. 1992. Calcification in marine mollusks - how costly is it. Proceedings of the National Academy of Sciences of the United States of America **89**:1379-1382.

Pigliucci, M. 2005. Evolution of phenotypic plasticity: where are we going now? Trends in Ecology & Evolution **20**:481-486.

Relyea, R. A. 2001. Morphological and behavioral plasticity of larval anurans in response to different predators. Ecology **82**:523-540.

Sih, A. 1987. Predators and prey lifestyles: an evolutionary and ecological overview. Pages 203-224 *in* W. C. Kerfoot and A. Sih, editors. Predation: Direct and Indirect Impacts on Aquatic Communities. University Press of New England, London.

Smith, D. C. and J. Vanbuskirk. 1995. Phenotypic design, plasticity, and ecological performance in 2 tadpole species. American Naturalist **145**:211-233.

Spitze, K. 1992. Predator-mediated plasticity of prey life-history and morphology - Chaoborus americanus predation on *Daphnia pulex*. American Naturalist **139**:229-247.

Tollrian, R. 1993. Neckteeh formation in *Daphnia pulex* as an example of continuous phenotypic plasticity - morphological effects of *Chaoborus* kairomone concentration and their quantification. Journal of Plankton Research **15**:1309-1318.

Tollrian, R. and C. D. Harvell, editors. 1998. The Ecology and Evolution of Inducible Defenses. Princeton University Press, Princeton, NJ.

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

Tufto, J. 2000. The evolution of plasticity and nonplastic spatial and temporal adaptations in the presence of imperfect environmental cues. American Naturalist **156**:121-130.

West-Eberhard, M. J. 2003. Developmental Plasticity and Evolution. Oxford University Press, New York.

References for Chapter 2

Appleton RD, Palmer AR (1988) Water-borne stimuli released by predatory crabs and damaged prey induce more predator-resistant shells in a marine gastropod. Proceedings of the National Academy of Sciences of the United States of America 85:4387-4391

Avery R, Etter RJ (2006) Microstructural differences in the reinforcement of a gastropod shell against predation. Marine Ecology-Progress Series 323:159-170

Bamber SD, Naylor E (1997) Sites of release of putative sex pheromone and sexual behaviour in female *Carcinus maenas* (Crustacea: Decapoda). Estuarine Coastal and Shelf Science 44:195-202

Benard MF (2006) Survival trade-offs between two predator-induced phenotypes in Pacific treefrogs (*Pseudacris regilla*). Ecology 87:340-346

Bourdeau PE (2009) Prioritized phenotypic responses to combined predators in a marine snail. Ecology

Bronmark C, Pettersson LB (1994) Chemical cues from piscivores induce a change in morphology in crucian carp. Oikos 70:396-402

Brown GE, Dreier VM (2002) Predator inspection behaviour and attack cone avoidance in a characin fish: the effects of predator diet and prey experience. Animal Behaviour 63:1175-1181

Chivers DP, Smith RJF (1998) Chemical alarm signalling in aquatic predator-prey systems: A review and prospectus. Ecoscience 5:338-352

Chivers DP, Wisenden BD, Smith RJF (1996) Damselfly larvae learn to recognize predators from chemical cues in the predator's diet. Animal Behaviour 52:315-320

Colgan DJ, Ponder WF, Beacham E, Macaranas J (2007) Molecular phylogenetics of Caenogastropoda (Gastropoda : Mollusca). Molecular Phylogenetics and Evolution 42:717-737

Dalesman S, Rundle SD, Bilton DT, Cotton PA (2007) Phylogenetic relatedness and ecological interactions determine antipredator behavior. Ecology 88:2462-2467

Freeman AS (2007) Specificity of induced defenses in *Mytilus edulis* and asymmetrical predator deterrence. Marine Ecology-Progress Series 334:145-153

Griffiths CL, Richardson CA (2006) Chemically induced predator avoidance behaviour in the burrowing bivalve *Macoma balthica*. Journal of Experimental Marine Biology and Ecology 331:91-98

Hagen NT, Andersen A, Stabell OB (2002) Alarm responses of the green sea urchin, *Strongylocentrotus droebachiensis*, induced by chemically labelled durophagous predators and simulated acts of predation. Marine Biology 140:365-374

Hardege JD et al. (2002) Novel behavioural assay and partial purification of a female-derived sex pheromone in *Carcinus maenas*. Marine Ecology-Progress Series 244:179-189

Jacobsen HP, Stabell OB (1999) Predator-induced alarm responses in the common periwinkle, *Littorina littorea*: dependence on season, light

conditions, and chemical labelling of predators. Marine Biology 134:551-557

Jacobsen HP, Stabell OB (2004) Antipredator behaviour mediated by chemical cues: the role of conspecific alarm signalling and predator labelling in the avoidance response of a marine gastropod. Oikos 104:43-50

Kats LB, Dill LM (1998) The scent of death: Chemosensory assessment of predation risk by prey animals. Ecoscience 5:361-394

Kristensen EA, Closs GP (2004) Anti-predator response of naive and experienced common bully to chemical alarm cues. Journal of Fish Biology 64:643-652

Laforsch C, Beccara L, Tollrian R (2006) Inducible defenses: The relevance of chemical alarm cues in *Daphnia*. Limnology and Oceanography 51:1466-1472

Lima SL, Dill LM (1990) Behavioral decisions made under the risk of predation - a review and prospectus. Canadian Journal of Zoology-Revue Canadienne De Zoologie 68:619-640

Lively CM (1986) Canalization versus developmental conversion in a spatially-variable environment. American Naturalist 128:561-572

McCollum SA, VanBuskirk J (1996) Costs and benefits of a predator-induced polyphenism in the gray treefrog *Hyla chrysoscelis*. Evolution 50:583-593

Miner BG, Sultan SE, Morgan SG, Padilla DK, Relyea RA (2005) Ecological consequences of phenotypic plasticity. Trends in Ecology & Evolution 20:685-692

Moran NA (1992) The evolutionary maintenance of alternative phenotypes. American Naturalist 139:971-989

Palmer AR (1982) Growth in marine gastropods - a non-destructive technique for independently measuring shell and body-weight. Malacologia 23:63-73

Relyea RA (2001) Morphological and behavioral plasticity of larval anurans in response to different predators. Ecology 82:523-540

Rittschof D, Cohen JH (2004) Crustacean peptide and peptide-like pheromones and kairomones. Peptides 25:1503-1516

Schoeppner NM, Relyea RA (2005) Damage, digestion, and defence: the roles of alarm cues and kairomones for inducing prey defences. Ecology Letters 8:505-512

Slusarczyk M (1999) Predator-induced diapause in *Daphnia magna* may require two chemical cues. Oecologia 119:159-165

Smee DL, Weissburg MJ (2006) Hard clams (*Mercenaria mercenaria*) evaluate predation risk using chemical signals from predators and injured conspecifics. Journal of Chemical Ecology 32:605-619

Smith ME, Belk MC (2001) Risk assessment in western mosquitofish (*Gambusia affinis*): do multiple cues have additive effects? Behavioral Ecology and Sociobiology 51:101-107

Stabell OB, Lwin MS (1997) Predator-induced phenotypic changes in crucian carp are caused by chemical signals from conspecifics. Environmental Biology of Fishes 49:145-149

Stabell OB, Ogbebo F, Primicerio R (2003) Inducible defences in *Daphnia* depend on latent alarm signals from conspecific prey activated in predators. Chemical Senses 28:141-153

Taylor HH, Taylor EW (1991) The dorsoventral muscles of *Carcinus maenas* - evidence for hydrostatic pressure control in a crab. Physiological Zoology 64:1110-1129

Teplitsky C, Plenet S, Joly P (2004) Hierarchical responses of tadpoles to multiple predators. Ecology 85:2888-2894

Tollrian R, Harvell CD (eds) (1998) The Ecology and Evolution of Inducible Defenses. Princeton University Press, Princeton, NJ

Trussell GC, Ewanchuk PJ, Bertness MD (2002) Field evidence of trait-mediated indirect interactions in a rocky intertidal food web. Ecology Letters 5:241-245

Trussell GC, Nicklin MO (2002) Cue sensitivity, inducible defense, and trade-offs in a marine snail. Ecology 83:1635-1647

Turner AM, Bernot RJ, Boes CM (2000) Chemical cues modify species interactions: the ecological consequences of predator avoidance by freshwater snails. Oikos 88:148-158

Van Buskirk J (2001) Specific induced responses to different predator species in anuran larvae. Journal of Evolutionary Biology 14:482-489

Vermeij GJ (1987) Evolution and Escalation: An Ecological History of Life. Princeton Univesrity Press, Princeton, NJ

Verschoor AM, Vos M, van der Stap I (2004) Inducible defences prevent strong population fluctuations in bi- and tritrophic food chains. Ecology Letters 7:1143-1148

Yamada SB, Boulding EG (1996) The role of highly mobile crab predators in the intertidal zonation of their gastropod prey. Journal of Experimental Marine Biology and Ecology 204:59-83

References for Chapter 3

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

Arendt, J. D. 1997. Adaptive intrinsic growth rates: An integration across taxa. Quarterly Review of Biology **72**:149-177.

Boulding, E. G., M. Holst, and V. Pilon. 1999. Changes in selection on gastropod shell size and thickness with wave-exposure on Northeastern Pacific shores. Journal of Experimental Marine Biology and Ecology **232**:217-239.

Bourdeau, P. E. 2009. Prioritized phenotypic responses to combined predators in a marine snail. Ecology **in press**.

Bronmark, C. and L. A. Hansson. 2000. Chemical communication in aquatic systems: an introduction. Oikos **88**:103-109.

Caro, A. U. and J. C. Castilla. 2004. Predator-inducible defences and local intrapopulation variability of the intertidal mussel *Semimytilus algosus* in central Chile. Marine Ecology-Progress Series **276**:115-123.

Collins, T. M., K. Frazer, A. R. Palmer, G. J. Vermeij, and W. M. Brown. 1996. Evolutionary history of northern hemisphere *Nucella* (Gastropoda, Muricidae): Molecular, morphological, ecological, and paleontological evidence. Evolution **50**:2287-2304.

Currey, J. D. and J. D. Taylor. 1974. Mechanical-behavior of some molluscan hard tissues. Journal of Zoology **173**:395-406.

Dalziel, B. and E. G. Boulding. 2005. Water-borne cues from a shell-crushing predator induce a more massive shell in experimental populations of an intertidal snail. Journal of Experimental Marine Biology and Ecology **317**:25-35.

Dodson, S. I., T. A. Crowl, B. L. Peckarsky, L. B. Kats, A. P. Covich, and J. M. Culp. 1994. Nonvisual communication in freshwater benthos - an overview. Journal of the North American Benthological Society **13**:268-282.

Edgell, T. C. and C. J. Neufeld. 2008. Experimental evidence for latent developmental plasticity: intertidal whelks respond to a native but not an introduced predator. Biology Letters **4**:385-387.

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

Gabriel, W., B. Luttbeg, A. Sih, and R. Tollrian. 2005. Environmental tolerance, heterogeneity, and the evolution of reversible plastic responses. American Naturalist **166**:339-353.

Getty, T. 1996. The maintenance of phenotypic plasticity as a signal detection problem. American Naturalist **148**:378-385.

Gosselin, L. A. 1997. An ecological transition during juvenile life in a marine snail. Marine Ecology-Progress Series **157**:185-194.

Gould, S. J. and E. S. Vrba. 1982. Exaptation - a missing term in the science of form. Paleobiology **8**:4-15.

Hampton, S. L. and C. L. Griffiths. 2007. Why *Carcinus maenas* cannot get a grip on South Africa's wave-exposed coastline. African Journal of Marine Science **29**:123-126.

Harding, J. M. 2003. Predation by blue crabs, *Callinectes sapidus*, on rapa whelks, Rapana venosa: possible natural controls for an invasive species? Journal of Experimental Marine Biology and Ecology **297**:161-177.

Harding, J. M., R. Mann, and C. W. Kilduff. 2007. The effects of female size on fecundity in a large marine gastropod *Rapana venosa* (Muricidae). Journal of Shellfish Research **26**:33-42.

Harvell, C. D. 1998. Genetic variation and polymorphism in the inducible spines of a marine bryozoan. Evolution **52**:80-86.

Hawkins, L. A., A. E. Magurran, and J. D. Armstrong. 2007. Innate abilities to distinguish between predator species and cue concentration in Atlantic salmon. Animal Behaviour **73**:1051-1057.

Hendrix, L. J., M. W. Carter, and D. T. Scott. 1982. Covariance analyses with heterogeneity of slopes in fixed models. Biometrics **38**:641-650.

Huitema, B. E. 1980. The Analysis of Covariance and Alternatives. John Wiley and Sons, Inc., New York.

Jensen, G. C. 1995. Pacific Coast crabs and shrimps. Sea Challengers, Monterey, CA, USA.

Kozloff, E. N. 1987. Marine Invertebrates of the Pacific Northwest. University of Washington Press, Seattle, WA, USA.

Kraft, P. G., C. E. Franklin, and M. W. Blows. 2006. Predator-induced phenotypic plasticity in tadpoles: extension or innovation? Journal of Evolutionary Biology **19**:450-458.

Laforsch, C., L. Beccara, and R. Tollrian. 2006. Inducible defenses: The relevance of chemical alarm cues in *Daphnia*. Limnology and Oceanography **51**:1466-1472.

Langerhans, R. B. and T. J. DeWitt. 2002. Plasticity constrained: over-generalized induction cues cause maladaptive phenotypes. Evolutionary Ecology Research **4**:857-870.

Leonard, G. H., M. D. Bertness, and P. O. Yund. 1999. Crab predation, waterborne cues, and inducible defenses in the blue mussel, *Mytilus edulis*. Ecology **80**:1-14.

Levins, R. 1968. Evolution in changing environments. Princeton University Press, Princeton, NJ.

Lima, S. L. and L. M. Dill. 1990. Behavioral decisions made under the risk of predation - a review and prospectus. Canadian Journal of Zoology-Revue Canadienne De Zoologie **68**:619-640.

Lively, C. M. 1986. Canalization versus developmental conversion in a spatially-variable environment. American Naturalist **128**:561-572.

Miner, B. G., S. E. Sultan, S. G. Morgan, D. K. Padilla, and R. A. Relyea. 2005. Ecological consequences of phenotypic plasticity. Trends in Ecology & Evolution **20**:685-692.

Moran, N. A. 1992. The evolutionary maintenance of alternative phenotypes. American Naturalist **139**:971-989.

Padilla, D. K. and S. C. Adolph. 1996. Plastic inducible morphologies are not always adaptive: The importance of time delays in a stochastic environment. Evolutionary Ecology **10**:105-117.

Palmer, A. R. 1982. Growth in marine gastropods - a non-destructive technique for independently measuring shell and body-weight. Malacologia **23**:63-73.

Palmer, A. R. 1990. Effect of crab effluent and scent of damaged conspecifics on feeding, growth, and shell morphology of the Atlantic dogwhelk *Nucella lapillus* (L). Hydrobiologia **193**:155-182.

Palmer, A. R. 1992. Calcification in marine mollusks - how costly is it. Proceedings of the National Academy of Sciences of the United States of America **89**:1379-1382.

Quinn, G. P. and M. J. Keough. 2002. Experimental Design and Data Analysis. Cambridge University Press, New York.

Relyea, R. A. 2003. How prey respond to combined predators: A review and an empirical test. Ecology **84**:1827-1839.

Robles, C., D. A. Sweetnam, and D. Dittman. 1989. Diel variation of intertidal foraging by *Cancer productus* L in British Columbia. Journal of Natural History **23**:1041-1049.

Scheiner, S. M. 1998. The genetics of phenotypic plasticity. VII. Evolution in a spatially-structured environment. Journal of Evolutionary Biology **11**:303-320.

Schoeppner, N. M. and R. A. Relyea. 2005. Damage, digestion, and defence: the roles of alarm cues and kairomones for inducing prey defences. Ecology Letters **8**:505-512.

Stachowicz, J. J. and M. Hay. 1999. Reduced mobility is associated with compensatory feeding and increased diet breadth of marine crabs. Marine Ecology-Progress Series **188**:169-178.

Taylor, G. M., A. R. Palmer, and A. C. Barton. 2000. Variation in safety factors of claws within and among six species of *Cancer* crabs (Decapoda : Brachyura). Biological Journal of the Linnean Society **70**:37-62.

Teplitsky, C., S. Plenet, and P. Joly. 2004. Hierarchical responses of tadpoles to multiple predators. Ecology **85**:2888-2894.

Tollrian, R. and C. D. Harvell, editors. 1998. The Ecology and Evolution of Inducible Defenses. Princeton University Press, Princeton, NJ.

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

Vermeij, G. J. 1987. Evolution and Escalation: An Ecological History of Life. Princeton Univesrity Press, Princeton, NJ.

von Elert, E. and G. Pohnert. 2000. Predator specificity of kairomones in diel vertical migration of *Daphnia*: a chemical approach. Oikos **88**:119-128.

West-Eberhard, M. J. 2003. Developmental Plasticity and Evolution. Oxford University Press, New York.

Yamada, S. B. and E. G. Boulding. 1996. The role of highly mobile crab predators in the intertidal zonation of their gastropod prey. Journal of Experimental Marine Biology and Ecology **204**:59-83.

Yamada, S. B. and E. G. Boulding. 1998. Claw morphology, prey size selection and foraging efficiency in generalist and specialist shell-breaking crabs. Journal of Experimental Marine Biology and Ecology **220**:191-211.

References for Chapter 4

Aigner, P. A. 2004. Floral specialization without trade-offs: Optimal corolla flare in contrasting pollination environments. Ecology **85**:2560-2569.

Anderson, K. E., J. B. Tharrington, P. A. Curtis, and F. T. Jones. 2004. Shell characteristics of eggs from historic strains of single comb white leghorm chickens and the relationship of egg shape to shell strength. International Journal of Poultry Science **3**:17-19.

Appleton, R. D. and A. R. Palmer. 1988. Water-Borne Stimuli Released by Predatory Crabs and Damaged Prey Induce More Predator-Resistant Shells in a Marine Gastropod. Proceedings of the National Academy of Sciences of the United States of America **85**:4387-4391.

Bertness, M. D. and C. Cunningham. 1981. Crab shell-crushing predation and gastropod architectural defense. Journal of Experimental Marine Biology and Ecology **50**:213-230.

Bookstein, F. L. 1989. Principal Warps - Thin-Plate Splines and the Decomposition of Deformations. Ieee Transactions on Pattern Analysis and Machine Intelligence **11**:567-585.

Daniel, T. L. 1984. Unsteady aspects of aquatic locomotion. American Zoologist **24**:121-134.

DeWitt, T. J. 1998. Costs and limits of phenotypic plasticity: Tests with predator-induced morphology and life history in a freshwater snail. Journal of Evolutionary Biology **11**:465-480.

DeWitt, T. J. and R. B. Langerhans. 2003. Multiple prey traits, multiple predators: keys to understanding complex community dynamics. Journal of Sea Research **49**:143-155.

DeWitt, T. J., B. W. Robinson, and D. S. Wilson. 2000. Functional diversity among predators of a freshwater snail imposes an adaptive trade-off for shell morphology. Evolutionary Ecology Research **2**:129-148.

DeWitt, T. J. and S. M. Scheiner, editors. 2003. Phenotypic Plasticity: Functional and Conceptual Approaches. Oxford University Press, New York, NY.

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

Edgell, T. C. and C. J. Neufeld. 2008. Experimental evidence for latent developmental plasticity: intertidal whelks respond to a native but not an introduced predator. Biology Letters **4**:385-387.

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

Freeman, A. S. 2007. Specificity of induced defenses in *Mytilus edulis* and asymmetrical predator deterrence. Marine Ecology-Progress Series **334**:145-153.

Gabriel, W., B. Luttbeg, A. Sih, and R. Tollrian. 2005. Environmental tolerance, heterogeneity, and the evolution of reversible plastic responses. American Naturalist **166**:339-353.

Hendrix, L. J., M. W. Carter, and D. T. Scott. 1982. Covariance analyses with heterogeneity of slopes in fixed models. Biometrics **38**:641-650.

Hoverman, J. T. and R. A. Relyea. 2007. The rules of engagement: how to defend against combinations of predators. Oecologia **154**:551-560.

Jacobsen, H. P. and O. B. Stabell. 1999. Predator-induced alarm responses in the common periwinkle, *Littorina littorea*: dependence on season, light conditions, and chemical labelling of predators. Marine Biology **134**:551-557.

Lima, S. L. 1992. Life in a multi predator environment: some considerations for antipredator vigilance. Annales Zoologici Fennici **29**:217-226.

Lima, S. L. and L. M. Dill. 1990. Behavioral decisions made under the risk of predation - a review and prospectus. Canadian Journal of Zoology-Revue Canadienne De Zoologie **68**:619-640.

Manly, B. F. J., P. Miller, and L. M. Cook. 1972. Analysis of a Selective Predation Experiment. American Naturalist **106**:719-&.

Marko, P. B. and A. R. Palmer. 1991. Responses of a Rocky Shore Gastropod to the Effluents of Predatory and Nonpredatory Crabs - Avoidance and Attraction. Biological Bulletin **181**:363-370.

Matsuda, H., M. Hori, and P. A. Abrams. 1996. Effects of predator-specific defense on community complexity in two-trophic-level communities. Evolutionary Ecology **10**:13-28.

Mauzey, K. P. 1966. Feeding Behavior and Reproductive Cycles in Pisaster Ochraceus. Biological Bulletin **131**:127-&.

Menge, B. A. and J. Lubchenco. 1981. Community Organization in Temperate and Tropical Rocky Inter-Tidal Habitats - Prey Refuges in Relation to Consumer Pressure-Gradients. Ecological Monographs **51**:429-450.

Miner, B. G., S. E. Sultan, S. G. Morgan, D. K. Padilla, and R. A. Relyea. 2005. Ecological consequences of phenotypic plasticity. Trends in Ecology & Evolution **20**:685-692.

Navarrete, S. A., B. A. Menge, and B. A. Daley. 2000. Species interactions in intertidal food webs: Prey or predation regulation of intermediate predators? Ecology **81**:2264-2277.

Padilla, D. K. and S. C. Adolph. 1996. Plastic inducible morphologies are not always adaptive: The importance of time delays in a stochastic environment. Evolutionary Ecology **10**:105-117.

Paine, R. T. 1974. Intertidal Community Structure - Experimental Studies on Relationship between a Dominant Competitor and Its Principal Predator. Oecologia **15**:93-120.

Palmer, A. R. 1982. Growth in Marine Gastropods - a Non-Destructive Technique for Independently Measuring Shell and Body-Weight. Malacologia **23**:63-73.

Palmer, A. R. 1990. Effect of crab effluent and scent of damaged conspecifics on feeding, growth, and shell morphology of the Atlantic dogwhelk *Nucella lapillus* (L). Hydrobiologia **193**:155-182.

Pigliucci, M. 2003. Phenotypic integration: studying the ecology and evolution of complex phenotypes. Ecology Letters **6**:265-272.

Pigliucci, M. 2005. Evolution of phenotypic plasticity: where are we going now? Trends in Ecology & Evolution **20**:481-486.

Ramos-Jiliberto, R., E. Frodden, and A. Aranguiz-Acuna. 2007. Pre-encounter versus post-encounter inducible defenses in predator-prey model systems. Ecological Modelling **200**:99-108.

Reimer, O. and M. Tedengren. 1996. Phenotypical improvement of morphological defences in the mussel *Mytilus edulis* induced by exposure to the predator Asterias rubens. Oikos **75**:383-390.

Relyea, R. A. 2003. How prey respond to combined predators: A review and an empirical test. Ecology **84**:1827-1839.

Relyea, R. A. 2004. Integrating phenotypic plasticity when death is on the line: Insights from predator-prey systems. Pages 176-193 *in* M. Pigliucci and K. Preston, editors. Phenotypic Integration: Studying the Ecology and Evolution of Complex Phenotypes. Oxford University Press, New York, NY.

Rohlf, F. J. 2006. tpsDig. Version 2.10.

Rohlf, F. J. and L. F. Marcus. 1993. A Revolution in Morphometrics. Trends in Ecology & Evolution **8**:129-132.

Sih, A., G. Englund, and D. Wooster. 1998. Emergent impacts of multiple predators on prey. Trends in Ecology & Evolution **13**:350-355.

Smith, L. D. and J. A. Jennings. 2000. Induced defensive responses by the bivalve *Mytilus edulis* to predators with different attack modes. Marine Biology **136**:461-469.

Spight, T. M. 1974. Sizes of Populations of a Marine Snail. Ecology **55**:712-729.

Teplitsky, C., S. Plenet, and P. Joly. 2004. Hierarchical responses of tadpoles to multiple predators. Ecology **85**:2888-2894.

Tollrian, R. and C. D. Harvell, editors. 1998. The Ecology and Evolution of Inducible Defenses. Princeton University Press, Princeton, NJ.

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

Van Buskirk, J. and M. Arioli. 2002. Dosage response of an induced defense: How sensitive are tadpoles to predation risk? Ecology **83**:1580-1585.

Vermeij, G. J. 1987. Evolution and Escalation: An Ecological History of Life. Princeton Univesrity Press, Princeton, NJ.

Vermeij, G. J. 1993. A Natural History of Shells. Princeton University Press, Princeton, NJ.

Watanabe, J. M. 1983. Anti-predator defenses of 3 kelp forest gastropods - contrasting adaptations of closely-related prey species. Journal of Experimental Marine Biology and Ecology **71**:257-270.

West, K., A. Cohen, and M. Baron. 1991. Morphology and behavior of crabs and gastropods from Lake Tanganyika, Africa - implicatons for lacustrine predator-prey coevolution. Evolution **45**:589-607.

West-Eberhard, M. J. 2003. Developmental Plasticity and Evolution. Oxford University Press, New York, NY.

References for Chapter 5

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

Avery, R. and R. J. Etter. 2006. Microstructural differences in the reinforcement of a gastropod shell against predation. Marine Ecology-Progress Series **323**:159-170.

Brookes, J. I. and R. Rochette. 2007. Mechanism of a plastic phenotypic response: predator-induced shell thickening in the intertidal gastropod *Littorina obtusata*. Journal of Evolutionary Biology **20**:1015-1027.

Christy, J. H. 1995. Mimicry, mate choice, and the sensory trap hypothesis. American Naturalist **146**:171-181.

Currey, J. D. and J. D. Taylor. 1974. Mechanical-behavior of some molluscan hard tissues. Journal of Zoology **173**:395-406.

Dalziel, B. and E. G. Boulding. 2005. Water-borne cues from a shell-crushing predator induce a more massive shell in experimental populations of an intertidal snail. Journal of Experimental Marine Biology and Ecology **317**:25-35.

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

Emerson, S. B. and S. K. Boyd. 1999. Mating vocalizations of female frogs: Control and evolutionary mechanisms. Brain Behavior and Evolution **53**:187-197.

Grunbaum, D. 1997. Hydromechanical mechanisms of colony organization and cost of defense in an encrusting bryozoan, *Membranipora membranacea*. Limnology and Oceanography **42**:741-752.

Harvell, C. D. 1992. Inducible defenses and allocation shifts in a marine bryozoan. Ecology **73**:1567-1576.

Huitema, B. E. 1980. The Analysis of Covariance and Alternatives. John Wiley and Sons, Inc., New York.

Lande, R. and S. J. Arnold. 1983. The measurement of selection on correlated characters. Evolution **37**:1210-1226.

Menge, B. A., E. L. Berlow, C. A. Blanchette, S. A. Navarrete, and S. B. Yamada. 1994. The keystone species concept - variation in interaction strength in a rocky intertidal habitat. Ecological Monographs **64**:249-286.

Miner, B. G., S. E. Sultan, S. G. Morgan, D. K. Padilla, and R. A. Relyea. 2005. Ecological consequences of phenotypic plasticity. Trends in Ecology & Evolution **20**:685-692.

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

Palmer, A. R. 1982. Growth in marine gastropods - a non-destructive technique for independently measuring shell and body-weight. Malacologia **23**:63-73.

Palmer, A. R. 1983. Relative cost of producing skeletal organic matrix versus calcification - evidence from marine gastropods. Marine Biology **75**:287-292.

Palmer, A. R. 1990. Effect of crab effluent and scent of damaged conspecifics on feeding, growth, and shell morphology of the Atlantic dogwhelk *Nucella lapillus* (L). Hydrobiologia **193**:155-182.

Palmer, A. R. 1992. Calcification in marine mollusks - how costly is it. Proceedings of the National Academy of Sciences of the United States of America **89**:1379-1382.

Pigliucci, M. 2005. Evolution of phenotypic plasticity: where are we going now? Trends in Ecology & Evolution **20**:481-486.

Relyea, R. A. and J. R. Auld. 2005. Predator- and competitor-induced plasticity: How changes in foraging morphology affect phenotypic trade-offs. Ecology **86**:1723-1729.

Rundle, S. D., J. I. Spicer, R. A. Coleman, J. Vosper, and J. Soane. 2004. Environmental calcium modifies induced defences in snails. Proceedings of the Royal Society of London Series B-Biological Sciences **271**:S67-S70.

Tollrian, R. and C. D. Harvell, editors. 1998. The Ecology and Evolution of Inducible Defenses. Princeton University Press, Princeton, NJ.

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

Trussell, G. C. 2000. Predator-induced plasticity and morphological trade-offs in latitudinally separated populations of *Littorina obtusata*. Evolutionary Ecology Research **2**:803-822.

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

Trussell, G. C. and L. D. Smith. 2000. Induced defenses in response to an invading crab predator: An explanation of historical and geographic

phenotypic change. Proceedings of the National Academy of Sciences of the United States of America **97**:2123-2127.

Van Buskirk, J. and B. R. Schmidt. 2000. Predator-induced phenotypic plasticity in larval newts: Trade-offs, selection, and variation in nature. Ecology **81**:3009-3028.

Vermeij, G. J. 1987. Evolution and Escalation: An Ecological History of Life. Princeton Univesrity Press, Princeton, NJ.

Vermeij, G. J. 2002. Characters in context: molluscan shells and the forces that mold them. Paleobiology **28**:41-54.

West-Eberhard, M. J. 2003. Developmental Plasticity and Evolution. Oxford University Press, New York.

Wimberger, P. H. 1991. Plasticity of jaw and skull morphology in the neotropical cichlids *Geophagus brasilensis* and *G. steindachneri*. Evolution **45**:1545-1563.

Windig, J. J., C. G. F. De Kovel, and G. De Jong. 2003. Genetics and Mechanics of Plasticity. Pages 31-49 *in* T. J. DeWitt and S. M. Scheiner, editors. Phenotypic Plasticity: Functional and Conceptual Approaches. Oxford University Press, New York, NY.

Zipser, E. and G. J. Vermeij. 1978. Crushing behavior of tropical and temperate crabs. Journal of Experimental Marine Biology and Ecology **31**:155-172.

References for Chapter 6

Abjornsson, K., L. A. Hansson, and C. Bronmark. 2004. Responses of prey from habitats with different predator regimes: Local adaptation and heritability. Ecology **85**:1859-1866.

Anderson, K. E., J. B. Tharrington, P. A. Curtis, and F. T. Jones. 2004. Shell characteristics of eggs from historic strains of single comb white leghorm chickens and the relationship of egg shape to shell strength. International Journal of Poultry Science **3**:17-19.

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

Bookstein, F. L. 1989. Principal Warps - Thin-Plate Splines and the Decomposition of Deformations. Ieee Transactions on Pattern Analysis and Machine Intelligence **11**:567-585.

Boulding, E. G., M. Holst, and V. Pilon. 1999. Changes in selection on gastropod shell size and thickness with wave-exposure on Northeastern Pacific shores. Journal of Experimental Marine Biology and Ecology **232**:217-239.

Bourdeau, P. E. 2009. Prioritized phenotypic responses to combined predators in a marine snail. Ecology **in press**.

Cotton, P. A., S. D. Rundle, and K. E. Smith. 2004. Trait compensation in marine gastropods: Shell shape, avoidance behavior, and susceptibility to predation. Ecology **85**:1581-1584.

Currey, J. D. and J. D. Taylor. 1974. Mechanical-behavior of some molluscan hard tissues. Journal of Zoology **173**:395-406.

Dalziel, B. and E. G. Boulding. 2005. Water-borne cues from a shell-crushing predator induce a more massive shell in experimental populations of an intertidal snail. Journal of Experimental Marine Biology and Ecology **317**:25-35.

DeWitt, T. J. 1998. Costs and limits of phenotypic plasticity: Tests with predator-induced morphology and life history in a freshwater snail. Journal of Evolutionary Biology **11**:465-480.

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

Edgell, T. C. and C. J. Neufeld. 2008. Experimental evidence for latent developmental plasticity: intertidal whelks respond to a native but not an introduced predator. Biology Letters **4**:385-387.

Hampton, S. L. and C. L. Griffiths. 2007. Why *Carcinus maenas* cannot get a grip on South Africa's wave-exposed coastline. African Journal of Marine Science **29**:123-126.

Karban, R. and J. H. Myers. 1989. Induced plant-responses to herbivory. Annual Review of Ecology and Systematics **20**:331-348.

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

Lima, S. L. and L. M. Dill. 1990. Behavioral decisions made under the risk of predation - a review and prospectus. Canadian Journal of Zoology-Revue Canadienne De Zoologie **68**:619-640.

Lively, C. M. 1986. Canalization versus developmental conversion in a spatially-variable environment. American Naturalist **128**:561-572.

Marko, P. B. and A. R. Palmer. 1991. Responses of a rocky shore gastropod to the effluents of predatory and nonpredatory crabs - avoidance and attraction. Biological Bulletin **181**:363-370.

Menge, B. A. and J. P. Sutherland. 1987. Community regulation: variation in disturbance, competition, and predation in relation to environmental-stress and recruitment. American Naturalist **130**:730-757.

Morris, W. F., M. B. Traw, and J. Bergelson. 2006. On testing for a tradeoff between constitutive and induced resistance. Oikos **112**:102-110.

Padilla, D. K. and S. C. Adolph. 1996. Plastic inducible morphologies are not always adaptive: The importance of time delays in a stochastic environment. Evolutionary Ecology **10**:105-117.

Paine, R. T. 1966. Food web complexity and species diversity. American Naturalist **100**:65-&.

Palmer, A. R. 1982. Growth in marine gastropods - a non-destructive technique for independently measuring shell and body-weight. Malacologia **23**:63-73.

Palmer, A. R. 1990. Effect of crab effluent and scent of damaged conspecifics on feeding, growth, and shell morphology of the Atlantic dogwhelk *Nucella lapillus* (L). Hydrobiologia **193**:155-182.

Rohlf, F. J. 2006. tpsDig. Version 2.10.

Rohlf, F. J. and L. F. Marcus. 1993. A Revolution in Morphometrics. Trends in Ecology & Evolution **8**:129-132.

Rundle, S. D. and C. Bronmark. 2001. Inter- and intraspecific trait compensation of defence mechanisms in freshwater snails. Proceedings of the Royal Society of London Series B-Biological Sciences **268**:1463-1468.

Stone, J. R. 1999. Using a mathematical model to test the null hypothesis of optimal shell construction by four marine gastropods. Marine Biology **134**:397-403.

Tollrian, R. and C. D. Harvell, editors. 1998. The Ecology and Evolution of Inducible Defenses. Princeton University Press, Princeton, NJ.

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

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

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

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

Van Buskirk, J. and S. A. McCollum. 1999. Plasticity and selection explain variation in tadpole phenotype between ponds with different predator composition. Oikos **85**:31-39.

Vermeij, G. J. 1987. Evolution and Escalation: An Ecological History of Life. Princeton Univesrity Press, Princeton, NJ.

References for Chapter 7

Abrams, P. A. and L. Rowe. 1996. The effects of predation on the age and size of maturity of prey. Evolution **50**:1052-1061.

Anholt, B. R. and E. E. Werner. 1995. Interaction between food availability and predation mortality mediated by adaptive behavior. Ecology **76**:2230-2234.

Arendt, J. D. 1997. Adaptive intrinsic growth rates: An integration across taxa. Quarterly Review of Biology **72**:149-177.

Bertness, M. D. 1977. Behavioral and ecological aspects of shore-level size gradients in *Thais lamellosa* and *Thais emarginata*. Ecology **58**:86-97.

Bourdeau, P. E. 2009. Prioritized phenotypic responses to combined predators in a marine snail. Ecology **in press**.

Collins, T. M., K. Frazer, A. R. Palmer, G. J. Vermeij, and W. M. Brown. 1996. Evolutionary history of northern hemisphere *Nucella* (Gastropoda, Muricidae): Molecular, morphological, ecological, and paleontological evidence. Evolution **50**:2287-2304.

Connell, J. H. 1961. Influence of interspecific competition and other factors on distribution of barnacle *Chthamalus stellatus*. Ecology **42**:710-&.

Dalziel, B. and E. G. Boulding. 2005. Water-borne cues from a shell-crushing predator induce a more massive shell in experimental populations of an intertidal snail. Journal of Experimental Marine Biology and Ecology **317**:25-35.

De Block, M., M. A. McPeek, and R. Stoks. 2007. Winter compensatory growth under field conditions partly offsets low energy reserves before winter in a damselfly. Oikos **116**:1975-1982.

Emlen, D. J. and H. F. Nijhout. 2000. The development and evolution of exaggerated morphologies in insects. Annual Review of Entomology **45**:661-708.

Gotthard, K. 2000. Increased risk of predation as a cost of high growth rate: an experimental test in a butterfly. Journal of Animal Ecology **69**:896-902.

Hahn, T. and M. Denny. 1989. Tenacity-mediated selective predation by oystercatchers on intertidal limpets and its role in maintaining habitat

partitioning by *Collisella scabra* and *Lottia digitalis*. Marine Ecology-Progress Series **53**:1-10.

Harding, J. M., R. Mann, and C. W. Kilduff. 2007. The effects of female size on fecundity in a large marine gastropod *Rapana venosa* (Muricidae). Journal of Shellfish Research **26**:33-42.

Hendrix, L. J., M. W. Carter, and D. T. Scott. 1982. Covariance analyses with heterogeneity of slopes in fixed models. Biometrics **38**:641-650.

Huitema, B. E. 1980. The Analysis of Covariance and Alternatives. John Wiley and Sons, Inc., New York.

Ludwig, D. and L. Rowe. 1990. Life-history strategies for energy gain and predator avoiance under time constraints. American Naturalist **135**:686-707.

McCoy, M. W. 2007. Conspecific density determines the magnitude and character of predator-induced phenotype. Oecologia **153**:871-878.

McPeek, M. A. 2004. The growth/predation risk trade-off: So what is the mechanism? American Naturalist **163**:E88-E111.

Menge, B. A. and J. P. Sutherland. 1976. Species diversity gradients: synthesis of roles of predation, competition, and temporal heterogeneity. American Naturalist **110**:351-369.

Menge, B. A. and J. P. Sutherland. 1987. Community regulation: variation in disturbance, competition, and predation in relation to environmental stress and recruitment. American Naturalist **130**:730-757.

Palmer, A. R. 1980. A comparative and experimental study of feeding and growth in thaidid. PhD Dissertation. University of Washington, Seattle, WA, USA.

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

Palmer, A. R. 1982. Growth in marine gastropods - a non-destructive technique for independently measuring shell and body-weight. Malacologia **23**:63-73.

Palmer, A. R. 1985. Adaptive value of shell variation in *Thais lamellosa* - effect of thick shells on vulnerability to and preference by crabs. Veliger **27**:349-356.

Palmer, A. R. 1990. Effect of crab effluent and scent of damaged conspecifics on feeding, growth, and shell morphology of the Atlantic dogwhelk *Nucella lapillus* (L). Hydrobiologia **193**:155-182.

Pigliucci, M. 2003. Phenotypic integration: studying the ecology and evolution of complex phenotypes. Ecology Letters **6**:265-272.

Rilov, G., Y. Benayahu, and A. Gasith. 2004. Life on the edge: do biomechanical and behavioral adaptations to wave-exposure correlate with habitat partitioning in predatory whelks? Marine Ecology-Progress Series **282**:193-204.

Robles, C., D. A. Sweetnam, and D. Dittman. 1989. Diel variation of intertidal foraging by *Cancer productus* L in British Columbia. Journal of Natural History **23**:1041-1049.

Rowe, L. and D. Ludwig. 1991. Size and timing of metamorphosis in complex life-cycles: time constraints and variation. Ecology **72**:413-427.

Schiesari, L., S. D. Peacor, and E. E. Werner. 2006. The growth-mortality tradeoff: evidence from anuran larvae and consequences for species distributions. Oecologia **149**:194-202.

Skelly, D. K. 1995. A behavioral trade-off and its consequences for the distribution of *Pseudacris* treefrog larvae. Ecology **76**:150-164.

Sokal, R. R. and F. J. Rohlf. 1995. Biometry: the principles and practice of statistics in biological research. 3rd edition. W.H. Freeman and Co., New York.

Sorte, C. J. B. and G. E. Hofmann. 2005. Thermotolerance and heat-shock protein expression in Northeastern Pacific *Nucella* species with different biogeographical ranges. Marine Biology **146**:985-993.

Stoks, R. and M. A. McPeek. 2003. Antipredator behavior and physiology determine Lestes species turnover along the pond-permanence gradient. Ecology **84**:3327-3338.

Tollrian, R. and C. D. Harvell, editors. 1998. The Ecology and Evolution of Inducible Defenses. Princeton University Press, Princeton, NJ.

Trussell, G. C. 2000. Predator-induced plasticity and morphological trade-offs in latitudinally separated populations of *Littorina obtusata*. Evolutionary Ecology Research **2**:803-822.

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

Vermeij, G. J. 1987. Evolution and Escalation: An Ecological History of Life. Princeton Univesrity Press, Princeton, NJ.

Vermeij, G. J. and J. D. Currey. 1980. Geographical variation in the strength of Thaidid snail shells. Biological Bulletin **158**:383-389.

Werner, E. E. and B. R. Anholt. 1993. Ecological consequences of the trade-off between growth and mortality rates mediated by foraging activity. American Naturalist **142**:242-272.

West-Eberhard, M. J. 2003. Developmental Plasticity and Evolution. Oxford University Press, New York.

References for Chapter 8

Agrawal, A. A., C. Laforsch, and R. Tollrian. 1999. Transgenerational induction of defences in animals and plants. Nature **401**:60-63.

Chivers, D. P. and R. J. F. Smith. 1998. Chemical alarm signalling in aquatic predator-prey systems: A review and prospectus. Ecoscience **5**:338-352.

DeWitt, T. J. and S. M. Scheiner, editors. 2003. Phenotypic Plasticity: Functional and Conceptual Approaches. Oxford University Press, New York, NY.

Grosberg, R. K. and C. W. Cunningham. 2001. Genetic structure in the sea: from populations to communities. Pages 61-84 *in* M. D. Bertness, S. D. Gaines, and M. E. Hay, editors. Marine Community Ecology. Sinauer, Sunderland, MA.

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

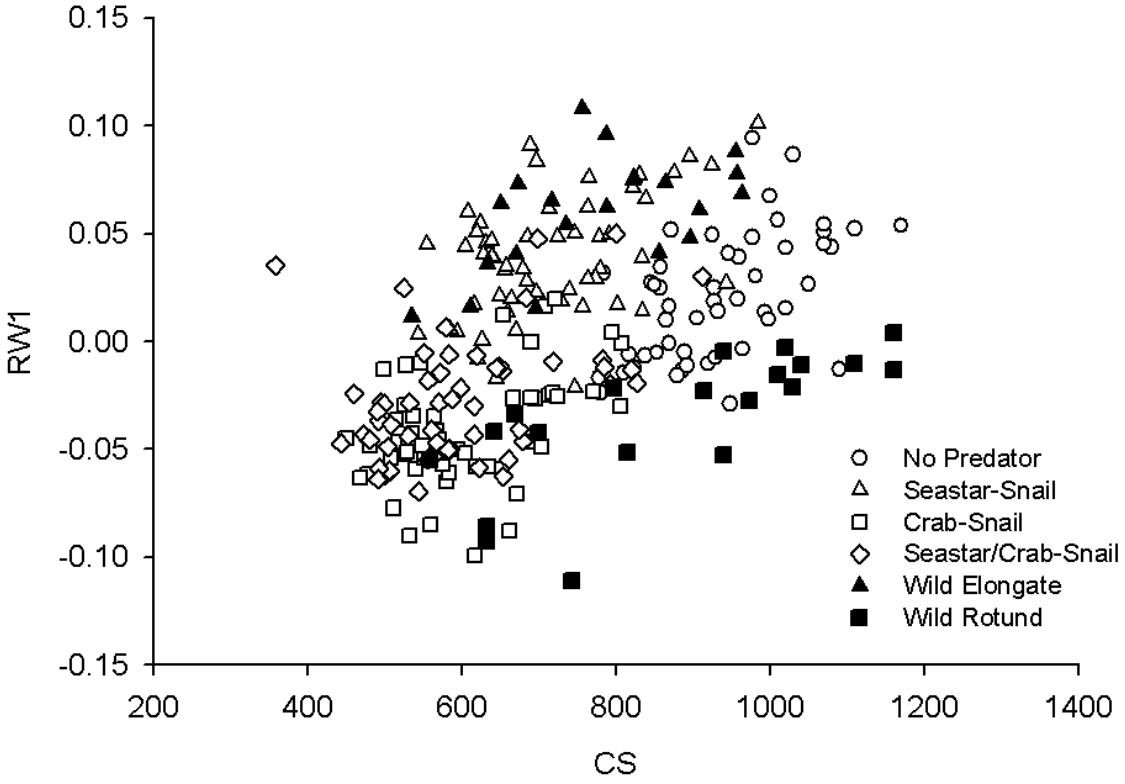
Trussell, G. C. and L. D. Smith. 2000. Induced defenses in response to an invading crab predator: An explanation of historical and geographic phenotypic change. Proceedings of the National Academy of Sciences of the United States of America **97**:2123-2127.

Verschoor, A. M., M. Vos, and I. van der Stap. 2004. Inducible defences prevent strong population fluctuations in bi- and tritrophic food chains. Ecology Letters **7**:1143-1148.

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

West-Eberhard, M. J. 2003. Developmental Plasticity and Evolution. Oxford University Press, New York.

Appendices



Appendix 1. Pseudo-allometry plot of centroid size (the square root of the sum of squared distance from each landmark to their centroid) and relative warp 1 (RW1) for experimental *Nucella lamellosa* reared in no predator (No Predator), Seastar fed conspecific snails (Seastar-Snail), crab fed conspecific snails (Crab-Snail), and both predators fed conspecifics (Seastar/Crab-Snail) treatments and wild-caught snails used in predation trials (Wild Elongate and Wild Rotund).

Appendix 2. Analyses of morphological responses to predators by *Nucella lamellosa* after 70 days of exposure to experimental conditions with body weight as a covariate for shell weight and aspect ratio and aperture area as a covariate for unoccupied volume. Replicate aquaria nested within treatments. All data were $\log_{10}$-transformed prior to analyses, with the exception of aperture area, which was square root transformed.

Source	*F*	Df	*P*
Shell weight			
Body weight	4630.916	1,199	<0.0001
Treatment	39.435	3,8	<0.0001
Aquarium (Treatment)	6.924	8,199	<0.0001
Aspect ratio			
Body weight	8.02	1,199	0.0051
Treatment	34.32	3,8	<0.0001
Aquarium (Treatment)	1.62	8,199	0.1197
Unoccupied volume			
Aperture area	64.00325	1,198	<0.0001
Treatment	8.84393	3,8	0.0025
Aquarium (Treatment)	1.79656	8,198	0.0796

Appendix 3. Results of MANCOVA on shape variables: two uniform components (U1 and U2) and the nine main (explaining 92.04% of the overall variation) non-uniform estimates (RW1-RW9) with centroid size (CS) as a covariate and replicate aquaria nested within treatment.

Source	*F*	Df	*P*
CS	1596.396	11,189.00	<0.0001
Treatment	80.084	33,557.533	<0.0001
Aquarium (Treatment)	3.452	88,1248.815	<0.0001

Appendix 4. Analysis of shell and body growth variation: ANCOVA with initial shell length and body weight as respective covariates and replicate aquaria nested within treatment. All data were $\log_{10}$-transformed prior to analysis.

Source	*F*	df	*P*
Shell growth			
Initial length	18.2872	1,191	<0.0001
Treatment	48.5774	3,8	<0.0001
Aquarium (Treatment)	6.0190	8, 191	<0.0001
Body growth			
Initial weight	1053.931	1,196	<0.0001
Treatment	89.658	3,8	<0.0001
Aquarium (Treatment)	6.900	8,196	<0.0001

Appendix 5. Final size ranges (shell length, mm) of reference samples of *Nucella* species (*N. ostrina*, *N. canaliculata*, *N. lamellosa*) raised in the presence or absence of chemical cues from crabs, which are known to induce morphological defenses in *Nucella*.

	No cue	Risk cue
N. ostrina	18.80-25.84	17.09-23.09
N. canaliculata	25.19-32.56	20.58-27.57
N. lamellosa	26.67-41.59	22.52-38.63

Appendix 6. Results of analyses of covariance on shell strength for experimental and reference samples of *Nucella* species (*N. ostrina*, *N. canaliculata*, *N. lamellosa*) raised in the presence or absence of cues associated with predation risk.

Effect	SS	Df	MS	*F*	*P*
Treatment (T)	0.016276	1	0.016276	1.06171	0.303926
Species (S)	0.103626	5	0.020725	1.35192	0.243562
Log shell length	0.919662	1	0.919662	59.99017	0.000000
T*S	0.044919	5	0.008984	0.58602	0.710699
T*log shell length	0.035614	1	0.035614	2.32310	0.128864
S*log shell length	0.086423	5	0.017285	1.12749	0.346563
T*S*log shell lgth	0.043396	5	0.008679	0.56615	0.725902
Error	3.464627	226	0.015330		