MORPHOLOGICAL, BEHAVIORAL AND REPRODUCTIVE INDUCIBLE DEFENSES IN TWO SPECIES OF INTERTIDAL GASTROPODS

By

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Accepted in Partial Completion
Of the Requirements for the Degree
Master of Science

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MASTER'S THESIS

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MORPHOLOGICAL, BEHAVIORAL AND REPRODUCTIVE INDUCIBLE DEFENSES IN TWO SPECIES OF INTERTIDAL GASTROPODS

A Thesis
Presented to
The Faculty of
Western Washington University

In Partial Fulfillment
Of the Requirements for the Degree
Masters in Science

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February 2014

ABSTRACT

An organism's ability to respond to threats in its environment can influence its fitness, as well as the community in which that organism lives. A common type of threat that organisms face is the threat of predation and there are many ways prey species respond to this threat. Some common ways include altering morphology, behavior or life- history strategy. Changes in morphology can make it harder for predators to consume prey while changes in behavior can decrease the probability of prey encountering a predator. Shifts in life-history strategy can alter when organisms are exposed to predation and increase the likelihood of survival in the event of a predator-prey encounter.

Marine gastropods can respond to a wide array of threats by altering their morphology, behavior, or life history. Because marine gastropods are highly plastic, they are a promising clade to further our understanding of inducible defenses and how these defenses relate to the surrounding community. For my thesis, I conducted two studies to examine how two species of marine gastropods respond to predators in their environment.

In the first study, I ran two experiments to test whether cues from predators induce plastic defenses in *Littorina sitkana*. In the first experiment, snails where exposed to either a predator treatment, which consisted of waterborne cues from the predatory crab *Hemigrapsus nudus* and crushed conspecific snails, or a control treatment, which consisted of cues from uncrushed conspecific snails. Shell thickness, strength, and size

were measured after nine months of exposure to treatments. Snails exposed to the combination of crabs and crushed conspecifics showed no change in shell thickness or strength but did have both narrower and shorter shells than control snails. In the second experiment, I tested whether cues from *H. nudus* or crushed conspecifics altered the behavior or feeding patterns of *L. sitkana*. Snails were exposed to waterborne cues from either *H. nudus* only, crushed conspecific snails only, a combination of *H. nudus* and crushed conspecific snails, or uncrushed conspecific snails only. Crushed conspecifics and the combination of crushed conspecifics and *H. nudus* caused an increase in an escape response in snails while crushed conspecifics only caused a reduction in snail grazing.

In the second study, I completed three experiments to test how *Nucella lamellosa* alters its reproductive behavior in response to different combinations of organisms that are predatory and non-predatory on encapsulated *N. lamellosa* embryos. In the first experiment, I tested how adult *N. lamellosa* responded to cues from the crab *Hemigrapsus oregonensis* and the isopod *Idotea wosnesenskii* (both of which consume encapsulated embryos but not adult snails). Adult snails were exposed to waterborne cues from *H. oregonensis* only, *I. wosnesenskii* only, both *H. oregonensis* and *I. wosnesenskii*, or a control with no crabs or isopods. I measured how these treatments altered the timing and rate at which egg capsules were laid, as well as the physical and energetic characteristics of the capsules. I found that adult whelks delayed depositing capsules and reduced the rate of capsule deposition in response to predatory crabs, and to isopods, but only if isopods were present in combination with crabs. No change was

observed in the physical or energetic characteristics of the deposited capsules. The results of this experiment showed that crabs had a strong effect on snail reproduction. This led to our second experiment, which examined whether snails respond differently to crab species that do or do not pose a threat to encapsulated snails. In this experiment I measured the timing and rate of capsule deposition as well as the strength required to pierce the wall of an egg capsule. Snails were exposed to cues from *H. oregonensis, Petrolisthes eriomerus, Pugettia* spp., *Pagurus granosimanus* or a control with no crabs. *P. eriomerus*, a species that does not prey on encapsulated snails, was the only species to induce a delay in capsule deposition. *P. eriomerus* and two other crabs, one that does (*H. oregonensis)* and one that does not (*P. granosimanus)* consume capsules, lowered the rate of deposition. In the third experiment, I tested whether different densities of conspecific adult snails alter snail reproductive behavior as previous studies have shown that cues from conspecifics can alter traits associated with inducible defenses. In this experiment I measured the timing and the rate of capsule deposition, as well as the energy invested into capsules in response to different densities of conspecific snails. Adult snails were exposed to either a high density of conspecific snails, a low density of conspecific snails or a control with no additional snails. I found that both treatments with additional snails accelerated the timing of capsule deposition relative to the control, but that the low and the high density treatments were not different from each other. I observed no difference in the rate of capsule deposition or in the energy invested into the capsules between the treatments.

I show that *L. sitkana* does not alter shell thickness in response to predation, but it does decrease its shell size. It is possible that thick shells have become a fixed as opposed to a plastic trait in *L. sitkana.* Additionally, *L. sitkana* decreases its grazing and increases its crawl away behavior in response to cues from predators. These findings are consistent with responses found in previous studies and suggest that *Cancer productus* may be the predator driving selection for inducible defenses in *L. sitkana*. I also show that adult *N. lamellosa* can alter their reproductive behavior in response to organisms that pose a threat to their offspring but do not threaten the adult snails themselves. However, in some cases, the level of threat posed by a species of crab was a poor predictor of how adult snails would respond. Adult *N. lamellosa* also alter their reproductive behavior in response to elevated densities of conspecific snails. All shifts in reproductive timing by adults were in the same direction as shifts in embryonic snail's time of hatching seen in other studies. Cues from *H. oregonensis* delayed time to hatching in embryonic *N. lamellosa* and delayed capsule deposition by adults. Similarly, elevated levels of conspecific snails accelerated both time to hatching and the time at which capsules were deposited. This highlights the importance of studying how biotic cues affect multiple life-history switch points of the same organism.

ACKNOWLEDGEMENTS

I would like to thank my advisors Deb Donovan and Ben Miner whose patience and guidance made all of this possible. I would also like to thank Brian Bingham for his thoughtful insights on several aspects of this work. Special recognition also goes to Geoff Mayhew. Without his hard work and dedication to this project I would never have been able to get all of this done. I also need to thank Brielle Kemis, Ashley Hill, and Erin Stehr who all put in long hours making these studies happen. Jeannie Gilbert and Peter Thut were also incredibly helpful making sure that I was always adequately supplied to conduct my research. I would also like to thank Gene McKeen for helping me with my collection process. Funding was provided by the Pacific Northwest Shell Society and the WWU Biology Department.

TABLE OF CONTENTS

LIST OF FIGURES

exposed to cues from caged *H. oregonensis* (Crab), *I. wosnesenskii* (Isopod), both crabs and isopds (Both), or neither crabs nor isopods (Control). Each treatment had six replicates. Error bars represent one standard error.

Figure 11. Mean caloric value (kJ mg^{-1}) of embryos extracted from egg capsules. Whelks were exposed to cues from caged *H. oregonensis* (Crab), *I. wosnesenskii* (Isopod), both crabs and isopds (Both), or neither crabs nor isopods (Control). Each treatment had six replicates. Error bars represent one standard error.

Figure 12. A) Mean number of days until capsules were first observed, and B) mean rate of capsule deposition by whelks exposed to cues from different species of crab. Whelks were exposed to cues from *H.oregonensis*, *Pugettia spp.*, *P. granosimanus*, *P. eriomerus,* and no crabs (control). Each treatment had five replicates. Error bars represent one standard error.

Figure 13. Mean force (N) required to pierce A) intact-capsules and B) punctured-capsules deposited by whelks exposed to cues from different species of crab. Whelks were exposed to cues from *H. oregonensis*, *Pugettia spp.*, *P. granosimanus*, *P. eriomerus,* and no crabs (control). Each treatment had five replicates. Error bars represent one standard error.

Figure 14. A) Mean number of days until capsules were first observed, and B) mean rate of capsule deposition by whelks exposed to cues from three densities of adult

conspecifics. Whelks were exposed to cues from 20 additional adult whelks (high density), 10 additional adult whelks (low density), or no additional whelks (control). Each treatment had eight replicates. Error bars represent one standard error.

Figure 15. Mean energy density (kJ mg^{-1}) of embryos extracted from egg capsules. Whelks were exposed to cues from 20 additional adult whelks (high density), 10 additional adult whelks (low density), or no additional whelks (control). Each treatment had eight replicates. Error bars represent one standard error.

LIST OF TABLES

CHAPTER 1: THE SIGNIFICANCE OF INDUCIBLE DEFENSES

Phenotypic plasticity is an organism's ability to express multiple phenotypes in response to different environments. Different abiotic and biotic factors provide cues that trigger organisms to alter their morphology, behavior, physiology or life-history. These plastic responses are often adaptive and can increase an organism's fitness within a variable environment. This may cause organisms to shift phenotypes in conjunction with shifting selective pressures in their surroundings. Common mechanisms that can drive plastic responses include climatic cycles, food availability, inter and intra-specific competition, and predator-prey interactions (Via et al. 1995, Mousseau and Fox 1998, Agrawal 2001). Responses that occur when prey organisms adaptively alter their phenotype in response to predators are called inducible defenses.

From an ecological perspective there are many reasons to study inducible defenses. Inducible defenses allow organisms to express a range of phenotypes in response to a variable presence or type of predatory threat, and reduce the risk of death or injury from that predatory threat (Warkentin 1995, Agrawal 2001, Agrawal et al. 2002, Lagerhans and DeWitt 2002, Buckley et al. 2005). As inducible defenses can alter an organism's fitness, there can be subsequent changes to the biotic community that the prey organism exists within (Lively 1986, Harvell 1990, Fortin et al. 2005). Plastic defenses can occur at a wide range of life history stages, from encapsulated embryos to adults, making them important processes throughout an organism's lifetime (Palmer 1990, Warkentin 1999, Relyea 2003, Beladjal et al. 2007, Gomez-Mestre et al. 2008a, Aranguiz-Acuna et al.

2011). Because inducible defenses alter an organism's fitness, and can be found at all points in an organism's lifetime, it is important to understand how these process work in order to gain a better understanding of community dynamics.

Two common ways organisms employ inducible defenses is through altering their morphology and through altering their behavior. Prey can alter their morphology by developing large spines (Harvell 1992, Gowda 1997), thick shells (Appleton and Palmer 1998), or by attaining a size refuge (Teplitsky et al. 2004). Some common ways prey alter their behavior is by avoiding the areas frequented by predators or by increasing the amount of time they spend in sheltered habitat (Marko and Palmer 1991, Turner and Montgomery 2003, Gochfeld 2004, Fortin et al. 2005). These changes in phenotype can reduce the risk of an encounter with a predator and maximize the fitness of the prey if an encounter does occur. For my thesis, I examined how two species of marine gastropods respond to different predatory and non-predatory organisms that they would be likely to encounter in their environment.

CHAPTER 2: INDUCIBLE DEFENSES IN SHELL MORPHOLOGY AND FORAGING BEHAVIOR IN A LITTORINE SNAIL

INTRODUCTION

In many species of marine gastropods, increased shell thickness is a common response to feeding predators (Appleton and Palmer 1988, Lakowitz et al. 2008, Bourdeau 2009, Freeman et al. 2009, Bourdeau 2010). There is evidence that this response provides protection by making it harder for predatory crabs to crush a snail's shell (Pakes and Boulding 2010). Many species of marine gastropods also alter their behavior in response to feeding predatory crabs and these responses too provide protection from predation (Marko and Palmer 1991, Turner and Montgomery 2003, Gochfeld 2004, Fortin et al. 2005). As these plastic responses can change the snail's survival, changes in shell thickness and snail behavior can cause subsequent changes in the community that the organism exists within (Nagarajan et al. 2008).

Littorina is a common genus of marine gastropod that influences community dynamics through their grazing and as a common prey item for other intertidal animals (Norton et al. 1990). *Littorina* species express a wide array of behavioral and morphological inducible defenses. *L. obtusata, L. subrodundata, L. littorea, L. sitkana, L. saxatilis* and *L. scutulata* all alter their shell morphology in response to waterborne cues from predatory crabs feeding on conspecific snails (Fig. 1) (Trussell 1996, DeWolf et al. 1997,

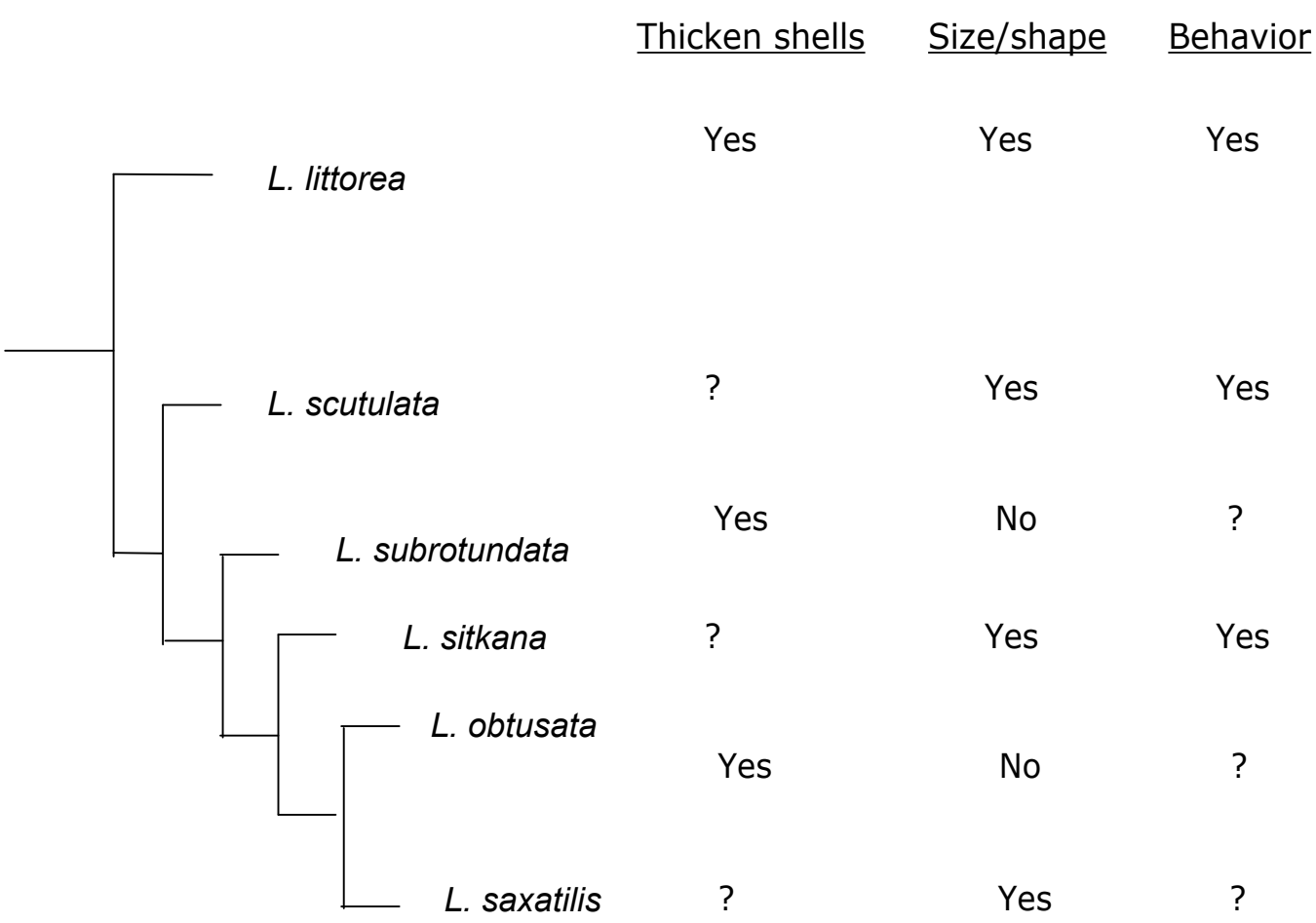

Figure 1. A phylogeny of the genus *Littorina* showing the species that have known morphological and behavioral inducible defenses. Question marks mean this response has not yet been investigated. Based on the phylogenetic work of Reid et al. (2012).

Yamada et al. 1998, Jacobsen and Stabell 1999, Rochette and Dill 2000, Trussell 2000, Keppel and Scrosati 2004, Dalziel and Boulding 2005, Hollander et al. 2006, Brookes and Rochette 2007, Vaughn 2007, Hollander and Butlin 2010). Some species, such as *L. saxatilis* and *L. littorea*, alter their shell shape and size while *L. obtusata* and *L subrotundata* thicken their shells in response to cues from predators. *L. scutulata* veligers decrease aperture size if exposed to predator cues (Vaughn 2007). *L. littorea, L. sitkana*, and *L. scutulata* behaviorally avoid predators when exposed to these same cues (Yamada et al. 1998, Jacobsen and Stabell 1999, Rochette and Dill 2000, Keppel and Scrosati 2004).

Within the *Littorina* genus, only *L. littorea, L. obtusata* and *L. subrodundata* are known to increase their shell thickness in response to crabs feeding on conspecific snails (Trussell 1996, Dalziel and Boulding 2005, Bibby et al. 2007). These three species are found throughout the different branches of the *Littorina* genus (Fig. 1) (Reid et al. 2012). Little work has been done on whether other species within this genus also thicken their shells in response to predators. This raises the question of whether shell thickening is present throughout the *Littorina* genus.

L. sitkana is a species likely to express plastic shell thickening in response to predatory crabs as their thick shells can deter predation when compared to other thinner shelled *Littorina* species (Boulding et al. 1999). Additionally, *L. sitkana* responds both morphologically and behaviorally to crushed conspecifics by decreasing shell length and moving higher in the intertidal (Yamada et al. 1998, Rochette and Dill 2000). Having

smaller shells and moving higher in the intertidal decreases the likelihood of an encounter with the predatory crab *Cancer productus*, that selects for larger shelled snails in the mid to low intertidal zone (Yamada and Boulding 1998, Yamada et al. 1998). However, it is unknown whether *L. sitkana* can plastically thicken its shell. Additionally, it is unlikely that *C. productus* would induce thicker shells in *L. sitkana* as *C. productus* selects for the snails with the largest shells, which are likely to be the thickest. *Hemigrapsus nudus* crabs are also known to prey on *L. sitkana*, but little work has been done to determine how the *L. sitkana* respond to them (Yamada and Boulding 1998). *L. obtusata* and *L. subrotundata*, two species very closely related to *L. sitkana* (Fig. 1), both plastically thicken their shells in response to predatory crabs and *L. subrotundata* does so in response to *H. nudus*. Because *L. sitkana* alters its shell size in response to predators and shell thickening is present in closely related species, I hypothesized that *L. sitkana* would thicken its shell in response to cues from *H. nudus* crabs feeding on conspecifics.

In this study I investigated whether predatory crabs feeding on crushed conspecifics induce a change in the shell thickness, shell morphology, or behavior of *L. sitkana*. To test for morphological responses, I exposed *L. sitkana* to *H. nudus* crabs feeding on conspecific snails for nine months, and recorded changes in shell morphology and structure. To test for behavioral responses, I exposed *L. sitkana* to crabs feeding on conspecific snails and recorded snail feeding rates and habitat use.

METHODS

In this study, I performed two experiments. In the first experiment, I exposed *Littorina sitkana* to either waterborne cues from *Hemigrapsus nudus* and crushed conspecifics or to water without these cues and measured the effect on shell morphology and shell strength. In the second experiment, I exposed *L. sitkana* to one of four treatments: cues from crushed conspecifics, cues from *H. nudus*, cues from *H. nudus* + crushed conspecifics or a no-cue control. I measured the effect of these treatments on snail grazing and the amount of time snails spent utilizing different microhabitats within the aquaria.

In both studies *L. sitkana* and *H. nudus* were collected from Shannon Point Beach in Anacortes, WA. Snails and crabs were transferred from Shannon Point to the Biology Department of Western Washington University in separate five gallon buckets. Once in the lab, snails and crabs were held in separate 80 L aquaria in a 10 ^{o}C cold room until they were transferred to their experimental aquaria.

Effect of crabs on shell morphology and strength

This study began in May of 2010. After snails had been transferred into the cold room and had a week to acclimatize, twenty experimental snails were placed as a group in 175 ml tea strainers in 20 L aquaria. This ensured that snails would be exposed to waterborne cues from the treatments, yet prevented them from leaving their experimental enclosure.

These snails were exposed to one of two treatments. The first treatment consisted of two *H. nudus* crabs fed five mechanically crushed and five non-crushed *L. sitkana* every two weeks. Crabs were held in a plastic container with holes drilled in it. These holes allowed waterborne cues from the crabs and crushed conspecifics to flow freely throughout the tank but prevented the crabs from actually disturbing the experimental snails. The other treatment was a control that had ten uncrushed *L. sitkana*, and no *H. nudus* within the plastic containers. There were 18 replicate aquaria per treatment.

Experimental snails were fed ulvoid algae *ad libitum* during the experiment. Each aquarium had an electric filter to remove particulate matter. The carbon socks were removed from the filters in an effort to prevent absorption of the chemical cues from the crabs. Snails were exposed to their treatment for nine months.

In March of 2011, all snails were frozen in an -80 °C freezer for later analysis of shell length, width, lip thickness, and strength. I haphazardly selected ten snails for measurements from each aquarium and the values were averaged to yield one value per aquarium. Shell length was measured from the base of the siphonal canal to the apex of the shell. Shell width was measured at the widest part of the shell across the first whorl, perpendicular to the length measurement. Lip thickness was measured on the midsection on the apertural lip. Measurements of shell length, width, and lip thickness were all made using digital calipers accurate to 0.01 mm. Shell strength was measured using a Mechanical Test Systems (MTS) load cell that was sensitive to 0.004 N. Snails were placed aperture down and the load cell applied pressure on the first whorl of the shell

until the shell cracked. The force at which the shell cracked was recorded as the strength of the shell. Treatments were compared using ANOVA except for shell strength, which was analyzed with ANCOVA with length as a covariate to account for the fact that larger shells were overall stronger than smaller shells independent of treatment.

Effect of crabs and crushed conspecifics on feeding and habitat use

This study was carried out in February 2012. After snails had been transferred into the cold room and had one week to acclimatize, six snails were placed as a group in a 15-cm diameter glass bowl covered by a screen inside a 20 L aquarium. The screen ensured that snails would be exposed to waterborne cues from the treatments yet could not leave their experimental enclosure. Experimental snails were exposed to one of four treatments: a crushed conspecifics treatment consisting of five mechanically crushed *L. sitkana*; a *H. nudus* treatment that contained two live *H. nudus* crabs; a treatment with both *H. nudus* and crushed conspecifics; and a control with five uncrushed conspecifics and no crabs. All treatment organisms were held in a plastic container that had holes drilled into it, allowing waterborne cues to flow freely through the tank while preventing any of the treatment organisms from actually disturbing the experimental snails. A small rock was also placed in the watch glass to serve as shelter. This allowed us to measure how frequently snails would use shelter when exposed to cues from predators. Each aquarium had an individual electric filter to remove particulate matter. The carbon socks were removed from the filters in an effort to prevent absorption of chemical cues. Snails were exposed to their treatment for three days and there were six replicates per treatment.

The experimental snails were given a pre-weighed piece of ulvoid algae (0.2 g to 0.5 g) and allowed to eat *ad libitum* for three days while exposed to their treatment. Each tank also had a second piece of algae that was not subject to snail consumption. This second piece of algae allowed me to calculate the rate of growth for algae within a tank with no snail grazing. Mass and area were recorded for both pieces of algae at the beginning and end of three days. Algal mass was determined using a digital scale sensitive to 0.001g and all algae was blotted dry before weighing. To calculate the growth of the control piece of algae, I used the equation:

$$final\ ulvoid\ mass = initial\ ulvoid\ mass * e^{(g*t)}$$

where g is an algal growth parameter and t is the time of algal growth, in this case 3 days. This yielded the equation

$$g = (ln(final\ ulvoid\ mass / initial\ ulvoid\ mass))/t$$

With the growth parameter for each control piece of algae, I calculated the rate of grazing for the experimental piece of algae using the equation:

$$final\ ulvoid\ mass = initial\ ulvoid\ mass * e^{(g-gr*t)}$$

Where *g* is the growth parameter calculated for the control piece of algae and *gr* is the grazing parameter (the effect of snail grazing on algal growth). The grazing parameter is a unit-less number that is a relative quantification of how much algae snails in a tank consumed. I then solved for gr yielding the equation:

gr=g-(ln(final ulvoid mass / initial ulvoid mass))/t

Snail habitat use was also recorded by noting where snails were inside the watch glass. The location of the snails was documented three times a day throughout the course of the three-day experiment. Observations were made roughly at 9am, 12pm and 3pm. The possible places that snails could use were: the bottom of the glass, the bottom corner of the glass, the side of the glass, the top corner where the glass met the mesh, the mesh top, the ulvoid algae, on the rock, or under the rock (Fig. 2). I then totaled the number of observations that occurred at a given location over the course of the three day experiment and divided it by the total number of possible observations per tank (9 observations per snail and 54 observations per tank). This allowed me to calculated the percentage of observations that occurred at a given location. There were also three meta-categories used when recording habitat use. These categories were feeding, exposed, and sheltered. A snail was only considered feeding when it was found on the algae. A snail was considered exposed if it was on the bottom of the glass, the side of the glass, the top mesh, or on the rock. A snail was also considered exposed if it was feeding, thus feeding a subcategory of exposed. A snail was considered hiding

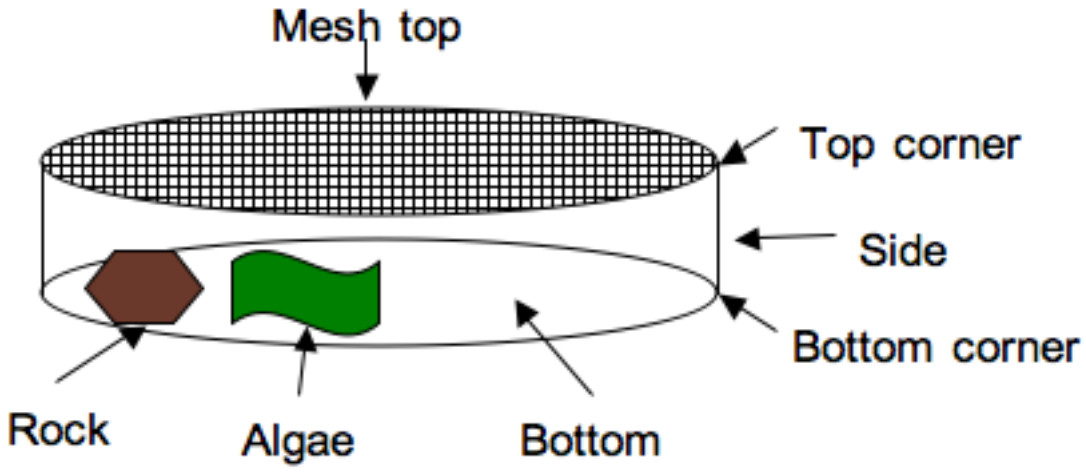

Figure 2. Possible habitats within the glass watch glass that snails could utilize.

if it was found in the bottom corner of the glass, the top corner of the glass or underneath the rock. I calculated the percentage of observations within these three meta-categories the same way that I calculated the individual categories.

Differences in the grazing parameter as well as percentage of observations classified as hiding within the tank were analyzed using a one-tailed two-sample T test. Additionally to be consistent with other studies, I tested to see if snails exposed to cues from predators utilized the upper corner of the watch glass more frequently. These studies have found that when exposed to predation cues, *L. sitkana* snails will move upwards within their habitat (Yamada et al. 1998 Rochette and Dill 2000). All treatments were compared against the control and a one-tailed t-test was used because I expected cues from predators to reduce the amount of algae consumed and increase the use of habitat considered hiding.

RESULTS

Effect of crabs on shell morphology and strength

There were no differences in shell lip thickness between control snails and snails that were exposed to predation cues (Table 1). Snails that were exposed to predation cues had both shorter and narrower shells compared to control snails (Table 1). Snails exposed to *Hemigrapsus nudus* + crushed conspecifics had an average shell width of 9.62 mm (± 0.10 SE) and length of 9.99 mm (± 0.11 SE) while control snails had an average shell width of 10.09 mm (± 0.09 SE) and length of 10.62 mm (± 0.12 SE) (Fig. 3). Thus, snails that were exposed to predation cues had 6.0% shorter and 4.6% narrower shells than control snails.

I found no difference in strength between treatments when shell size was taken into account (Table 1). Larger shells were harder to break than smaller shells independent of treatment and there was no difference in shell strength between treatments when shell size was used as a covariate. The ANCOVA assumption of parallel lines between treatments was met as the interaction between shell length and treatments was not significant. On average, it took 116.8 N (± 3.6 SE) to crush a snail shell.

Table 1. Results of ANOVA for lip thickness, shell width, shell length and shell strength. ANCOVA for shell strength is shown with shell length as a covariate.

Effect	df	Sum of Squares	F value	*P* value
Lip Thickness				
Treatment	1	0.00066	0.25	0.62
Residuals	34	0.091		
Shell Width				
Treatment	1	2.02	12.76	0.001
Residuals	34	5.37		
Shell Length				
Treatment	1	3.53	14.63	0.0005
Residuals	34	8.21		
Shell Strength				
Length	1	176.74	19.5	0.0001
Treatment	1	3.21	0.355	0.55
Residuals	33	299.03		

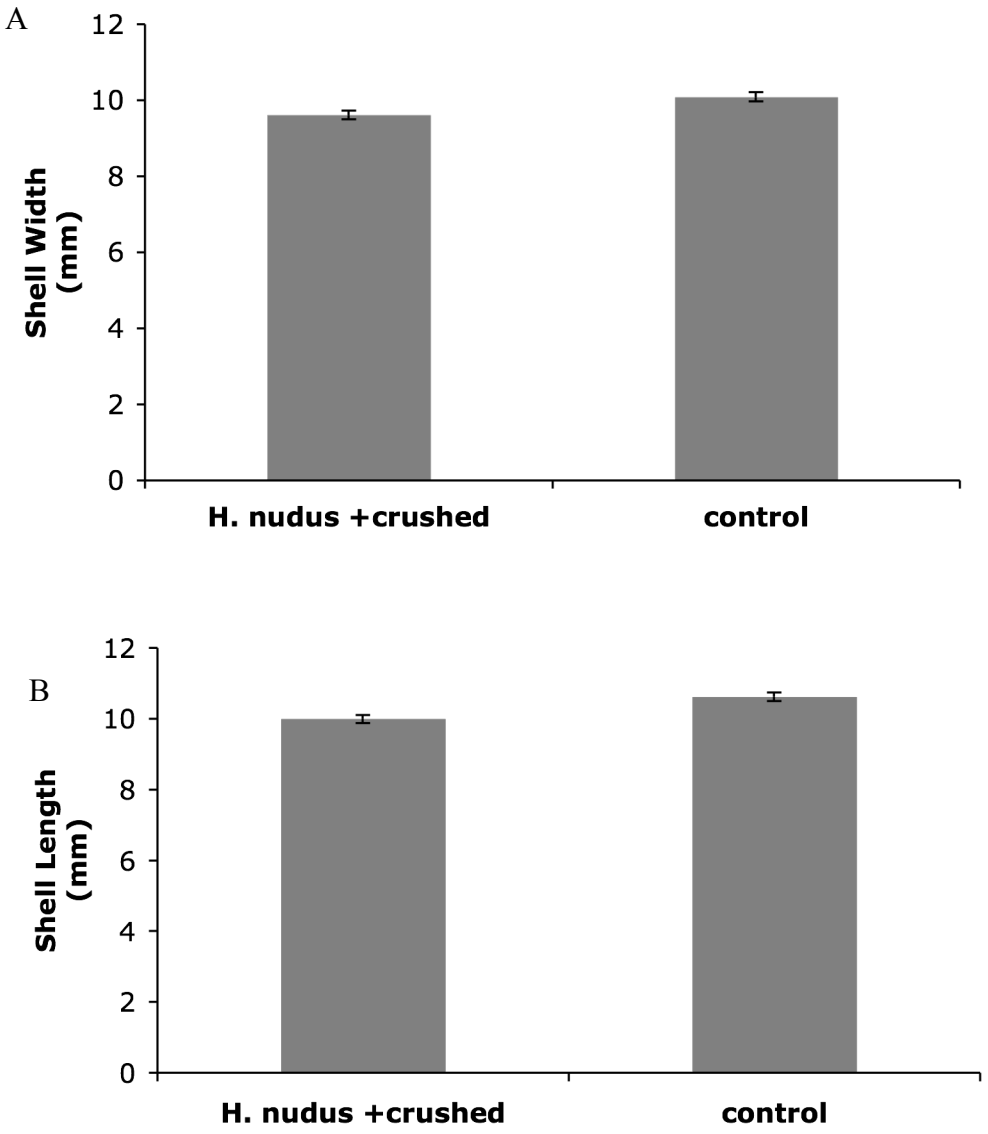

Figure 3. A) Snail shell width and B) shell length when *L. sitkana* was exposed to cues from *H. nudus* + crushed conspecifics , or to a no cue control. Each treatment had 18 replicates. Error bars denote standard error.

Effect of crabs and crushed conspecifics on feeding and habitat use

Snails exposed to just crushed conspecifics consumed less algae than control snails. The grazing parameter for control snails was 0.13 (± 0.037 SE) and 0.046 (± 0.027 SE) for snails exposed to cues from crushed conspecifics (Fig. 4). Snails that were exposed to *H. nudus*, as well as the combination of *H. nudus* + crushed conspecifics, were not significantly different from the control (Table 2).

Snails that were exposed to crushed conspecifics or cues from *H. nudus* +crushed conspecifics utilized the upper corner of the watch glass more often than control snails did (Fig. 5). Snails that were exposed to cues from crushed conspecifics and *H. nudus* +crushed conspecifics were found in the upper corner of the watch glass for 49% (±4.3 SE) and 47% (±3.3 SE) of the observations respectively while control snails were found in the upper corner of the watch glass for 33.74% (±4.1 SE) of the observations. Snails that were exposed to *H. nudus* were not significantly different from the control (Table 3). There was no difference in the frequency at which snails utilized hiding habitat between treatments (Table 3).

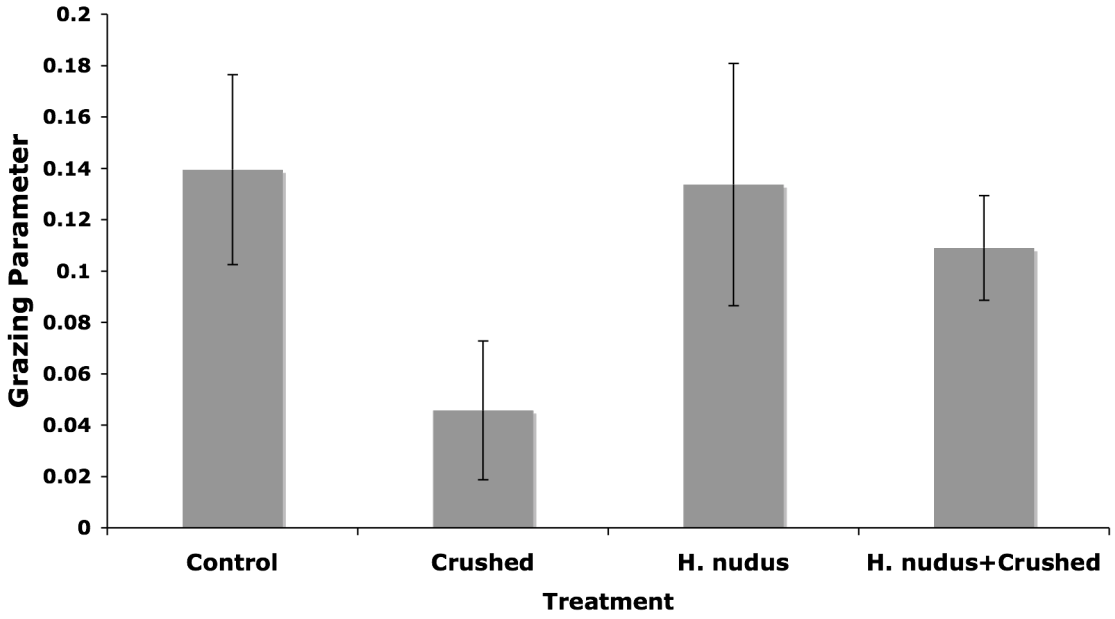

Figure 4. Grazing parameters calculated for grazing *L. sitkana.* Snails were exposed to crushed conspecific snails, caged *H. nudus* crabs, both crabs and crushed snails, or a control with no crabs and uninjured snails. Each treatment had six replicates. Error bars denote standard error.

Table 2. One tailed treatment t-tests comparing grazing parameters of snails in the three treatments to the control.

Effect	t	df	*P* value
Grazing Parameter			
Crushed vs. Control	-2.6	6.1	0.035
Crab vs. Control	-1.5	10	0.46
Crushed + Crab vs. Control	-1.07	9.84	0.25

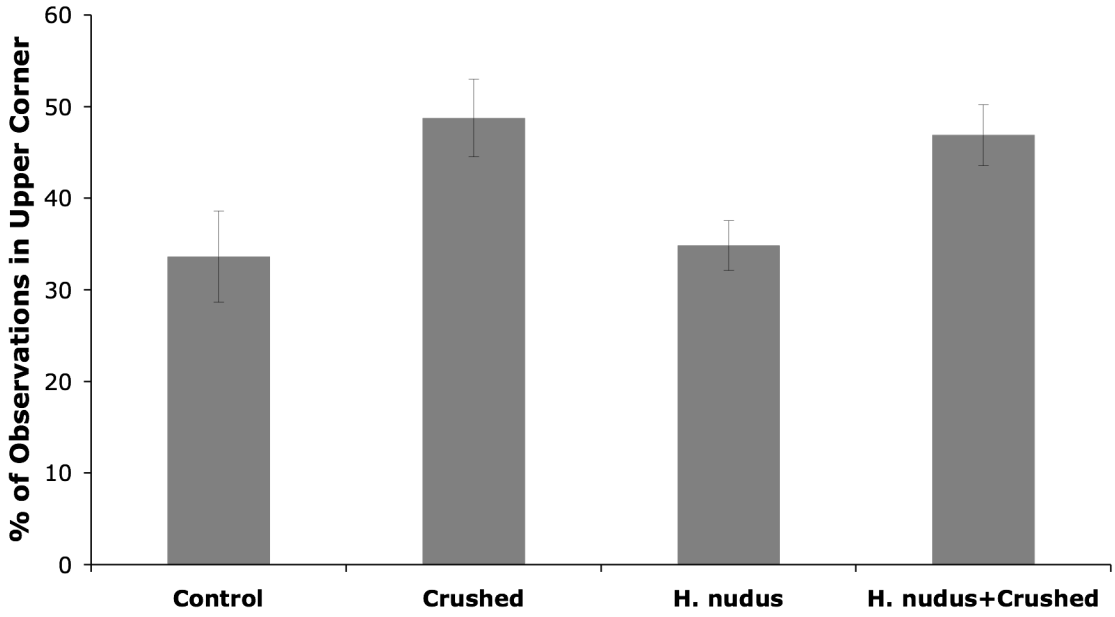

Figure 5. Percent of observations recorded in the upper corner of the watch class by *L. sitkana*. Snails were exposed to crushed conspecific snails, caged *H. nudus* crabs, both *H. nudus* crabs and crushed snails, or a control with no crabs and uninjured snails. Each treatment had six replicates. Error bars denote standard error.

Table 3. One tailed treatment t-tests comparing the percent of observations recorded at a particular location to the control.

Effect	t	df	*P* value
Hiding			
Crushed vs. Control	1.74	10	0.056
Nudus vs. Control	-1.06	8.74	0.84
Crushed+*Nudus* vs. Control	1.07	9.83	0.15
Upper Corner			
Crushed vs. Control	-2.33	9.74	0.021
Nudus vs. Control	-0.21	7.72	0.42
Crushed+*Nudus* vs. Control	-2.21	8.71	0.027

DISCUSSION

In this study, I found that *Littorina sitkana* does not alter its shell thickness in response to *Hemigrapsus nudus* feeding on *L. sitkana*. One possible explanation as to why I did not see shell thickening in *L. sitkana* is that many of the snails used in this study had a shell length greater than 10 mm, the threshold beyond which *Hemigrapsus nudus* can no longer effectively consume *L. sitkana* (Yamada and Boulding 1998). Thus many of the snails in this study did not need a plastic defense from *H. nudus* as they already had a constitutive one. In other systems, studies have shown and models predicted that some prey species of different size classes will lose their plastic defenses as they develop more constitutive ones (Riessen and Trevett-Smith 2009, Rabus and Laforsch 2011). It is possible that *L. sitkana* employs a similar strategy and future studies would need to look specifically at how snails of different size classes respond to feeding *H. nudus* crabs.

It is also possible that shell thickening has become a fixed trait in *L. sitkana*. Both *L. subrotundata* and *L. obtusata*, two species that are adjacent to *L. sitkana* on the *Littorina* phylogenetic tree, show plastic shell thickening (Trussel 2000, Dalziel and Boulding 2005, Reid et al. 2012). This suggests that the common ancestor of these species would be able to plastically thicken its shell. Additionally, *L. sitkana* is generally thicker shelled than *L. subrotundata*, and that thicker shell can deter predation (Trussel 2000, Boulding et al. 1999). Because, *L. sitkana* has a thicker shell than a closely related species that does show plastic shell thickening, and that thicker shell increases *L.*

sitkana's fitness, it seems possible that having a thick shell has become a fixed as opposed to a plastic trait in *L. sitkana*.

The lack of shell thickening is also of note because it occurred in response to the same treatments that produced smaller shells and a reduction in feeding behavior. In other species of intertidal gastropods, it has been argued that changes in shell size and thickness are passive results from a change in snail feeding behavior. Bourdeau (2009) found that there was no difference in shell thickening between whelks that were exposed to predatory crabs and whelks that were starved. He also found that as size decreased shell thickness increased, suggesting that a reduction in feeding may causes a change in shell size and thickness. In my study, snails that were exposed to cues from crushed conspecifics + *H. nudus* crabs decreased shell growth but showed no difference in lip thickness among treatments. This shows that decreased shell growth does not always correlate to increased shell thickness. Additionally, in the feeding and behavior study, snails that were exposed to crushed conspecifics + *H. nudus* crabs showed no change in feeding behavior. Although these data were recorded in a separate study from the morphology data, this suggests that changes in morphology may be occurring separately from changes in feeding behavior.

L. sitkana decreased shell length and width in response to the *H. nudus* + crushed conspecific treatment. Based on *H. nudus*'s preference for small size classes of *L. sitkana,* this is not the response I was expecting to see. However, this response is consistent with those found in other studies looking at the effect of *Cancer productus* on

L. sitkana shell growth. These studies found that *L. sitkana* decreases shell growth in response to *C. productus* + crushed conspecifics, and that smaller snails were less likely to be consumed by *C. productus* (Yamada et al. 1998). Although the time required for *C. productus* to break *L. sitkana* shells increased with snail size, larger snails, which have stronger shells, are still the preferred prey of *C. productus* (Yamada and Boulding 1998). Because *L. sitkana* alters its shell size in a way that reduces its risk to *C. productus* and not to *H. nudus*, it suggests that *C. productus* may pose more of a threat to *L. sitkana* than *H. nudus*.

The effect of crushed conspecifics on snail feeding and habitat use in this study is similar to those found in previous experiments using different crab predators. In one such study, *L. sitkana* decreased foraging in response to the combination of crushed conspecifics + *C. productus* (Yamada et al. 1998) but not in response to non-feeding *C. productus*. These responses suggest that there is some type of alarm cue released by crushed conspecifics that activates the behavioral defenses in *L. sitkana*. I found a similar decrease in snail feeding, but only when snails are exposed to just the crushed conspecific cue. When snails were exposed to a combination of crushed conspecifics + *H. nudus* crabs, no change in feeding behavior was recorded. *L. sitkana* habitat use changed when snails were exposed to either crushed conspecifics or the combination of *H. nudus* + crushed conspecifics. Although there was no overall change in the frequency with which snails utilized habitat that was considered hiding, cues from predators did cause snails to spend more time in the upper corner of their watch glass. This observation is consistent with previous work showing *L. sitkana* move upward within their habitat when exposed to

cues from *C. productus* feeding on *L. sitkana* snails (Yamada 1998, Rochette and Dill 2000). Based on *C. productus*'s occupancy of the mid to lower intertidal, moving up in the intertidal seems to be the appropriate response for *L. sitkana* to avoid *C. productus.* It may be that crushed conspecifics serve as an indicator for *L. sitkana* to the presence of feeding *C. productus*.

The effect of crushed conspecifics on snail feeding was nullified if the crushed conspecific cue was combined with a cue from *H. nudus*. This finding is interesting as it raises the question of why *L. sitkana* would employ an inducible defense in the presence of crushed conspecifics but not employ that same defense when the crushed conspecific cue is combined with the presence of a potential predator. One possible reason is that large *L. sitkana* may use the presence of *H. nudus* to determine whether its environment is safe. Marco and Palmer (1991) found that the whelk *Nucella lamellosa* may use the presence of *H. nudus* to detect a safe environment and it is possible that *L. sitkana* is making a similar assessment. Additionally, it would be maladaptive for the large *L. sitkana* to alter their behavior in response to conspecifics being crushed by *H. nudus* as only small snails can be consumed by *H. nudus*. If *L. sitkana* senses crushed conspecifics anything could be consuming snails in the adjacent environment, including *C. productus*. This would be a high-risk situation for all *L. sitkana* snails. However if *L. sitkana* senses crushed conspecifics and cues from *H. nudus*, this would represent a situation in which only small snails could be consumed. This would represent a low risk environment for large snails and they would not be under selective pressure to respond plastically. As many of the snails used in this study had a shell length of larger than 10mm, many of the

snails I used would not be under selective pressure to plastically respond. However, this hypothesis is at odds with the reduction in shell size and change in habitat use I found when snails were exposed to a crushed conspecific + *H. nudus* cue. If snails employ a morphological and crawl away defense in the presence of a certain combination of cues, it would seem logical for it to employ its feeding counterpart in response to those same cues. Future studies could investigate how different size classes of snails alter their behavior to a range of cues associated with different levels of predation risk.

CHAPTER 3: PREDATORS AND CONSPECIFICS ALTER THE TIME AND RATE AT WHICH A MARINE SNAILS DEPOSITS EMBRYO CAPSULES

INTRODUCTION

Many organisms alter their behavior, morphology, physiology, and life history in response to environmental threats (Agrawal 2001, Oyarzun and Strathman 2011). The ability to properly assess and respond to threats in the environment can significantly increase an organism's fitness. Many studies have shown encapsulated embryos alter the time at which they hatch from their encapsulated state in response to predators (Warkentin 1999, Relyea 2003, Bernard 2006, Gomez-Mestre and Warkentin 2007, Gomez-Mestre et al. 2008b, Miner et al. 2010, Oyarzun and Strathmann 2011, Warkentin 2011). Changing the timing at which an individual transitions between early life-history stages can also have strong influences on an organism's fitness later in life (Buckley et al. 2005).

Fitness at early life history stages can also be strongly influenced by paternal effects. Parents can alter many aspects of their offspring's early life history, which can have profound consequences on offspring fitness later in life (Morgan and Christy 1994, Bridges and Heppel 1996, Sinervo and Doughty 1996, Dziminski and Roberts 2006). Parental behaviors can potentially alter offspring development rate, access to food, ability to avoid predators, and mate choice (Fox et al. 1996, Fox et al. 1997, Mousseau and Fox 1998, Allen et al. 2008). One of the ways adults influence their offspring's fitness is by

altering the time at which they deposit their offspring (Lambrechts and Perret 2000, Hipfner et al. 2005, Visser et al. 2009, Ahola et al. 2012). If cues from predators cause juveniles to alter aspects of their early life history, adults might tune into the same predator cues that juveniles respond to and manipulate their reproductive behavior accordingly.

Changing the timing of egg deposition could potentially confer a fitness advantage similar to those associated with juveniles altering their time-to-hatching. If adults can sense and respond to organisms that prey on their offspring, shifting the timing of egg deposition could alter when the juveniles switch from one life stage to another (Fig. 6). This shift could alter the type of predation risk juveniles are exposed to, and when they are exposed to that risk. It is also possible that changing time-to-deposition could compound the effects of a change in time-to-hatching. For example, in the case where predator presence delays hatching, any delay in depositing embryos would further delay when the juveniles enter the environment with the predators.

The intertidal snail *Nucella lamellosa* is a prime organism to test whether parents can alter timing of egg deposition in response to predators that consume only embryos and juveniles. *Nucella lamellosa* is an intertidal whelk that deposits numerous capsules with ~20-60 embryos per capsule, attaching the capsules to rocks or other hard surfaces (Spight and Emlen 1976). Larval development is direct and occurs exclusively within the capsule over the course of 1-2 months (Strathmann 1987). Crawling juveniles hatch

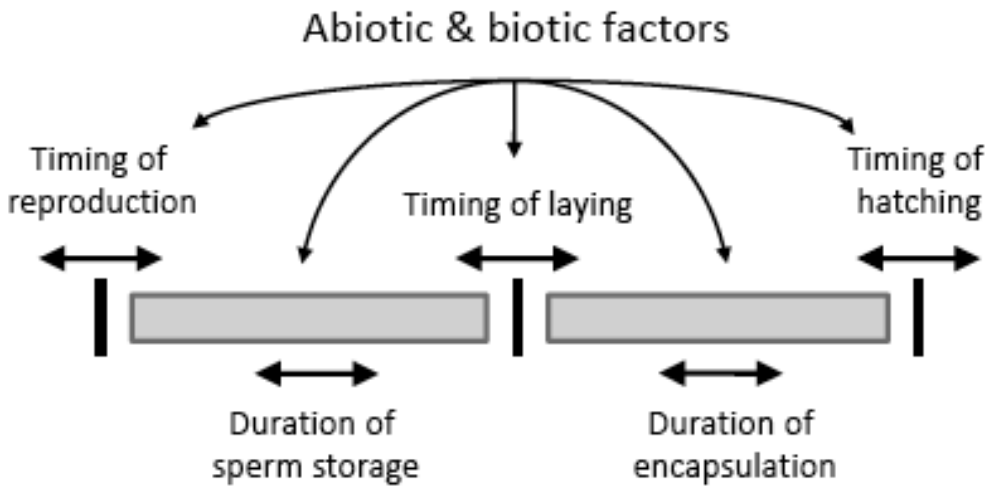

Figure 6. Flow chart depicting possible switch-points during reproduction and embryonic development. Abiotic and biotic factors could affect multiple reproductive and developmental stages, yielding cumulative shifts in time-to-hatching.

directly from the capsule, which limits their ability to relocate to a potentially safer environment. Additionally, juvenile *N. lamellosa* exhibit hatching plasticity in response to predatory crabs, suggesting that plastic timing at early life-history stages is advantageous (Miner et al. 2010). It may also be advantageous for adults to alter the timing of capsule deposition in response to these same predatory crabs. With such limited mobility in the larval state, the parental effects might be paramount to the survival of the encapsulated juveniles. Finally, adult *N. lamellosa* express inducible defenses in both their morphology and behavior, which indicates that adults can detect and respond to predators.

Besides changing when egg capsules are deposited, adult *N. lamellosa* may also alter other aspects of reproductive effort, such as the number of capsules deposited, the energy invested into those capsules or the morphology of the capsules in response to predators. By lowering the number of capsules deposited in the presence of egg predators, an adult would potentially conserve energy that could go towards reproduction at a later date. Previous work has shown that predatory crabs alter the amount of time *N. lamellosa* embryos spend within their capsules (Miner et al. 2010). Thus, parents may provide their embryos with variable levels of yolk nutrition to see them through different durations of embryo encapsulation. Also, the capsules could be made with tougher walls or a particular shape to deter predators. Adults of a closely related species, *Nucella ostrina* (formerly *N. emarginata*), produce egg capsules with walls of different thickness, and these thick-walled capsules are less likely to be preyed upon by isopods than thinner-

walled capsules (Rawlings 1990). It is possible that variation in the thickness of capsule walls is a plastic response to cues from isopods and we might expect to see a similar response in *N. lamellosa*.

It is also possible that different densities of conspecific snails may alter some of these same characteristics, as *N. lamellosa* embryos alter their time to hatching in response to the presence of adult conspecifics (Miner et al. 2010). Snail embryos exposed to elevated numbers of conspecifics hatched from the encapsulated state earlier than control embryos. In this situation, adult snails might provision their offspring with less energy in an egg, as embryos will spend less time within the capsules. Adults could then divert the "saved" energy into creating more offspring. Additionally, elevated levels of conspecifics could indicate that the capsule environment is relatively safe. If this were the case, parents may put less energy into capsule walls, as protection from predators would be less necessary.

In this study, I investigated whether adult *N. lamellosa* alter the timing of capsule deposition, the number of capsules deposited, the energy invested into those capsules and the morphometry of the capsules. I tested these factors in response to the environmental conditions known to induce hatching plasticity in embryonic *N. lamellosa*. Specifically, I tested whether exposure to predators that only pose a threat to juveniles (*Hemigrapsus oregonensis* crabs and *Idotea wosnesenskii* isopods), non-predatory crabs, and adult conspecifics altered capsule time-to-deposition, number of capsules deposited, capsule energetics, and capsule size, shape, and wall strength.

METHODS

I conducted three experiments to test whether different predators, non-predatory crabs, and adult conspecifics affect the reproductive strategy of *Nucella lamellosa*. I measured time to deposition of the first capsule, rate of capsule deposition, capsule morphometry, capsule-wall strength, capsule-wall thickness and the caloric content of the capsules and embryos.

General experimental design

All three experiments had a similar design. Experimental units were 10 L glass aquaria containing either 10 or 20 experimental adult *N. lamellosa* (depending on the experiment, details below) and a plastic container enclosing the treatment organisms (either predators, conspecifics, or no organisms). Each aquarium was equipped with a filter that contained a sponge and pumice blocks to remove large particulate matter and provide surface area for microbes that convert nitrogenous waste to less toxic forms. I removed the activated-carbon filter, which might bind organic compounds released by predators or snails that serve as cues for adult whelks. All aquaria were held in a 10°C cold room at Western Washington University, Bellingham, WA.

Prior to each experiment, adult snails were collected when they were aggregating to reproduce from a local rocky beach (Marine Park, Bellingham, WA). Crabs

(*Hemigrapsus oregonensis* and *Pagurus granosimanus*) and isopods (*Idotea wosnesenskii*) were collected from adjacent areas of the same beach. Two other species of crabs (*Pugettia* spp., either *P. producta* or *P. gracilis,* and *Petrolisthes eriomerus*) were collected from a beach at Shannon Point Marine Center, Anacortes, WA. Predators and whelks were transported to the Western Washington University campus in separate containers and held in separate 20 L aquaria until the start of the experiments (1-3 days).

Experimental adult *N. lamellosa* were haphazardly added to each aquarium. I then randomly assigned each aquarium a treatment and added the appropriate individuals (crabs, isopods, or adult conspecific snails) for that treatment. Predators or conspecifics used to produce cues were enclosed in a mesh-lined plastic container to prevent them from directly contacting the experimental whelks and the egg capsules they produced. Control treatments had the plastic container with nothing in them.

At the beginning of each experiment, I carefully inspected aquaria for capsules every day. When the first capsules were found, I removed them from their attachment point with a razor blade and saved them for future measurements. I subsequently removed capsules every four days to minimize disrupting adults depositing capsules while maximizing temporal resolution. I recorded the number of capsules removed from each tank, which allowed me to determine when whelks started depositing capsules and the average rate they laid capsules during the experiment.

I measured morphometry and wall strength of the first 10 capsules deposited in each tank. If 10 or more capsules were initially collected, 10 capsules were chosen haphazardly and measured. If fewer than 10 capsules were collected, I measured all capsules present on the first collection, and used capsules gathered on subsequent collections to total 10 capsules. Capsule length and width were measured by taking digital photographs of each capsule and analyzing the images with Image J software (NIH, http://rsbweb.nih.gov/nih-image). Capsule length was measured from the tip of the capsule plug to the base of the capsule where the stalk began (the stalk was not measured) and capsule width was measured at the midpoint of the length (Fig. 7). I determined the shape of each capsule by calculating the ratio of length to width.

Capsule wall strength was measured in two ways after the capsule was photographed. I first measured intact capsules and then I measured previously punctured capsules because we assumed these measurements would provide us information about different aspects of capsule strength. The force required to puncture an intact capsule was likely influenced by the strength of the capsule wall, capsule shape, and internal pressure of the capsule. I predicted that this value would be more representative of how much force a predator would need to apply in the field to pierce a capsule. The force required to pierce a punctured capsule was likely influenced primarily by the capsule wall strength because the internal fluid and eggs had been removed. This value only tells us whether or not the material used to produce the capsule is resistant to puncturing. Measuring the force required to puncture an intact capsule vs. a punctured capsule allowed me to determine whether or not it is that capsule wall material itself or the morphology of the intact

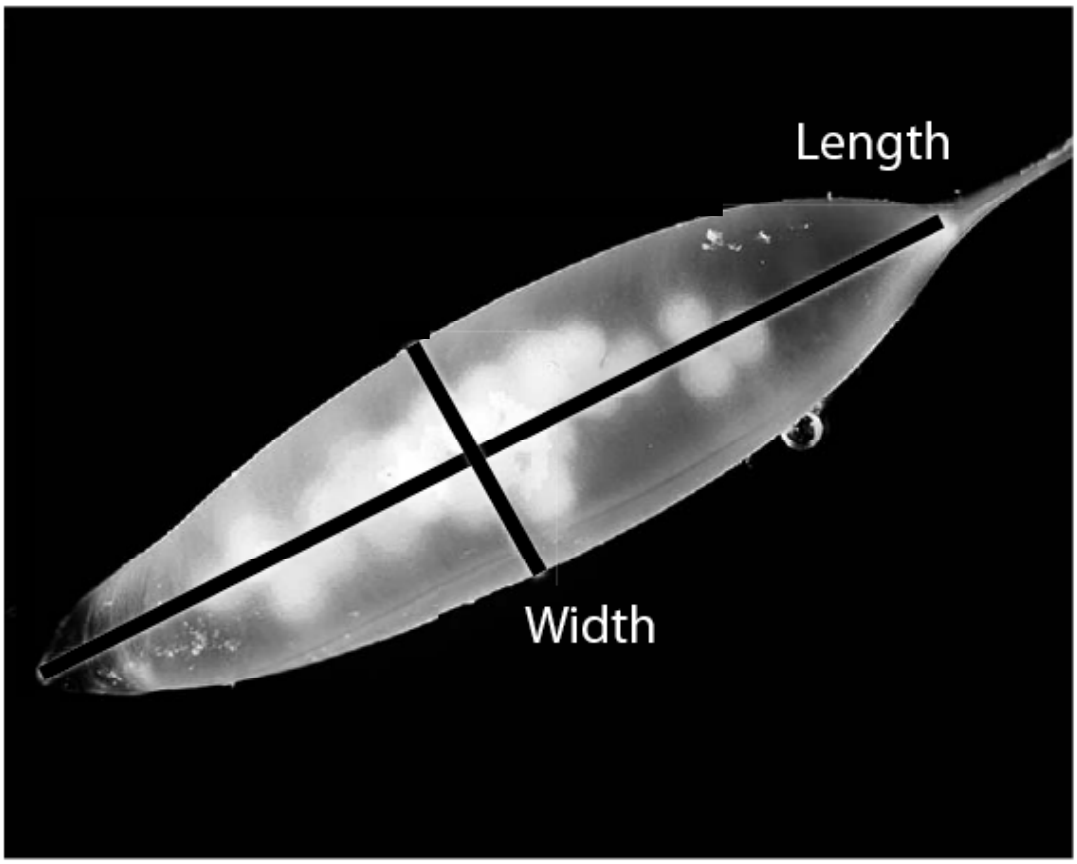

Figure 7. Digital photograph of a *Nucella lamellosa* embryo capsule showing the placement of the length and width measurements. Width was measured at the point on half the total length. "Shape" was calculated as width/length. Capsules were punctured where the width and length measurement lines crossed.

capsule that account for an increase in force required to puncture an intact capsule. To measure force needed to puncture intact capsules, each capsule was punctured with a blunt probe (0.75 mm in diameter) attached to a force transducer sensitive to 0.3 N. To hold the capsule steady but not compress it, I used two small sheets of Plexiglas, one of which had a small capsule-sized well carved into it. The sheets were separated with spacers. A hole, 1 mm in diameter was drilled through the sheets immediately over and under the well, which allowed us to insert the probe through the hole and puncture the capsule. Each capsule was placed in the well and held between the two Plexiglas sheets. I then inserted the probe and depressed it with the force transducer until the probe punctured the capsule. All capsules were pierced near the middle of the capsule where the length and width measurements intersected (Fig. 7). The maximum force generated was recorded. To measure the force needed to pierce a punctured capsule (i.e., a capsule not filled with fluid), each capsule was punctured again in the middle of the capsule but a new location using the above methods, with the exception that I removed the spacers from between the Plexiglas sheets. Without the spacers, the capsule was compressed between the two Plexiglas sheets and the internal contents were expelled before the capsule walls were punctured.

After measurements were made on the first 10 capsules, all remaining capsules were frozen in an -80°C freezer for caloric analysis at a later date. To measure the caloric density of embryos, embryos were removed from 5 thawed capsules using scissors and forceps and then dried to a constant mass at 60°C. Dried embryos were then compressed

into pellet form. Pellet masses ranged from approximately 2-15 mg. These values were within the range of masses that the Phillipson microbomb calorimeter used in this study is designed to burn. After pellets were weighed, they were burned using the bomb calorimeter following the technique described by Phillipson (1964). The value for energy released during the embryo burning was compared to a benzoic acid standard that was used to calibrate the calorimeter. Three pellets where burned per treatment or control tank and these values were averaged within each tank.

Predator experiment

Intertidal shore crabs *Hemigrapsus oregonensis* and isopods *Idotea wosnesenskii* were used in this study because they both prey on embryo capsules of *Nucella ostrina* (Rawlings 1990), a species closely related to *N. lamellosa* and often found in the same habitat. Additionally, these same species are known to induce changes in time to hatching in encapsulated *N. lamellosa* (Miner et al. 2010). I also verified in a pilot study that both species consume capsules of *N. lamellosa*. I designed a fully factorial 2x2 experiment to test whether *H. oregonensis* and *I. wosnesenskii* affected the timing or the rate at which *N. lamellosa* deposited capsules, as well as the morphometry or strength of the capsules. Each factor, crab or isopod, had two levels, present or absent. This resulted in the following four treatments: 1) crab only 2) isopod only, 3) crab and isopod, and 4) a no predator control.

The crab treatment consisted of 4-5 *H. oregonensis* crabs contained within each plastic container, the isopod treatment consisted of 2-3 *I. wosnesenskii* isopods, and the no predator control had no crabs or isopods within the container. Each treatment had 6 replicates for a total of 24 experimental aquaria. All treatment organisms were starved except for the crabs in the *H. oregonensis* and "crab and isopod" treatment. Within these containers the crabs consumed several to all of the isopods present. This interaction was unintentional.

Twenty experimental *N. lamellosa* were placed in each aquarium and the aquaria were monitored for a total of 16 days after the first capsules were laid. Capsules were collected every four days yielding four separate collections of egg capsules per tank. I recorded the time to first capsule deposition, the rate of capsule deposition, capsule morphometry, capsule wall strength and caloric value of the embryos for each aquarium.

Crab species experiment

To test whether different taxa of crabs induce reproductive plasticity in *Nucella lamellosa*, I designed a one-factor experiment with five treatment levels. The five levels included: 1) *H. oregonensis* (seven crabs per aquarium), 2) *Pugettia* spp. (*P. gracilis* and *P. producta*; five crabs per aquarium), 3) *Petrolisthes eriomerus* (five crabs per aquarium), 4) *Pagurus granosimanus* (seven crabs per aquarium) and 5) a control treatment with no crabs present. Crab numbers varied in an effort to have roughly equal crab biomass in each aquarium. In a pilot study, I verified that *H. oregonensis* and

Pugettia spp. consume capsules and that *P. eriomerus* and *P. granosimanus* do not. Each treatment had five replicates yielding 25 experimental aquaria.

20 experimental *N. lamellosa* were added to each aquarium, and aquaria were monitored for 16 days after the first capsules were deposited. Capsules were collected every four days yielding four separate collections of egg capsules per tank. I measured the time elapsed in each aquarium until the first capsule was laid, average rate of capsule deposition, and capsule wall strength. Capsule morphometry and the caloric value of the embryos were not measured because they were not significantly affected by the presence of predators in the predator study (see Results). Unlike the previous experiment, we directly measured capsule wall thickness. This was done by cutting a cross section from the middle of a capsule using a sharp, single-edged razor blade and taking a digital picture of it using a compound microscope at 1000X magnification. Capsule wall thickness of each cross section was measured using imaging Image J software. These measurements were done with remaining capsules that were not used for other measurements at the end of the study. I measured up to ten capsules per aquaria, depending on how many capsules were available, and averaged these values within a tank.

Conspecific density experiment

To test whether different densities of conspecifics induce reproductive plasticity in *N. lamellosa*, I designed a one-factor experiment with three levels. The three levels were

different densities of adult *N. lamellosa*: 1) high density (20 additional snails held in the plastic container), 2) low density (10 additional snails in the container), and 3) control (no additional snails in the container). Each of the three treatments had 8 replicates, for a total of 24 aquaria.

10 experimental *N. lamellosa* were in each aquarium and aquaria were monitored for 20 days. Capsules were collected every four days yielding five separate collections of egg capsules per tank. I measured the time elapsed in each tank until the first capsule was laid, rate of capsule deposition, as well as the caloric value of the embryos. Wall strength and capsule morphometry were not measured in this study because I expected changes in morphometry to be adaptations to predation risk and thus not affected by the presence of conspecifics.

Analyses

For all analyses of the predator experiment, I had two predictor variables: crab (two levels) and isopod (two levels). For the crab species and conspecific density experiments, I had a single predictor variable: crab species (5 levels) or conspecific density (3 levels).

For the analyses of size, shape, thickness of wall, strength of capsules and capsule caloric value, I used ANOVA. The response variables length, width, width/length, wall thickness of capsules, capsule energetics, force to pierce intact or punctured capsules

were averaged for each aquarium—the averages per tank were used because capsules were not independent of aquaria.

I analyzed when whelks started depositing capsules with generalized linear models (GLM) with a Poisson error and an identity link. The Poisson distribution was used to model the error because the response variable was an integer (number of days) and the variance increased with the mean. The response variable was number of days to first observed capsules in an aquarium and was measured in 4 day intervals. The first time I observed capsules during an experiment was assigned day 0, and I added 1 d to each observation because some treatments were all zero (with a variance of zero). After analyses were complete, the 1 day shift was taken away to show the data in the form it was collected. For the predator experiment, I used AIC values to compare the fully-crossed model with reduced models to determine which factors best fit the data given the number of parameters in the model. For the conspecific density and crab species experiment, both of which had a single factor with more than 2 levels, I tested whether each treatment differed from the control with *a priori* contrasts. For the crab species experiment, I used one-tailed contrasts because whelks delayed depositing capsules when exposed to cues from predators, and I expected the same direction of response for other crab species.

I analyzed the data for the rate at which whelks deposited capsules with generalized linear mixed-effects models (GLME) with a Gaussian error and an identity link—to account for repeatedly sampling each aquarium through time, I modeled aquarium as a

random variable. The response variable was number of capsules collected at each 4-day interval. I used the same methods as with the analysis of when whelks started depositing capsules to determine which factors were important predictors and which treatments differed from controls.

RESULTS

Predator experiment

Crabs and the combination of crabs and isopods, but not isopods alone, affected the timing of capsule deposition (Fig. 8A). The full model, which included the interaction between crabs and isopods, fit the data best for the number of parameters (Table 4). Compared to the control (no isopods or crabs), whelks delayed depositing capsules by 6.7 d when exposed to cues from both crabs and isopods and 2.7 d when exposed to only crabs. Snails exposed to just isopods did not delay timing of capsule deposition (Table 5).

Cues from crabs, but not isopods, affected the rate that whelks deposited capsules (Fig. 8B). The average rate of laying for the experiment was 12.8 capsules d^{-1} (± 1.8 SE) and whelks that were exposed to crabs laid capsules at a rate 50% less than those not exposed to crabs. The model that best fit the data included crabs only (Table 6). Compared to the treatments without crabs, whelks deposited fewer capsules when exposed to cues from crabs (Table 7).

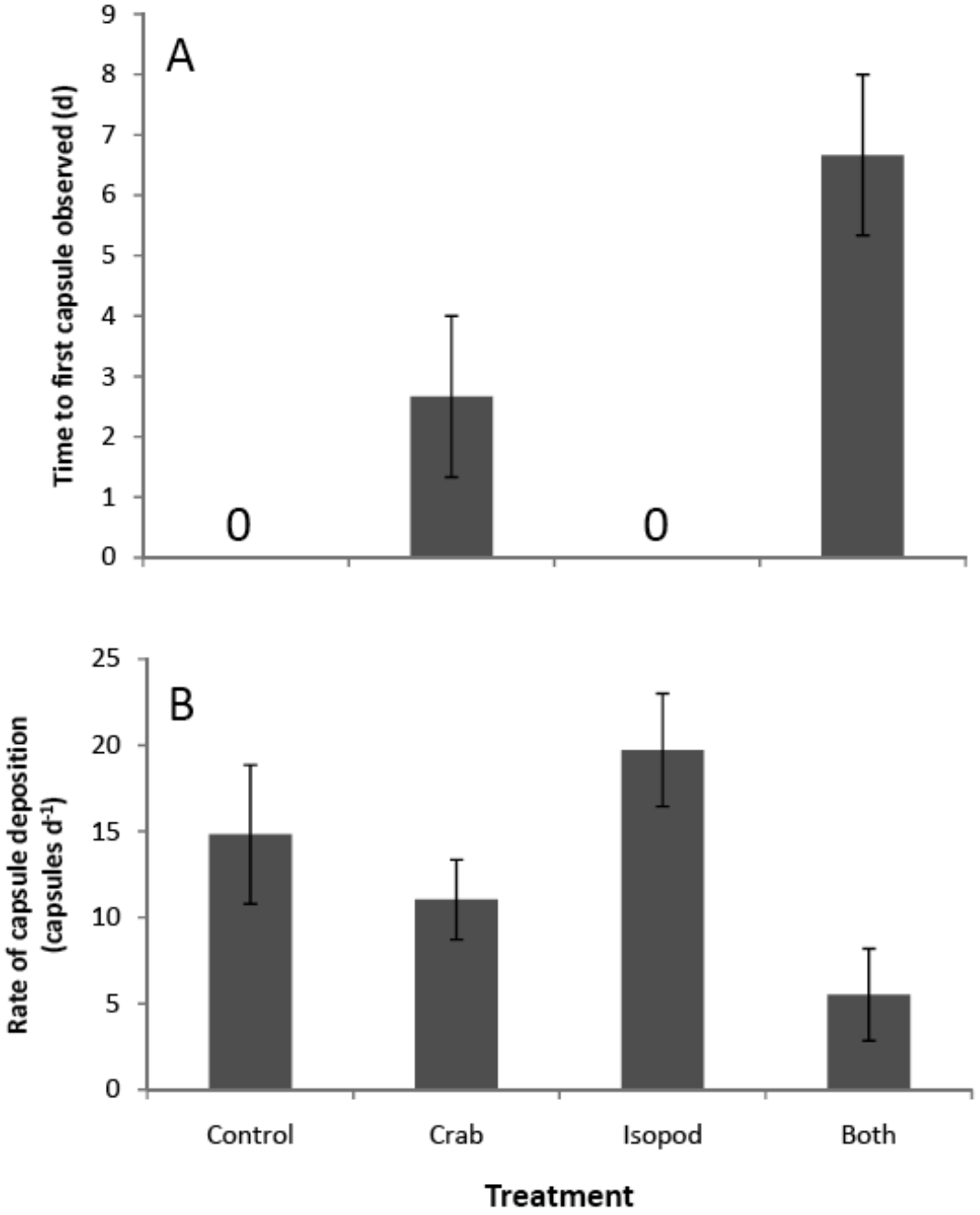

Figure 8. A) Mean number of days until capsules were first observed, and B) mean rate of capsule deposition by whelks exposed to cues from crabs and isopods. Whelks were exposed to cues from caged *H. oregonensis* (Crab), *I. wosnesenskii* (Isopod), both crabs and isopds (Both), or neither crabs nor isopods (Control). Each treatment had six replicates. Error bars represent one standard error.

Table 4. Model comparisons for when whelks started to deposit capsules for the predator experiment.

Model	AIC*
Predator experiment	
Crab × Isopod	93.2
Crab	97.9
Isopod	133.8
Common intercept	139.2

*The smallest Akaike information criteria (AIC) value indicates the most appropriate model given the number of parameters.

Table 5. T-test contrasts between treatments for when whelks started to deposit capsules within each of the three experiments.

Effect	Estimate	SE	z value	*P* value
Predator experiment				
Crab *vs*. no Crab	2.67	0.82	3.02	0.003
Isopod *vs*. no Isopod	≈ 0.00	0.58	≈ 0.0	1.000
Crab × Isopod *vs*. Crab + Isopod	4.00	0.1.49	2.68	0.007
Crab species experiment				
H. oregonensis vs. Control	≈ 0.00	0.85	≈ 0.00	0.50*
Pugettia spp. vs. Control	-0.80	0.75	-1.07	0.86*
P. granosimanus vs. Control	0.80	0.94	0.85	0.20*
P. eriomerus vs. Control	4.00	1.23	3.24	0.0006*
Conspecific density experiment				
Low density vs. Control	-1.50	0.75	-2.00	0.045
High vs. Control	-1.50	0.75	-2.00	0.045

*One-tailed tests, alternative hypothesis crab species > control.

Table 6. Model comparisons for the rate at which whelks deposited capsules for the predator experiment.

Model	AIC
Crab & isopod experiment	
Crab × Isopod	990.4
Crab + Isopod	991.5
Crab	989.5
Isopod	996.9
Common intercept	994.9

Table 7. T-test contrasts between treatments for the rate at which whelks deposited capsules within each of three experiments.

Effect	Estimate	SE	*t* value	df	*P* value
Predator experiment					
Crab *vs*. no Crab	-36.0	12.3	-2.94	22	0.010
Crab species experiment					
H. oregonensis vs. Control	-14.8	8.38	-1.77		0.046*
Pugettia spp. vs. Control	-3.5	8.38	-0.42		0.34*
P. granosimanus vs. Control	-16.4	8.38	-1.96		0.032*
P. eriomerus vs. Control	-17.5	8.38	-2.09		0.024*
Conspecific density experiment					
Low density vs. Control	-2.90	10.87	-0.27	21	0.79
High vs. Control	-4.20	10.87	-0.39	21	0.70

*One-tailed tests, alternative hypothesis crab species < control.

Cues from crabs, isopods, and the combination of crabs and isopods did not affect the size or shape of capsules deposited during the experiment (Fig. 9) (Table 8). Cues from crabs, isopods, and both crabs and isopods did not affect capsule strength (Fig. 10). On average, the force required to pierce an intact capsule was 4.17 N (± 0.35 SE), and the force required to pierce a punctured capsule was 5.97 N (± 0.30 SE). Force to pierce intact or punctured capsules was not significantly different for capsules exposed to crabs, isopods, or the combination of crab and isopod (Table 9). Cues from crabs, isopods and crabs and isopods did not affect the energy invested into embryos (Fig 11) (Table 10).

Crab species experiment

Only cues from *Petrolisthes eriomerus* affected the timing of capsule deposition (Fig. 12A). Whelks started depositing capsules 4 d later when exposed to cues from *P. eriomerus* (Table 6). Whelks exposed to the other three crabs, *Hemigrapsus oregonensis*, *Pugettia* spp., and *Pagurus granosimanus*, did not differ significantly from the control (Table 6). Whelks exposed to cues from *H. oregonensis* started depositing capsules at the same time as whelks in the control, which was contrary to what we observed in the crab and isopod experiment.

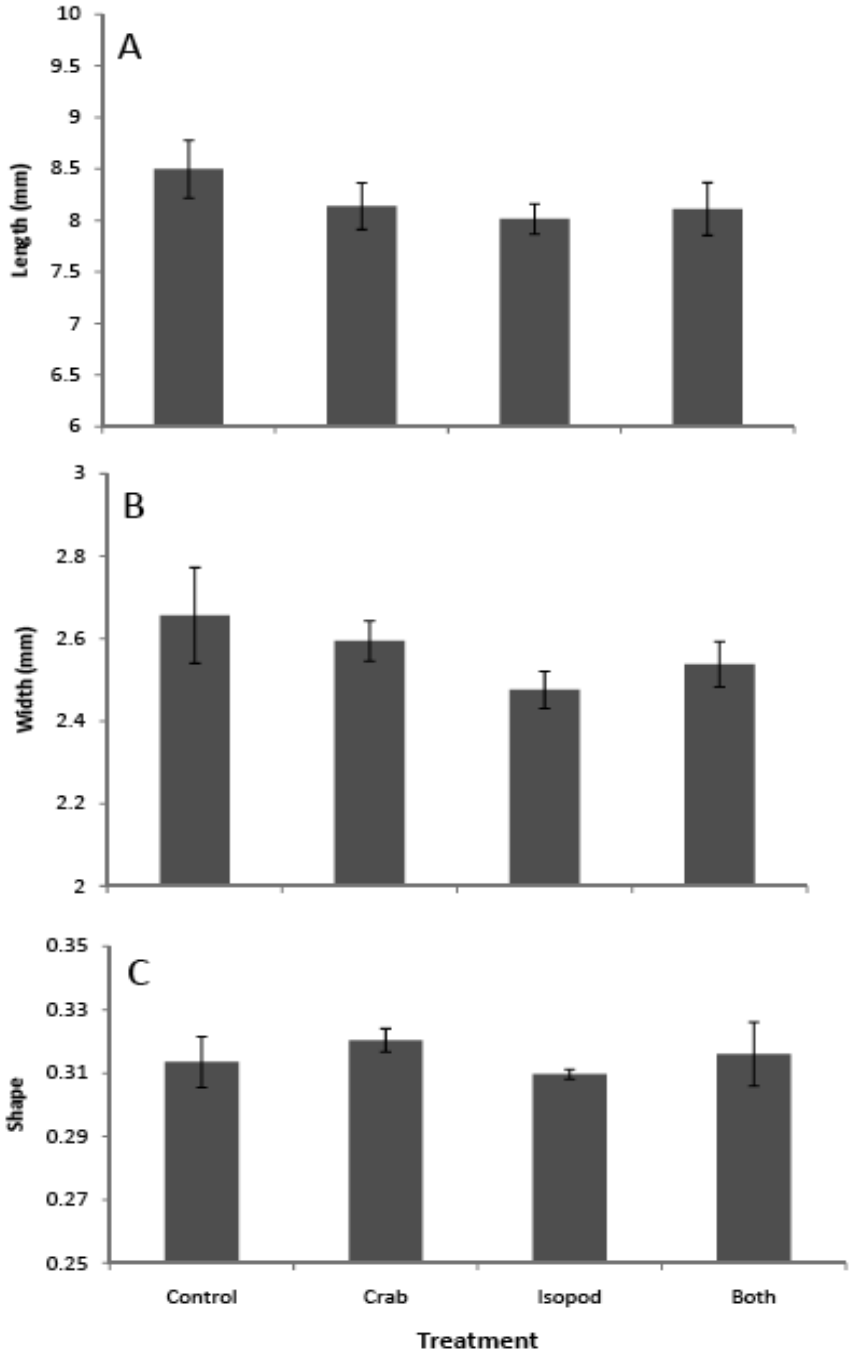

Figure 9. A) Mean length (mm), B) mean width (mm), and C) mean width/length or shape of capsules deposited by whelks exposed to cues from crabs and isopods. Whelks were exposed to cues from caged *H. oregonensis* (Crab), *I. wosnesenskii* (Isopod), both crabs and isopds (Both), or neither crabs nor isopods (Control). Each treatment had six replicates. Error bars represent one standard error.

Table 8. Results of ANOVA for size and shape data of capsules deposited by whelks exposed to crabs and isopods.

Effect	df	Sum of Squares	F value	*P* value
Length				
Crab	1	0.39	1.27	0.27
Isopod	1	0.11	0.37	0.55
Crab × Isopod	1	0.30	0.97	0.34
Residuals	19	5.84		
Width				
Crab	1	0.08	2.73	0.11
Isopod	1	0.00005	0.002	0.97
Crab × Isopod	1	0.02	0.71	0.41
Residuals	19	0.60		
Width/Length				
Crab	1	0.0001	0.49	0.49
Isopod	1	0.0003	1.12	0.30
Crab × Isopod	1	< 0.0001	0.002	0.96
Residual	19	0.004		

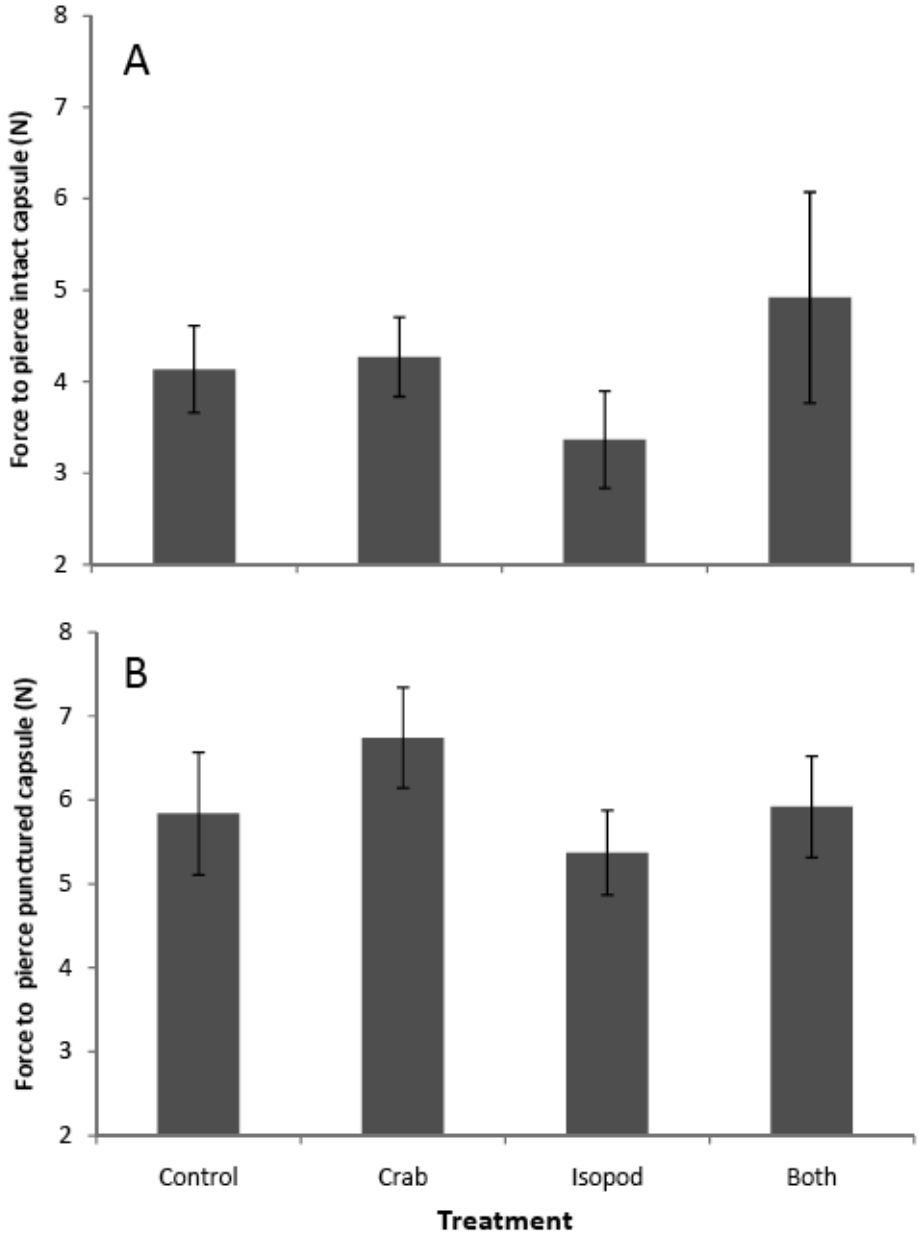

Figure 10. Mean force (N) required to pierce A) intact-capsules and B) punctured-capsules deposited by whelks exposed to cues from crabs and isopods. Whelks were exposed to cues from caged *H. oregonensis* (Crab), *I. wosnesenskii* (Isopod), both crabs and isopds (Both), or neither crabs nor isopods (Control). Each treatment had six replicates. Error bars represent one standard error.

Table 9. Results of ANOVA for strength of capsules deposited by whelks exposed to crabs and isopods.

Effect	df	Sum of Squares	F value	*P* value
Intact capsules				
Crab	1	4.27	1.40	0.25
Isopod	1	0.022	0.007	0.93
Crab × Isopod	1	3.01	0.99	0.33
Residuals	20	60.98		
Punctured capsules				
Crab	1	3.15	1.39	0.25
Isopod	1	2.50	1.10	0.31
Crab × Isopod		0.19	0.08	0.77
Residuals	20	45.29		

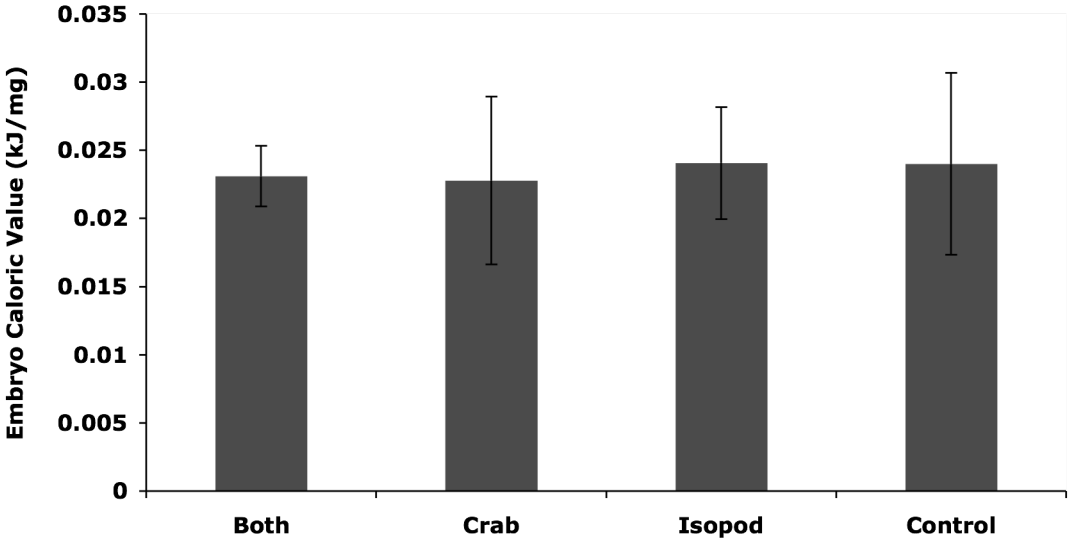

Figure 11. Mean caloric value (kJ mg^{-1}) of embryos extracted from egg capsules. Whelks were exposed to cues from caged *H. oregonensis* (Crab), *I. wosnesenskii* (Isopod), both crabs and isopds (Both), or neither crabs nor isopods (Control). Each treatment had six replicates. Error bars represent one standard error.

Table 10. Results of ANOVA for energetic investment by whelks exposed to crabs and isopods.

Effect	df	Sum of Squares	F value	*P* value
Predators				
Treatment	2	0.00004	0.63	0.54
Residuals	21	0.0006		

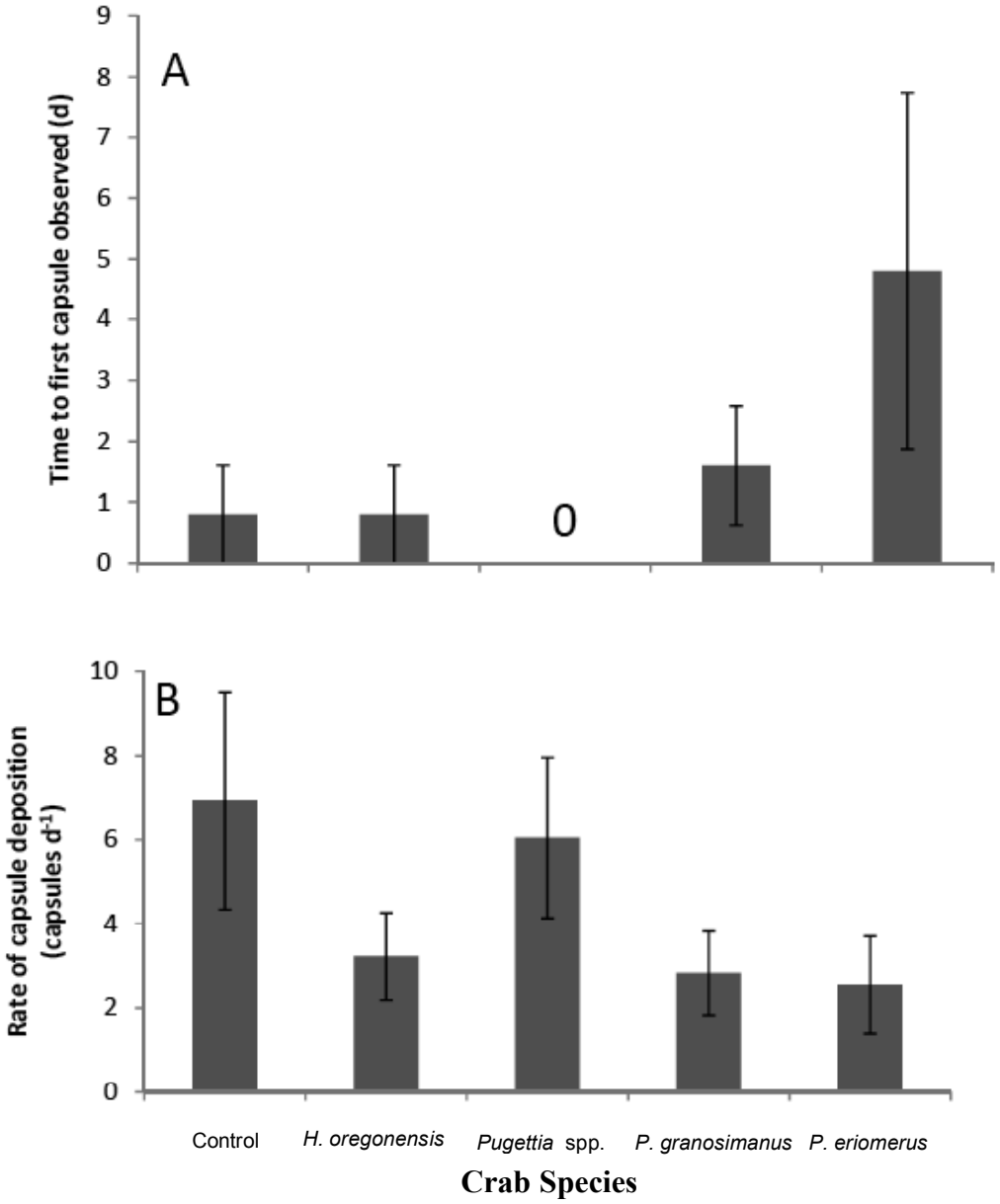

Figure 12. A) Mean number of days until capsules were first observed, and B) mean rate of capsule deposition by whelks exposed to cues from different species of crab. Whelks were exposed to cues from *H. oregonensis*, *Pugettia spp.*, *P. granosimanus*, *P. eriomerus,* and no crabs (control). Each treatment had five replicates. Error bars represent one standard error.

Cues from crab species affected the rate that whelks deposited capsules (Fig 12B). The average rate of laying for the experiment was 4.3 capsules d^{-1} (± 0.8 SE). Compared to the control, *H. oregonensis*, *P. granosimanus*, and *P. eriomerus* reduced the rate of capsule deposition by 50%, 61%, and 65%, respectively (Table 8). *Pugettia* spp. had no effect on the rate of capsule deposition (Table 8).

Cues from crab species did not affect the strength of capsules (Fig. 13). The average force to pierce an intact capsule was 4.60 N (± 0.36 SE) and to pierce a punctured capsule was 4.36 N (± 0.37 SE). Force to pierce intact or punctured capsules was not significantly different for the different crab treatments (Table 11).

Conspecific density experiment

Conspecific density affected the timing of capsule deposition (Fig. 14A). Whelks started depositing capsules 4 d sooner when exposed to cues from caged conspecifics (low and

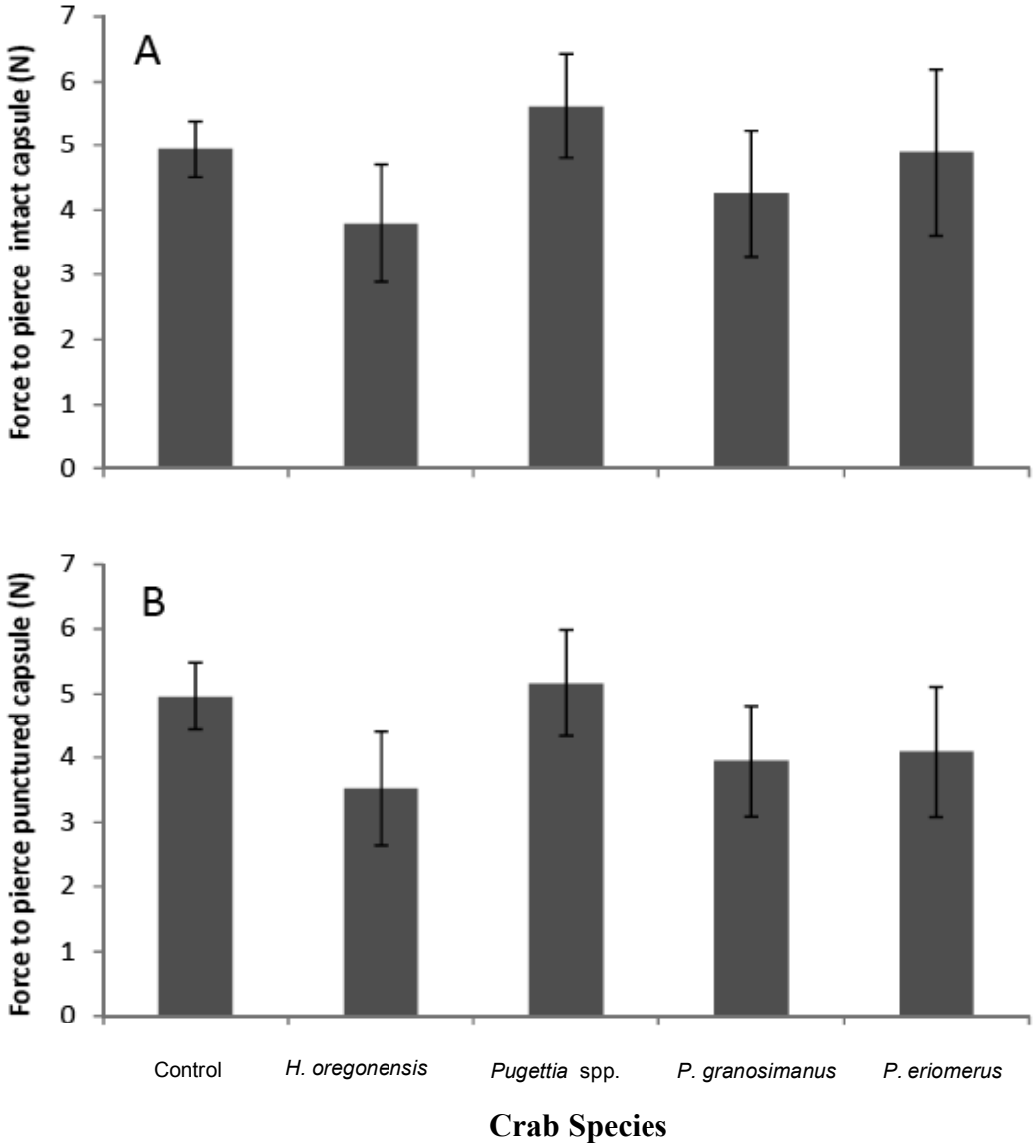

Figure 13. Mean force (N) required to pierce A) intact-capsules and B) punctured-capsules deposited by whelks exposed to cues from different species of crab. Whelks were exposed to cues from *H. oregonensis*, *Pugettia spp.*, *P. granosimanus*, *P. eriomerus,* and no crabs (control). Each treatment had five replicates. Error bars represent one standard error.

Table 11. Results of ANOVA model for strength of capsules deposited by whelks exposed to different species of crab.

Effect	df	Sum of Squares	F value	*P* value
Intact capsules				
Treatment	4	9.72	0.63	0.65
Residuals	18	69.30		
Punctured capsules				
Treatment	4	9.56	0.72	0.59
Residuals	18	59.74		

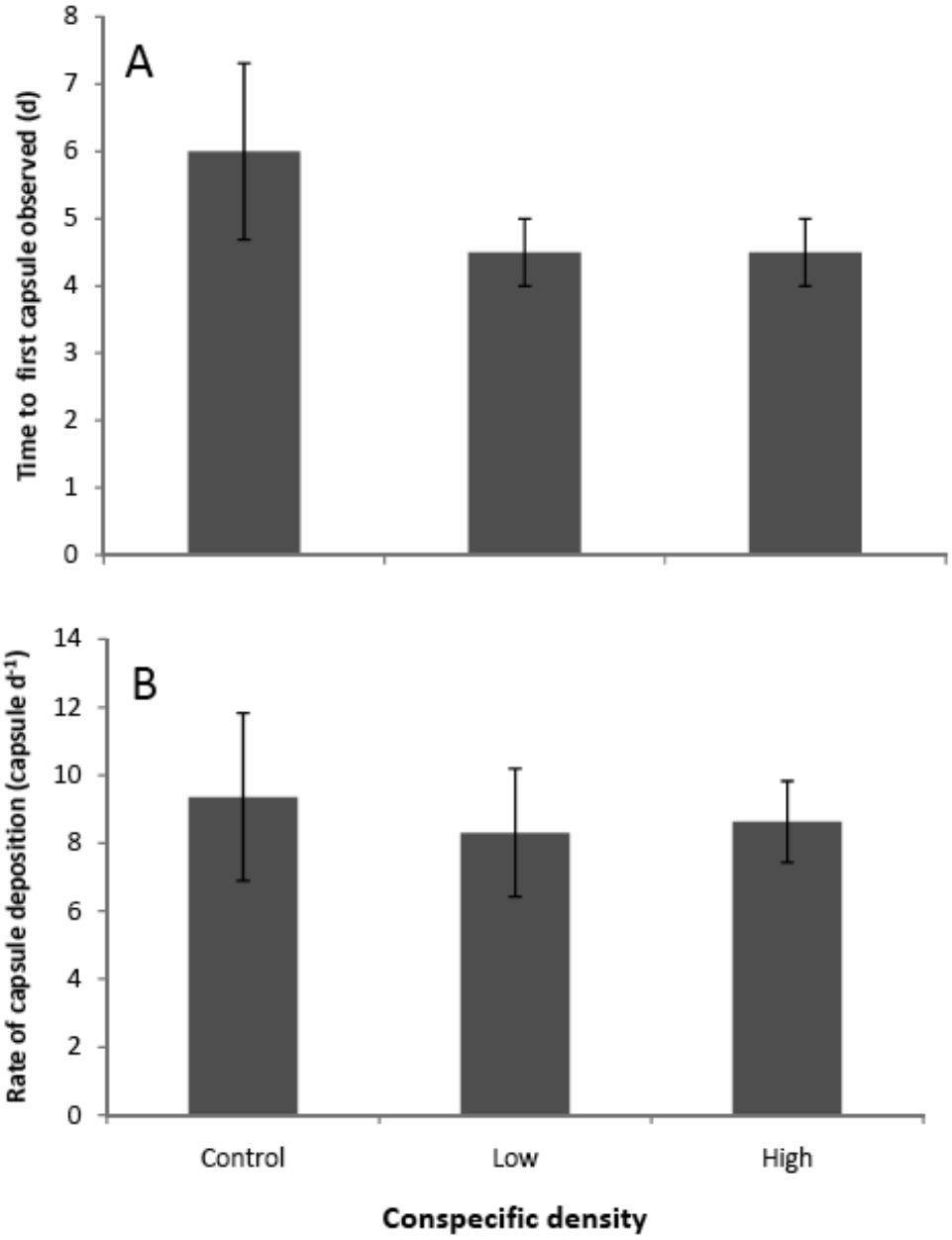

Figure 14. A) Mean number of days until capsules were first observed, and B) mean rate of capsule deposition by whelks exposed to cues from three densities of adult conspecifics. Whelks were exposed to cues from 20 additional adult whelks (high density), 10 additional adult whelks (low density), or no additional whelks (control). Each treatment had eight replicates. Error bars represent one standard error.

high density treatments). Both the low and high density treatment differed significantly from the control but were not different from each other (Table 6).

Cues from conspecifics did not affect the rate at which whelks deposited capsules (Fig. 14B). The average rate of laying for the experiment was 8.75 capsules d^{-1} (± 1.0 SE). Both the low and high density treatment did not differ significantly from the control (Table 8).

Cues from conspecifics also did not affect the energy invested into embryos (Fig. 15). On average, embryos had an energy density of 0.024 kJ mg^{-1} (± 0.0011 SE). Neither the low nor high density treatments were significantly different from the control (Table 12).

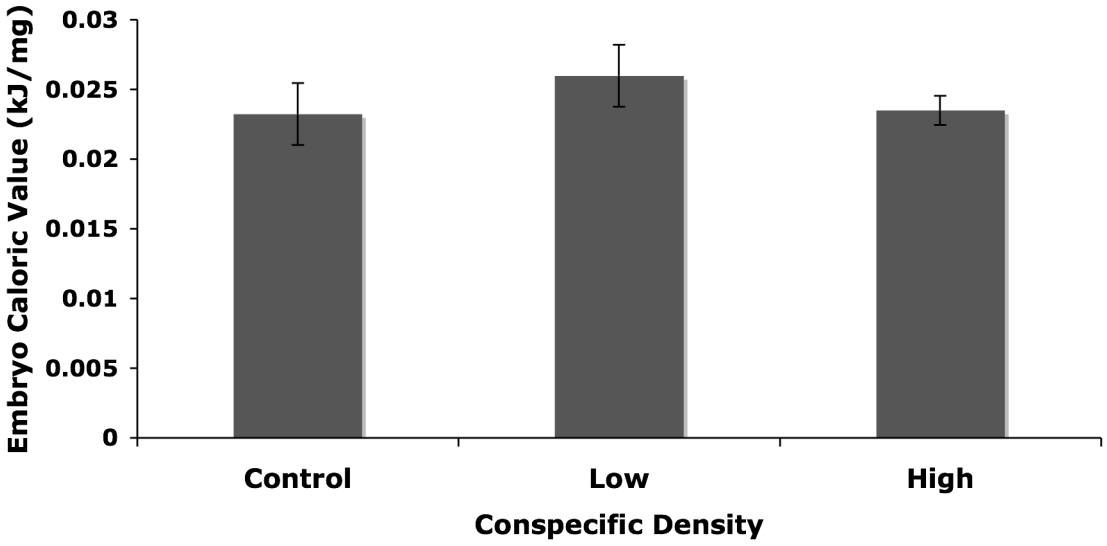

Figure 15. Mean energy density (kJ mg^{-1}) of embryos extracted from egg capsules. Whelks were exposed to cues from 20 additional adult whelks (high density), 10 additional adult whelks (low density), or no additional whelks (control). Each treatment had eight replicates. Error bars represent one standard error.

Table 12. Results of ANOVA for energetic investment by whelks exposed to different densities of conspecifics.

Effect on Caloric Density	df	Sum of Squares	F value	*P* value
Conspecifics				
Treatment	3	0.00002	0.3	0.83
Residuals	15	0.0004		

DISCUSSION

Cues from crabs affected the timing and rate at which whelks deposited capsules. However, the threat posed by crabs to capsules poorly predicted which species of crab would alter when, and at what rate, whelks deposited capsules. Of the two species I tested that consume capsules, *Hemigrapsus oregonensis* and *Pugettia* spp., only *H. oregonensis* affected deposition of capsules. In the predator experiment, cues from the crab *H. oregonensis* delayed when whelks began to deposit capsules by 2.7 d and reduced the rate of deposition by 50%. In the crab species experiment, cues from *H. oregonensis* also reduced the rate of deposition by 50%, but did not alter the timing of deposition. Both of the species I tested that do not consume capsules, *Petrolisthes eriomerus* and *Pagurus granosimanus*, reduced the rate of deposition, and *P. eriomerus* also delayed when whelks deposited capsules.

The responses to cues from crabs and whelks that I observed during our experiments are similar to the responses juveniles exhibit when they hatch from the capsules (Miner et al. 2010). Cues from the crab *H. oregonensis*, one of the same species used in this study, delayed juvenile hatching by about 4 d. Cues from adult conspecifics accelerated juvenile hatching by about 6 d. If densities of crabs and adult conspecifics are constant over an extended period of time, the effects on plastic time to hatching and time to deposition might be additive as both maternal capsule deposition and juvenile time-to-hatching were shifted in the same direction (Fig. 5). This emphasizes the need to study

how biotic cues affect multiple switch points before hatching to determine the magnitude and direction that these factors will alter hatching in nature.

The isopod, *Idotea wosnesenskii* affected the timing and rate of capsule deposition, but only synergistically with crabs. Whelks deposited capsules at a similar time and rate in the control and isopod treatments. However, the delay of capsule deposition and reduction of deposition rate were both significantly stronger in the crab and isopod treatment than in the crab treatment alone. A likely explanation for these results is that crabs consumed isopods while in the enclosure. Prey often show a greater response to feeding predators than non-feeding predators (e.g., Appleton and Palmer 1988; Griffiths and Richardson 2006; Smee and Weissburg 2006; Schoeppner and Relyea 2009a and b). During our experiment, crabs consumed many of the isopods in the treatment that included both predators, and likely induced a stronger response from whelks than in the starved crab treatment.

There was no evidence that the isopod *I. wosnesenskii,* or any of the four species of crabs, induced a response in capsule size, shape or strength. This is an interesting finding as other species of *Nucella* deposit capsules with wall thicknesses that directly correlate to the presence of predatory isopods (Rawlings 1990). Additionally, thicker walls make encapsulated embryos more resistant to predation (Rawlings 1994). My findings suggest that variation in capsule wall thickness and strength are not influenced by cues from predators present at capsule deposition. Variation in capsule wall thickness may be caused by selection in genetically isolated snail populations exposed to different levels of

capsule predation or influenced by other environmental factors prior to capsule deposition. A previous study showed that *N. lamellosa* is capable of increasing the number of eggs that it lays when it is given access to elevated levels of food (Spight and Emlen 1976). It is possible that nutrition could also influence capsule wall properties.

Cues from conspecifics affected the timing but not rate at which whelks deposited capsules. Whelks in the low- and high-density treatments deposited capsules 4 d sooner than whelks in the control treatment. The similar effect in the low- and high-density treatments suggests that *N. lamellosa* is sensitive to lower densities than those used in our experiment. Because whelks aggregate to reproduce and the timing of this aggregation varies greatly among sites in the inland waters of Washington (Spight and Emlen 1976; Strathmann 1987), there is very likely chemical communication among whelks to signal reproduction. Additionally, high densities of adult whelks may indicate that there will be high levels of competition among juveniles upon hatching.

Previous studies showed that: a) encapsulated *N. lamellosa* will alter the time that they spend in an encapsulated state in response to crabs, isopods and conspecifics and that b) there is no change in the growth rate of encapsulated snails between the above treatments (Miner et al. 2010). Due to this change in time to hatching and no change in growth rate, it seemed likely that parents would provide different levels of energy to the encapsulated embryos to provision them through their plastic encapsulation time. However, this study shows that there is no difference in the amount of energy invested into each capsule. Further studies investigating the metabolic rate of encapsulated snails, the energy

provided to individual embryos, the number of embryos per capsule or the size of embryos at hatching could clarify how adult snails invest a similar amount of energy into capsules to see their embryos through different durations of encapsulation or the cost of delayed hatching.

This study shows that adults can respond to potential predators their offspring may encounter but pose no threat to the adults themselves. I show that snails can respond to these threats by altering the time of capsule deposition as well as how many capsules are deposited. Adult snails also responded to organisms that do not pose a threat to themselves or their offspring. Some of the species of crabs used in the crab study do not prey on juvenile snails or snail capsules, yet induced a change in adult snails reproductive behavior. I also show that adult *N. lamellosa* alter their reproductive behavior in response to elevated densities of conspecific snails. All shifts in reproductive timing by adults were in the same direction as shifts in embryonic snails' time of hatching seen in other studies. This highlights the importance of studying how biotic cues affect multiple life-history stages of the same organism.

CHAPTER 4: CONCLUSION

In this work I tested two marine gastropods' responses to cues from predatory crabs and isopods, cues from non-predatory crabs, cues from crushed conspecifics, and cues from different densities of live conspecifics. I showed that these snails exhibit an array of responses to different cues from their environment. Some of these responses appear to confer a fitness advantage while others do not.

In my *Littorina* study, I tested how *Littorina sitkana* responds to *Hemigrapsus nudus* and crushed conspecifics. I showed that *L. sitkana* snails alter their shell size in response to the combined cues of *H. nudus* and crushed conspecifics but do not alter their shell thickness. I also found that snails crawl upward within their habitat and decrease feeding in response to cues associated with predation. This study also showed that a decrease in shell size does not necessarily cause an increase in shell thickness as previous studies have suggested.

In my *Nucella* study, I tested how adult *Nucella lamellosa* alter their reproductive behavior in response to cues from organisms that do and do not prey on encapsulated juvenile snails, as well as how these same adults respond to different densities of conspecific snails. I show that adults will delay when capsules are deposited as well as the rate at which these capsules are deposited in response to cues from some capsule predators. This study also demonstrates that adult *N. lamellosa* are capable of altering

their reproductive behavior in response to organisms that pose no threat to themselves, but do pose a serious threat to their offspring. However, adult *N. lamellosa* will employ these same responses to some species of crabs that are not capsule predators. The reason for employing these responses in unclear. Its possible that there are other factors involved with this response that were not taken into consideration in this study, such as the fitness of later life history stages. It is also possible that these are simply over-generalized, maladaptive responses. I also show that *N. lamellosa* will accelerate the time at which they deposit their egg capsules when exposed to elevated levels of conspecifics.

These findings show that *N. lamellosa* responds to biotic cues that can affect the fitness of their offspring and alter some aspects of their reproductive behavior accordingly. Additionally, juveniles and adults shift hatching and capsule deposition in the same direction when exposed to the same biotic cues (Miner et al. 2010). This highlights the importance of looking at how certain cues affect multiple life history switch points of an organism.

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