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Alien vs. predator: effects of a native predator on two invasive oyster drills and oysters in Washington State

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ALIEN VS. PREDATOR: EFFECTS OF A NATIVE PREDATOR
ON TWO INVASIVE OYSTER DRILLS AND OYSTERS
IN WASHINGTON STATE.

By
Emily W. Grason

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

Moheb A. Ghali, Dean of the Graduate School

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MASTER’S THESIS

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Emily W. Grason

May 11, 2010
ALIEN VS. PREDATOR: EFFECTS OF A NATIVE PREDATOR ON TWO INVASIVE OYSTER DRILLS AND OYSTERS IN WASHINGTON STATE.

A Thesis
Presented to
The Faculty of
Western Washington University

In Partial Fulfillment
Of the Requirements for the Degree
Master of Science

By

Emily W. Grason

May 2010
I investigated the consumptive (CEs) and non-consumptive effects (NCEs) of a native crab predator (the red rock crab, *Cancer productus*) on a system of two invasive oyster drills (the Atlantic drill, *Urosalpinx cinerea*, and the Japanese drill, *Ocinebrina inornata*), a native oyster (the Olympia oyster, *Ostrea lurida*), and an introduced but commercially valuable oyster (the Pacific oyster, *Crassostrea gigas*). In the presence of chemical effluent from crabs eating conspecifics, drills increased hiding behavior by 2 to 6 times, and reduced the number of oysters consumed by 50 to 67%. This is consistent with an adaptive behavioral response and has the potential to transmit large positive indirect NCEs of the crab to oysters. Neither species of drill uses a density-dependent risk assessment, as the effect of predation effluent was similar at both high and low densities of conspecific drills.

The response specificity of the Atlantic drill was further investigated to determine which cue sources (i.e., predator or prey) were eliciting these defenses. Drills responded equivalently to the consumptive predation cue (predators consuming injured conspecific prey) and injured conspecific cue alone, suggesting injured conspecifics alone are sufficient to elicit the greatest magnitude of defense. A smaller defense was elicited by cue from an unfed crab, indicating that drills have the ability to recognize a relatively novel predator. Cues of injured conspecifics and unfed predators do not have an additive effect on drill behavior. Finally, digestive byproducts, both general chemicals produced by the crab and altered cues from conspecifics, did not increase the magnitude of drills’ defenses.
I explored the consumptive effects of crabs on drills and oysters by estimating crab feeding rates on and preferences among all prey types. Crabs consumed both species of oyster at similar rates and did not express a preference for either oyster. However, while crabs can consume both drills and Pacific oysters at similar rates, they expressed a strong preference for Pacific oysters over either species of drill. As a result, it is likely that crabs can have strong negative consumptive effects on oysters, and that drills may be released from crab predation in oyster beds.

I have identified several mechanisms which might have facilitated invasion by drills: (1) inducible defenses against a novel predator, (2) general cue recognition strategy, and (3) simultaneous introduction of prey preferred by native predators. All three mechanisms weaken the efficacy of biotic resistance. Therefore, without greater human intervention, drills will continue to pose a problem for oyster culture and restoration efforts in Washington State. Behavioral defenses and crab preferences offer drills a refuge from predation that will enable them to persist in a stable trophic chain with red rock crabs and oysters. However, if the alarm cues that trigger defenses in Atlantic drills can be identified and isolated, application to oyster beds could reduce drill predation on oysters.

Taken together, these findings suggest that, in general, inducible defenses might facilitate invasion by defended prey. Further, the strong response by Atlantic drills to cues of injured conspecifics suggests a mechanism whereby these invasives, and potentially others, can recognize and respond appropriately to novel predators. Continued exploration of this system can offer opportunities to test hypotheses about the evolution of inducible defenses.
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# TABLE OF CONTENTS

ABSTRACT............................................................................................................................................. iv  

ACKNOWLEDGEMENTS ......................................................................................................................... vi  

LIST OF FIGURES .................................................................................................................................. x  

LIST OF TABLES .................................................................................................................................... xii  

CHAPTER I: INTRODUCTION ............................................................................................................... 1  

   Current Study .................................................................................................................................... 7  

CHAPTER II: MORE THAN MEETS THE CLAW: BEHAVIORAL PLASTICITY AND NON-CONSUMPTIVE EFFECTS IN A SYSTEM WITH INVASIVE PREDATORY WHELKS ................................................................................................................... 12  

   Introduction ....................................................................................................................................... 12  

   Materials and Methods ...................................................................................................................... 16  

   Results ............................................................................................................................................... 24  

   Discussion ......................................................................................................................................... 33  

CHAPTER III: CUE SPECIFICITY FOR INDUCIBLE DEFENSES OF AN INVASIVE MARINE SNAIL ............................................................................................................................ 42  

   Introduction ....................................................................................................................................... 42  

   Materials and Methods ...................................................................................................................... 49  

   Results ............................................................................................................................................... 57  

   Discussion ......................................................................................................................................... 61  

CHAPTER IV: PREFERENCE ALTERS CONSUMPTIVE EFFECTS OF PREDATORS: TOP DOWN EFFECTS OF A NATIVE CRAB ON A SYSTEM OF NATIVE AND INTRODUCED PREY ........................................................................................................ 71  

   Introduction ....................................................................................................................................... 71  

   Materials and Methods ...................................................................................................................... 76
Results .............................................................. 83
Discussion ........................................................... 87

CHAPTER V: DISCUSSION ........................................... 92
Relevance .................................................................. 94
Future Research ......................................................... 95
Washington Drills: Prognosis and Recommendations ...................... 100
Conclusions ................................................................ 102

WORKS CITED ................................................................ 104
LIST OF FIGURES

Figure 1.1. Trophic relationships (arrows) and terms used in this research to identify the role of organisms in this study (in parentheses). Arrows originate at predators and point in the direction of predation..........................8

Figure 2.1. Experimental bin set-up for experiment 1. Coupled flow-through bins allowed me to apply treatments to individual drills. Arrows indicate the direction of water flow through the system. Predation cue was manipulated in the upstream bin as either the presence or absence of a single rock crab. Two drills, which were of the same species as the focal drill, were also added to all upstream bins. The focal drill and 10 juvenile Pacific oysters were isolated from tactile cues of predation originating in the upstream bin. The focal drill was also offered a refuge in the downstream bin. Each treatment was replicated 12 times..........................19

Figure 2.2. Experimental bin set-up for experiment 2. Coupled flow-through bins allowed me to apply treatments to individual drills. Arrows indicate the direction of water flow through the system. Predation cue was manipulated in the upstream bin as either the presence or absence of a single rock crab. Two drills, which were of the same species as the focal drill, were also added to all upstream bins. The focal drill and 10 juvenile Pacific oysters were isolated from tactile cues of predation originating in the upstream bin. The focal drill was also offered a refuge in the downstream bin. Each treatment was replicated 12 times. Conspecific drill density was manipulated in the downstream bin, by enclosing a smaller, perforated bin that contained either 4 Japanese drills or 5 Atlantic drills in the high-density conspecific treatments. Perforated bins were empty in the low-density conspecific treatments.................................22

Figure 2.3. Mean proportion of times Atlantic drills (Urosalpinx cinerea) observed hiding (A) and per capita drill feeding rate (B) for drills in presence (n=12) and absence (n=12) of predation cue. Error bars indicate one SEM..........................25

Figure 2.4. Mean proportion of times Japanese drills (Ocinebrina inornata) observed hiding (A) and per capita drill feeding rate (B) for drills in presence (n=12) and absence (n=12) of predation cue. Error bars indicate one SEM.................................27

Figure 2.5. Mean proportion of times Atlantic drills (Urosalpinx cinerea) observed hiding (A) and per capita drill feeding rate (B) when subjected to the presence or absence of predation cue, and high or low conspecific drill densities. The experiment was conducted in two time blocks (gray bars = Block A; white bars = Block B). Error bars indicate one SEM.................................31

Figure 2.6. Mean proportion of times Japanese drill (Ocinebrina inornata) observed hiding (A) and per capita drill feeding rate (B) when subjected to the presence or absence of predation cue, and high or low conspecific drill densities. The experiment was conducted in two time blocks (gray bars = Block A; white bars = Block B). No oyster
mortality occurred any replicates in the predation cue, low density treatment in block A. Error bars indicate one SEM……………………………………………………32

**Figure 3.1** Bin set-up for cue specificity experiments. Coupled flow-through bins allowed me to apply treatments to individual drills. Solid arrows indicate the direction of water flow through the system. Cue sources were manipulated upstream of the focal drill as described in Methods. Dotted arrow indicates the mesh pouch containing injured drills, in appropriate treatments, located in the pipe between bins. The focal drill and 10 juvenile Pacific oysters offered as prey were isolated from tactile crab cues in the downstream bin. The crab, when present, was isolated from visual or chemical cues of the focal drills in the downstream bin. The drill was also offered a refuge in the downstream bin. Each treatment was replicated 8 times……………………………………………………54

**Figure 3.2** Mean proportion of times Atlantic drills were observed hiding (A) and per capita drill feeding rate (B) when subjected to six cue treatments. No focal drill mortality occurred in this experiment. Error bars represent 1 SEM…………………………………………………58

**Figure 4.1.** Schematic of treatments for experiment 1 - oyster preference experiment. One treatment for each species of oyster (Pacific or Olympia) offered only that species as prey, and a third treatment offered both species in equal abundance. A single crab was placed in each bin and allowed to feed freely on respective prey for 24 hours without replacement. Pacific oysters are marked with a “P”, and Olympia oysters are marked with an “O”……………………………………………………………………………78

**Figure 4.2.** Schematic of treatments for experiment 2 – drill/oyster preference experiment. One treatment for each species of prey (either Pacific oysters, Japanese drills, or Atlantic drills) offered only that species as prey, and a fourth treatment offered both species in equal abundance. A single crab was placed in each bin and allowed to feed freely on respective prey for 24 hours without replacement…………………………………………79

**Figure 4.3** Number (mean ± SE) of oysters consumed by crabs over 24 hours in experiment 1 - oyster preference experiment. Gray bars are Pacific oysters, and white bars are Olympia oysters. In the treatment labeled “Choice” both species were offered simultaneously in equal abundance………………………………………………………….84

**Figure 4.4.** Frequency distributions of P values of interaction terms generated by randomly pairing bins in the no-choice treatments of experiment 1, oyster preference (A), and experiment 2, drill and oyster preference (B). Note the difference in x-axis scales…………………………………………………………………………85

**Figure 4.5.** Number (mean ± SE) of prey consumed by crabs over 24 hours in experiment 2 - drill/oyster preference experiment. Black bars are Pacific oysters, gray bars are Japanese drills, and white bars are Atlantic drills. In the treatment labeled “Choice” all three species of prey were offered simultaneously to the crab in equal abundance…………………………………………………………………….86
LIST OF TABLES

Table 2.1 GLM (binomial error distribution, logit link) results for frequency of hiding behavior as a function of predation cue for Atlantic and Japanese drills. Bold values indicate statistical significance at \( \alpha \leq 0.05 \).................................................................26

Table 2.2 GLM (binomial error distribution, log link) results for oyster survivorship as a function of predation cue for Atlantic and Japanese drills. Bold values indicate statistical significance at \( \alpha \leq 0.05 \)..................................................................................28

Table 2.3 GLM (binomial error distribution, logit link) results for the frequency of hiding as a factor of predation cue, conspecific density, and time block for Japanese and Atlantic drills. For Japanese drills, the 3-way interaction term was removed from the model because it was not significant (P = 0.232). Bold values indicate statistical significance at \( \alpha \leq 0.05 \)..................................................................................30

Table 2.4. GLM (binomial error distribution, log link) results for oyster survivorship as a function of predation cue, conspecific density, and time block for Japanese and Atlantic drills. For Atlantic drills, the interaction terms for block were removed because they were not significant. For Japanese drills, one pseudo-mortality was added to one replicate of the predation cue, low density treatment in block A to permit the GLM to estimate parameters. Bold values indicate statistical significance at \( \alpha \leq 0.05 \)..................................................................................34

Table 3.1 Treatments used in 25 cue-specific inducible defense studies. Check marks in each column indicate treatments used, but studies might have used more than one of each treatment type (e.g., several treatments of different unfed predator species). Where multiple experiments appeared in a single publication, separate rows are used for each experiment. Response types measured include morphological (M), behavioral (B), and life history (LH)........................................................................................................45

Table 3.2. Cue sources present in each of the six treatments recommended for testing cue-specific inducible defenses. Shorthand cue notations appear in parentheses where the “X” indicates predators were fed indicated prey and “+” indicates that the cue of unfed predator was added to cue from injured conspecifics........................................51

Table 3.3. Hypotheses tested about which of cue sources induce defenses in Atlantic drills and the contrasts used to test predictions of hypotheses........................................52

Table 3.4. GLM (binomial error distribution, log link for oyster survivorship, logit link for hiding) results for contrasts conducted on oyster survivorship and hiding behavior. Bold values indicate statistical significance at \( \alpha \leq 0.05 \)..................................................................................59
CHAPTER I: INTRODUCTION

After centuries of transoceanic migration and commerce, we are only recently beginning to identify and understand the extent to which introduced species are impacting coastal marine environments worldwide (Carlton 1989, Grosholz 2002). Introductions can occur intentionally, to create new markets for commercially valuable organisms (e.g., fish or shellfish), or unintentionally, as a by-product of ship transport, traffic in seafood and ocean products, pet trade, or scientific research (Carlton 1987). Species are typically considered “invasive” when they establish a self-recruiting population that rapidly expands its range, and becomes dominant in the local community—corresponding to Colautti and MacIsaac’s (2004) stage V. While not all introduced species become invasive, those that do have the potential to damage native ecosystems in a variety of ways, including reducing biodiversity by competing with or consuming native species (Mack et al. 2000), altering nutrient cycles (Yelenik et al. 2007, Wardle et al. 2009), impairing ecosystem function (Vitousek 1990), and devastating economically valuable fisheries (Meacham 2001).

An important type of interaction in invaded systems is novel predator-prey relationships. These interactions can have large destabilizing effects on communities (Gherardi and Acquistapace 2007, Salo et al. 2007) that result from the lack of a shared evolutionary history. For instance, prey exposed to unfamiliar predators might not be equipped with appropriate defenses, and can experience population decline (Fritts and Rodda 1998, Smith et al. 2007, Sih et al. 2009). Alternatively, native predators might not immediately recognize
non-natives as a potential prey, thus allowing the non-native populations to increase, and become invasive. If the invader then competes with or displaces the predator’s native food, the native predator population will decline unless it can incorporate the invasive prey into its diet (Carlsson et al. 2009). These dynamics will tend to produce large fluctuations in populations at multiple trophic levels, and can result in extinctions of native species (Mack et al. 2000). However, because selective pressures can be strong, novel predator-prey relationships also offer opportunities to study the co-evolutionary effects of predator-prey arms races (Dawkins and Krebs 1979, Edgell and Rochette 2007).

For practical purposes, the dynamics of a novel predator-prey relationship are important to understand because they will, in part, determine whether a species will invade and persist in a habitat, and affect communities. Predators can cause two types of effects in their community. The best studied of the two occurs when predators attack and consume prey, removing them from the population. These consumptive effects (CEs) are transmitted, directly and indirectly, through the community by only those individuals that were directly involved in the interaction (i.e., only the predator that did the consuming and the prey that were consumed). Consumptive effects are also often referred to as density-mediated effects because the interaction strength between predator and prey, as well as the effects transmitted to the greater community, are predicted by the changes in density of the predator and prey populations (Abrams 2007).

The other type of effect does not rely on predation per se, but on prey responses to the threat of predation—non-consumptive effects (NCEs). Inducible anti-predator defenses are one
type of phenotypic plasticity that occurs when prey alter their morphology, behavior, or life history in response to cues of predator presence or past predation events (Havel 1987). For instance, many species of the cladoceran genus *Daphnia* develop morphological defenses, such as head spikes or neck-teeth, when they are exposed to chemical effluents of a predator (Tollrian 1995). Predators can induce defenses in any prey that can detect their cues. In this way, predators can affect whole populations of prey without actually consuming them (Miner et al. 2005, Kishida et al. 2010). As opposed to CEs, NCEs are transmitted by the individuals that are not consumed. In another example, cues from green crabs induced hiding by herbivorous, littorine snails (Trussell et al. 2003). Because they were hiding, snails consumed 460% less biomass of fucoid algae. This NCE reduces the growth of the snails, and indirectly improves survivorship of the algae. NCEs have also been termed trait-mediated effects, because the interaction strength and effect size depends on changes in trait values of organisms involved in the interaction. Increased recent attention to inducible defenses and NCEs/trait-mediated effects has demonstrated that they are widely influential in structuring predator-prey interactions, and entire communities (Kishida et al. 2010).

However, in invaded systems where predators and prey lack a common evolutionary history, the role of NCEs is less well known (Sih et al. 2009). This is likely because inducible defenses are not as well characterized in invaded systems as in native systems. Inducible defenses are thought to be less common in novel predator-prey interactions than in native systems since they can only occur if the prey recognizes a predation threat. Prey can be naïve to novel predators, reducing the potential for inducible defenses, and in turn, the potential for NCEs. Indeed, in several systems, non-native predators do not induce defenses
in native prey (Shave et al. 1994, Fritts and Rodda 1998, Cox and Lima 2006, Smith et al. 2007), but exceptions to this trend have recently been observed (Trussell and Smith 2000, Whitlow et al. 2003, Kristensen and Closs 2004, Freeman and Byers 2006). To date, there has been no research explicitly addressing NCEs in invaded systems. However, the inducible defenses documented in these latter studies suggest that non-native predators might indeed alter community dynamics by causing NCEs.

Interestingly, the inverse relationship, native predators inducing defenses in non-native prey, has received almost no attention from researchers. A notable exception to this is Engel & Tollrian’s (2009) recent study, which demonstrates that invasive Daphnia in the Great Lakes grow head spikes in the presence of native fish predator cues. These authors argue that this defense might have even facilitated the invasion by reducing biotic resistance of the native predators. If this is the case, then certainly the prevalence of inducible defenses in non-native prey deserves more study. Moreover, we might intuitively hypothesize that NCEs transmitted by invasive prey would result in qualitatively different indirect effects on other species. That is, if a non-native predator induces a defense in a native prey, the defense would likely be similar to one induced by a native predator. Therefore the NCEs that are transmitted might be of a different magnitude, but will probably be of a similar type. Conversely, the defense of a non-native prey might be completely novel to the community, and could therefore result in unpredictable dynamics.

Inducible defenses, like many other types of plasticity, are often context-dependent. For inducible defenses to be adaptive, prey should only express defenses when the benefits (e.g.,
increased probability of survivorship) outweigh the potential costs (e.g., carrying defensive armature, reduced feeding opportunities) (Harvell 1990, Lima 1998). The probability of survivorship will depend on the actual risk of predation to an individual prey. For instance, in a large group of prey, risk of predation on any individual is shared by the whole group (Peacor 2003, McCoy 2007). Therefore, theoretical models predict that prey should reduce the magnitude or frequency of defenses when prey are present in high densities (Peacor 2003). Density-dependent risk assessment has been demonstrated empirically in tadpoles (McCoy 2007) and lizards (Downes and Hoefer 2004). Density of introduced species often changes during the course of an invasion. Non-native species are often initially distributed patchily, at low overall densities, but can increase dramatically in a relatively short period of time (Colautti and MacIsaac 2004). Thus, if non-native prey defend themselves differently at different densities, then NCEs might also differ among stages of the invasion. Specifically, if high prey density attenuates NCEs, the relative strength of CEs to NCEs will increase with non-native prey density.

Where novel predators induce defenses in prey, it is informative to investigate the source of the inducing cues (Sih et al. 2009). Predation cues typically consist of a predator attacking and consuming conspecific prey. This type of cue has many potential sources of information including predator kairomones, digestive chemicals, prey alarm signals, contents of lysed prey cells and tissues, and digested prey. A subset of research on inducible defenses has tried to identify the cue sources that induce defenses (Appleton and Palmer 1988, Cheung et al. 2004, Schoeppner and Relyea 2005, Bourdeau 2009a, Fraker et al. 2009), and it seems probable that several risk-recognition strategies exist. If prey, such as the *Daphnia* in Engel
and Tollrian’s (2009) study, respond to novel predation cues, it begs the question: what sources of information in cues do non-native prey use to assess risk? The naïveté theory would suggest that prey are not responding to the predator itself, as they might not have evolved the ability to detect a novel predator in such a short time. Instead, general risk recognition strategies (i.e., response to conspecific injury or alarm cues) might benefit non-native prey by enabling them to respond to a broad range of predators, regardless of predator identity (Sih et al. 2009). Particularly where non-natives are involved, determining the proximal sources of cues that induce defenses will further inform models of when inducible defenses and NCEs are likely to play a role in invaded systems.

Investigation of the top-down effects of predators (CEs and context-dependent NCEs) is therefore crucial for an understanding of community dynamics in invaded systems. For instance, where the invader is a potential prey species, native predators might offer a measure of biotic resistance against the new species (Juliano et al. 2010) by exerting large consumptive effects on the non-native prey. Predators with generalist feeding strategies might be the most likely to take advantage of a novel food source (Symondson et al. 2002, Carlsson et al. 2009) and reduce novel prey populations. On the other hand, inducible defenses can stabilize trophic dynamics, and promote the coexistence of all levels of a trophic chain (Ives and Dobson 1987, Ruxton 1995, Miner et al. 2005, Ramos-Jiliberto et al. 2008). If native predators induce defenses in non-native prey, it could allow the prey to persist in the environment, and reduce the efficacy of biotic resistance. This would provide a mechanism for Engel & Tollrian’s (2009) argument that the inducible formation of head-spikes in *Daphnia* facilitated their invasion. In addition to predicting whether or not a non-
native prey will become invasive, a full understanding of native predator effects can inform models of the post-invasion dynamics. Empirical research that addresses novel predator-prey interactions is particularly important because assumptions based on research in native systems might not be met in invaded communities.

**Current Study**

I used the above framework to investigate the effects of a native crab predator, *Cancer productus*, on a system of two invasive oyster drills, *Urosalpinx cinerea* and *Ocinebrina inornata*, a native oyster, *Ostrea lurida*, and an introduced oyster, *Crassostrea gigas* (Figure 1.1). These species co-occur in various combinations in mature oyster beds in Puget Sound and Willapa Bay, WA (Holsman et al. 2006). While the direct consumptive effects of drills on oysters have been studied (Hanks 1957, Chew 1960, Wood 1968, Manzi 1970, Martel et al. 2004, Buhle and Ruesink 2009), the top-down effects of crab predators are not yet known.

This system is primarily of interest because both oyster species have commercial value. The Olympia oyster, *O. lurida*, is the only native oyster in the Pacific Northwest (Kozloff 1983). Due to over fishing and pollution, stocks of Olympia oysters declined dramatically during the late 1800’s and early 1900’s (McKernan et al. 1949, Baker 1995). At that time, oyster growers imported several non-native oyster species, including the Pacific oyster, *C. gigas*, to buoy up the industry (White et al. 2009). New species were imported as cultch, spat newly settled on mature oyster shell. With the cultch came an array of hitch-hiking invasive species
Figure 1.1. Trophic relationships (arrows) and terms used in this research to identify the role of organisms in this study (in parentheses). Arrows originate at predators and point in the direction of predation.
that have persisted – including both drills. Pacific oysters are now the backbone of the Washington oyster industry, and sustain self-recruiting populations in the wild outside of industry management (Ruesink et al. 2005). Commercial interest in Olympia oysters continues, but primarily as a boutique oyster. These oysters are only sold from cultured stocks. Efforts have also been made to restore wild Olympia oyster stocks in their native range with limited success (Cook et al. 2000, Trimble et al. 2009). It is possible that recovery of Olympia oyster is hampered by lack of habitat, competition with Pacific oysters, and predation from invasive oyster drills (Baker 1995, Buhle and Ruesink 2009, Trimble et al. 2009).

The Atlantic oyster drill, *U. cinerea*, is native to the east coast of the United States, and was imported with the Eastern oyster, *Crassostrea virginica* (Carlton 1979). The Japanese oyster drill, *O. inornata* (synonyms include *Ceratostoma inornatum*, *Ocinebrellus inornatus*, and *Tritonalia japonica*) originates in the western Pacific Ocean, on the coasts of Korea and Japan. This drill shares an evolutionary history with the Pacific oyster, and undoubtedly was imported with that species of oyster (Carlton 1979).

The direct consumptive effects of both drills on oysters have been well studied. Japanese and Atlantic drills consume one small (25-50 mm) Pacific or Olympia oyster every 4-10 d (Hanks 1957, Buhle and Ruesink 2009). These rates, however, can vary widely (Buhle and Ruesink 2009), and might be affected by temperature and salinity (Manzi 1970). Divergent results have been obtained for Japanese drill preference among oyster species: Buhle and Ruesink (2009) determined that Japanese drills prefer Pacific to Olympia oysters of similar size, but
Chew (1960) observed that drills rarely ate Pacific oysters and almost always selected Olympia oysters or other bivalve prey (*Mytilus edulis* and *Venerupis japonica = philippinarum*). Between the two species of oyster, Atlantic drills, prefer Pacific to Olympia oysters (Buhle and Ruesink 2009), but prefer barnacles to oysters (Wood 1968). In oyster beds, native predatory snails, *Nucella lamellosa*, are not a significant source of oyster mortality (Chapman and Banner 1949).

The effects of crabs on invasive drills and oysters are thought to be important, but are not yet known (Buhle and Ruesink 2009). Oyster growers consider crabs a pest and locate growing beds in areas that are not heavily populated with predatory crabs (Bill Dewey, Taylor Shellfish Farms, Pers. Comm.). Red rock crabs, *C. productus*, are one of several species of abundant cancrid crab that can be problematic for oyster growers. Red rock crabs are large-clawed generalist predators that structure intertidal habitats through predation (Yamada and Boulding 1996), and prey on native snails, *Nucella lamellosa* (Marko and Palmer 1991, Bourdeau 2009a, b). In addition, this crab might provide biotic resistance against the invasive European green crab, *Carcinus maenas*, through effects of predation (Hunt and Yamada 2003, Jensen et al. 2007). Because red rock crabs are found in mature oyster beds with invasive oyster drills (Holsman et al. 2006), I considered them to be a good candidate for a predator of the drills.

The addition of the native red rock crabs to this system of oyster drills and oysters could alter the effects of drills on oysters via both consumptive and non-consumptive effects. If crabs consume drills, they will indirectly benefit oysters by releasing them from drill predation.
However, it is also likely that because the red rock crab is a generalist predator, it will readily consume oysters directly. Therefore, the direct and indirect consumptive effects of crabs on oysters will depend on the feeding rates on and preferences for both drills and oysters.

Crabs could also alter the effects of drills on oysters by inducing a defense in drills. The native predatory snail *Nucella lamellosa* responds to predation cues from red rock crabs by increasing avoidance and hiding, reducing feeding, and producing thicker shells (Appleton and Palmer 1988, Marko and Palmer 1991, Bourdeau 2010). If invasive drills also behave defensively in the presence of predation cues, it is likely that the presence of crabs could reduce drill predation on oysters, thus transmitting a positive indirect NCE. Further, if the drills have an inducible defense that decreases in magnitude with increasing conspecific drill density, I would expect that the strength of indirect NCEs of crabs on oysters would decrease at high drill densities, where predation risk is shared by many drills.

To investigate these effects, I conducted experiments to answer the following questions:

1. Do drills respond to predation cues from a native crab predator?
2. Is this response different at low versus high drill densities?
3. Does the presence of the crab cause an indirect NCE to oysters?
4. What are the sources of the chemical cues that induce defenses in drills?
5. What are the consumptive effects of crabs on drills and oysters?

These five questions were addressed in laboratory studies conducted as my Master’s thesis research. They are presented in the following three chapters and discussed at the conclusion.
CHAPTER II: MORE THAN MEETS THE CLAW: BEHAVIORAL PLASTICITY AND NON-CONSUMPTIVE EFFECTS IN A SYSTEM WITH INVASIVE PREDATORY WHELKS

Introduction

It is unclear to what extent inducible defenses against predators affect invasion success (Sih et al. 2009). Constitutive defenses have been implicated in facilitating invasion by defended prey (Lages et al. 2006, Suttle and Hoddle 2006, Wikstrom et al. 2006), and so it is possible that inducible defense could have similar effects. Inducible defenses are very common in nature (Tollrian 1995, Karban and Baldwin 1997, Agrawal et al. 1999, DeWitt and Scheiner 2004), but effective inducible defenses require that prey can detect when they are at risk of being injured or killed (Harvell 1990). In the case of communities with non-native species, prey might not recognize risk of predation by a novel predator because they do not share an evolutionary history.

There is evidence that some native prey can detect non-native predators and mount effective defenses (Kiesecker and Blaustein 1997, Bryan et al. 2002, Pearl et al. 2003, Moore et al. 2004, Snyder and Evans 2006). In the Gulf of Maine, the invasive green crab, *Carcinus maenas*, induces shell thickening in native blue mussels, *Mytilus edulis* (Freeman and Byers 2006), deeper burying in soft-shell clams, *Mya arenaria* (Whitlow et al. 2003), and hiding in the whelk *Nucella lapillus* and the periwinkle *Littorina littorea* (Trussell et al. 2003). In the Great Lakes, the presence of the invasive cladoceran, *Bythotrephes longimanus*, alters
patterns of diel vertical migration in three species of native zooplankton prey (Pangle and Peacor 2006, Pangle et al. 2007). These examples suggest that native species can detect novel, non-native predators, and might be able to mount appropriate defenses that enable populations of native prey to persist.

The reverse scenario, non-native prey detecting and responding to native predators is less well studied, but does occur. For instance, native predatory fish cause the development of protective helmets in the invasive cladoceran, *Daphnia lumholtzi* (Engel and Tollrian 2009). In the Pacific Northwest, invasive bullfrog, *Rana catesbeiana*, larvae behave defensively in the presence of native fish predators (Pearl et al. 2003). By providing a refuge from predation, inducible defenses might reduce the ability of native predators to control introduced prey populations and therefore increase the probability or spread of invasion, as well as post-invasion community impacts.

If native or non-native prey with inducible defenses can detect novel predators, they have the potential to alter community dynamics. In particular, inducible defenses can transmit non-consumptive effects (NCEs) in the presence of a predator (Abrams 1995, Werner and Peacor 2003, Miner et al. 2005, Fievet et al. 2008). By inducing defensive phenotypes in prey, predators can alter prey behavior and population dynamics, which, in turn, can alter the strength and direction of interactions with other species in the community.

The strength of NCEs in a community depends on the magnitude and type of inducible defense (Turner 1997, Bolker et al. 2003, Abrams 2007). In addition to the presence of a
predator, conspecific prey density might influence the probability or magnitude of a defense (Jeschke and Tollrian 2000, Peacor 2003, Jeschke 2006, McCoy 2007). Prey with inducible defenses should evolve the ability to assess the risk of being consumed, which is likely a function of both predator and prey density. For predators with saturating functional responses (Holling’s type 2 and type 3), as conspecific prey density increases, the risk to each individual prey decreases (Holling 1961, Real 1979, Abrams et al. 1990). Prey should reduce investment in defenses as risk of predation decreases, since defenses carry costs (Harvell 1990, DeWitt et al. 1998). Density-dependent risk assessment is especially relevant to species invasions, because invasions typically begin with low densities of an introduced species that greatly increase over time. The strength of NCEs might therefore differ across stages of an invasion.

I investigated whether inducible defenses, and the NCEs they transmit, occur in two invasive oyster drills in the Pacific Northwest. The Atlantic oyster drill (*Urosalpinx cinerea*) and the Japanese oyster drill (*Ocinebrina inornata*, synonyms include *Ceratostoma inornatum*, *Ocinebrellus inornatus*, and *Tritonalia japonica*) are marine whelks introduced unintentionally to the Pacific Northwest in approximately the 1920’s along with a suite of novel commercial oyster species (Chapman and Banner 1949). Drills prey on barnacles and bivalves, and are particularly problematic for the oyster culture industry (Chapman and Banner 1949, Hanks 1957, Chew 1960, Wood 1968, Manzi 1970, Martel et al. 2004, Buhle and Ruesink 2009). In spite of targeted removal and eradication efforts, they remain a pest to the oyster-farming industry, and might also be inhibiting the recovery of native oyster (*Ostrea lurida*) stocks (Buhle and Ruesink 2009).
In Washington oyster beds, these two species of oyster drill interact with native red rock crabs (*Cancer productus*) and oysters (Holsman et al. 2006). A large-clawed, generalist crab known to prey on native snails, *C. productus* could provide potential biotic control for the drills. The red rock crab has also been implicated in reducing, through predation, populations of another invasive, the European green crab, *Carcinus maenas* (Hunt and Yamada 2003, Jensen et al. 2007). As a predator of the drills, the red rock crab could alter the effects of drills on oysters both by directly reducing drill populations through consumption, and by inducing defenses in the drills. Many snails mount defenses in the presence of predators (Appleton and Palmer 1988, Palmer 1990, Alexander and Covich 1991, Trussell and Nicklin 2002, Cotton et al. 2004, Jacobsen and Stabell 2004, Dalesman et al. 2006, Aizaki and Yusa 2010). Therefore, Atlantic and Japanese drills are good candidates for studying whether non-native prey can detect and respond to native predators, and generate non-consumptive effects in invaded communities.

I explored the role of induced drill defenses by performing experiments to answer the following questions:

1. Can non-native drills detect and respond to native crabs? If so,
2. Does this response transmit non-consumptive effects to oysters?
3. Does conspecific density of drills alter their response to crab predators?
Materials and Methods

To answer questions about the presence and context dependence of inducible defenses in invasive oyster drills, I designed two experiments. In the first experiment, I attempted to distinguish between two hypotheses about how drills react to effluent from predation: 1) invasive drills do not change their behavior in the presence of predation effluent, possibly because they are naïve to a relatively novel predator or because they do not mount defenses against any threats; and 2) invasive drills do exhibit defensive behavior by hiding and reducing their feeding rate in the presence of predation effluent, suggesting that they do recognize predation risk from a novel predator. I manipulated the presence and absence of predation cue, and recorded drill feeding rates and habitat use in laboratory mesocosm tanks over several weeks.

Additionally, I predicted that increasing drill density would reduce the effect of predation cue on drill behavior. Reducing investment in costly defense strategies as relative density of prey to predators increases is consistent with the hypothesis that drills factor conspecific density into assessments of predation risk. A density-dependent risk assessment strategy has been observed in lizards (Downes and Hoefer 2004) and tadpoles (McCoy 2007) and might also be used by drills. To test this hypothesis, I performed a second experiment and subjected focal drills to two densities of conspecific drills, both in the presence and absence of predation cue, and measured the same two behaviors.
**Collection and husbandry.** Japanese drills (*O. inornata*) were collected periodically (January-May 2009) from non-native Pacific oyster (*Crassostrea gigas*) beds managed by Taylor Shellfish Farms in West Samish Bay, WA. Atlantic drills (*U. cinerea*) were collected from naturally-recruiting Pacific oyster reefs off the southeast corner of Long Island in Willapa Bay, WA (April and June, 2009). Both species of drill were collected from sites where rock crabs were not known to be present. Red rock crabs (*C. productus*) were collected with ring nets and by hand during low tides from beaches and docks on and around Fidalgo Island, WA. Neither species of invasive drill was found at these locations. Juvenile Pacific oysters were purchased from Taylor Shellfish Farm hatchery as single diploids, 10-25 mm in length.

Each species of drill was maintained in a separate closed aquarium at approximately ambient sea surface temperature when not in use in an experiment. Drills were allowed to feed freely on Pacific oysters or mussels (*Mytilus* sp.) while in holding. Crabs and oysters were maintained in separate flow-through seawater aquaria and periodically fed mussels (*Mytilus* sp.) or frozen fish (*Tilapia* sp.). Oysters were fed Shellfish Diet 1800 (Reed Mariculture) at least once weekly.

**Experiment 1: The effect of predation cue on drill behavior.** To estimate the effect of predation effluent on the behavior of invasive oyster drills, feeding rate and frequency of hiding were recorded for individual drills subjected to either the presence or absence of predation effluent. Predator-cue experiments were performed in the flow-through seawater
system at Shannon Point Marine Center in Anacortes, WA, for either 38 days (April to May 2009) with Japanese drills, or 16 days (June 2009) with Atlantic drills. While I planned to run the experiments for approximately a month, the experiments with the Atlantic drills were terminated at 16 days when there appeared to be large divergence between treatments. Shortening the duration of Atlantic drill experiments allowed me to conduct additional experiments.

A coupled, flow-through bin system (Figure 2.1) allowed me to manipulate predation effluent, without exposing the focal drills to predation. Cues were manipulated in the upstream bin as the presence or absence of a single rock crab. In the downstream bin, an individual focal drill was physically isolated from the predation cues and provided with food and a refuge. The 2.2 cm pipe connecting the two bins was capped with plastic mesh on both ends to prevent any contact between organisms in the two bins.

The upstream bins were stocked with a single crab in the predation treatment, and lacked a crab in the control treatment. All upstream bins were also stocked with two conspecific drills. To simulate a realistic threat, crabs in the predation treatment were allowed to feed on these drills. Drills consumed by crabs were replaced every 48 hours. This effluent combination elicits the greatest magnitude of defense in prey (e.g., Appleton and Palmer 1988, Schoeppner and Relyea 2005). I placed drills in the upstream bins of the control treatment (no predation) to ensure that drill density was similar in all treatments. If a crab died or molted, I replaced it as soon as this change was noticed (< 48 h). In general this was
Figure 2.1. Experimental bin set-up for experiment 1. Coupled flow-through bins allowed me to apply treatments to individual drills. Arrows indicate the direction of water flow through the system. Predation cue was manipulated in the upstream bin as either the presence or absence of a single rock crab. Two drills, which were of the same species as the focal drill, were also added to all upstream bins. The focal drill and 10 juvenile Pacific oysters were isolated from tactile cues of predation originating in the upstream bin. The focal drill was also offered a refuge in the downstream bin. Each treatment was replicated 12 times.
very rare and occurred in, at most, 9 bins during any one experiment. Each treatment was replicated 12 times.

In each downstream bin, a single, isolated focal drill was allowed to feed freely on 10 juvenile (~10-25 mm) *C. gigas*. Every other day, I recorded the number of oysters consumed by the focal drill, and whether the drill was hiding. Oysters consumed by drills were easily identified by the presence of a funnel-shaped hole in one of the two valves. Any dead oysters lacking drill holes were excluded from analyses by adjusting the total number of oysters in the bin. Hiding was defined as a drill located above the water line or underneath structure in the bin.

**Experiment 2: The effect of conspecific density on drill behavior.** In the second experiment I tested whether prey density attenuates the drills’ perception of predation risk. I created a factorial cross of predation cue (present and absent) and conspecific density (“low” and “high” density), and each treatment combination had 6 replicates. The experiment was run in two time blocks to achieve 12 replicates per treatment.

In the Japanese drill experiments, four focal drills died of unknown causes. While the possibility of disease cannot be ruled out, a more likely cause of the mortality was periodic spikes in total dissolved gas levels in the sea water system. All replicates where the focal drill died were terminated and excluded from statistical analysis. This resulted in unbalanced replication for experiment 2 for the Japanese drills (predation + high density = 10, predation
+ low density = 11, no predation + high density = 11, no predation + low density = 12).

Predation cue in the upstream bins was manipulated as in the first experiment. To simulate
the effect of different densities of drills without introducing intraspecific competitive effects,
small, perforated enclosures were placed in each downstream bin (Figure 2.2). In the high-
density treatment, I added 4 conspecific drills to each enclosure in the Japanese drill
experiment. More Atlantic drills were available for use in this experiment, so to provide a
similar biomass of drills, I included 5 Atlantic drills to each density enclosure in the Atlantic
drill experiment. Drills in the density enclosure were not given any food during the
experiments. The density of drills, including the focal drill, was approximately 111 drills
m\(^{-2}\) for Japanese drill experiment, and 133 drills m\(^{-2}\) for Atlantic drill experiment. To
supplement the dissolved chemical cues provided by the density drills in the perforated
enclosure, all downstream bins in the high-density treatments were also “primed” with mucus
trails. Seven conspecific drills were allowed to freely roam in the downstream bin for two
days prior to the start of the experiment. Downstream bins were re-primed for approximately
1 hour weekly by temporarily releasing the density drills. In the low-density treatment, the
perforated bins were left empty, and the conspecific density was approximately 22 drills m\(^{-2}\)
in both experiments. Observations of drill hiding and oyster consumption were recorded
every other day, as in the first experiment. Experiments were run in two consecutive time
blocks of 38 days (May to August 2009) for Japanese drills, and 16 days (June to August
2009) for Atlantic drills.
Figure 2.2. Experimental bin set-up for experiment 2. Coupled flow-through bins allowed me to apply treatments to individual drills. Arrows indicate the direction of water flow through the system. Predation cue was manipulated in the upstream bin as either the presence or absence of a single rock crab. Two drills, which were of the same species as the focal drill, were also added to all upstream bins. The focal drill and 10 juvenile Pacific oysters were isolated from tactile cues of predation originating in the upstream bin. The focal drill was also offered a refuge in the downstream bin. Each treatment was replicated 12 times. Conspecific drill density was manipulated in the downstream bin, by enclosing a smaller, perforated bin that contained either 4 Japanese drills or 5 Atlantic drills in the high-density conspecific treatments. Perforated bins were empty in the low-density conspecific treatments.
**Analysis.** I used the statistical program R and analyzed the data with generalized linear models (GLM), with binomial error structure (R Development Core Team 2009). Models estimated the effect of predation cue (fixed factor), prey density (fixed factor), and time block (random factor) as predictor variables on the drill responses (total hiding behaviors per drill, and oyster survivorship). A binomial error structure is most appropriate because the response variables, number of oysters consumed and number of times drill were hiding, are each a set of Bernoulli trials (e.g., 4 out 10 oysters survived), and should be binomially distributed (Quinn and Keough 2002, Bolker 2008). A log-link was used for models of oyster survivorship because I was interested in modeling mortality, which can be estimated as $-\ln$ (proportion prey surviving) (Sih et al. 1998). This also allows an appropriate test for a non-additive effect between the factors in my experiment. However, a logit link was used for models of total hiding behaviors per drill since each recorded behavior is not affected by the previous behaviors.

In experiment 2 for Japanese drills, no oyster mortality occurred in any of the low density, predation cue treatments during block A. Because a log-linked GLM calculates the natural log of the odds, which for oyster survivorship is the number oysters survived divided by the total number of oysters, the absence of any mortality precluded maximum likelihood estimation for the low density, predation cue treatment mean for block A ($\ln 1 = 0$). I therefore inserted a single “pseudo-mortality” in this treatment to allow me to include data from block A. This makes the test more conservative because it adjusts the mean in the direction predicted by the null hypothesis.
Results

Experiment 1 – The effect of predation cue on drill behavior. Effluent from crabs eating conspecific drills increased the frequency with which both species of drill were observed hiding. Atlantic drills in the predation cue treatment hid 5.8 times more frequently than drills in the control (Figure 2.3A, Table 2.1). Japanese drills were observed hiding twice as often when exposed to predation cue (Figure 2.4A, Table 2.1).

Predation cue also significantly increased oyster survivorship compared to the control in both Japanese and Atlantic drill experiments. Atlantic drills in the control consumed oysters 3 times faster (0.078 oysters per day, one oyster every 12 days) than drills in the predation effluent treatment (0.026 oysters per day, one oyster every 38 days) (Figure 2.3B, Table 2.2). Japanese drills, on average, consumed oysters twice as fast in the control treatment as in the presence of predation effluent (Figure 2.4B, Table 2.2). In the control treatment, Japanese drills consumed 0.061 oysters per day, or one oyster every 16 days. Japanese drills ate only 0.031 oysters per day when exposed to predation cue, or approximately one oyster every 32.6 days. Observed feeding rates fell within the range of published values for both drill species (Buhle & Ruesink 2009).
Figure 2.3. Mean proportion of times Atlantic drills (*Urosalpinx cinerea*) observed hiding (A) and per capita drill feeding rate (B) for drills in presence (n=12) and absence (n=12) of predation cue. Error bars indicate one SEM.
Table 2.1 GLM (binomial error distribution, logit link) results for frequency of hiding behavior as a function of predation cue for Atlantic and Japanese drills. Bold values indicate statistical significance at $\alpha \leq 0.05$.

<table>
<thead>
<tr>
<th>Species</th>
<th>Factor</th>
<th>Estimate</th>
<th>SE</th>
<th>z</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atlantic drill</td>
<td>Predation cue</td>
<td>-2.57</td>
<td>0.394</td>
<td>-6.54</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Japanese drill</td>
<td>Predation cue</td>
<td>-0.99</td>
<td>0.202</td>
<td>-4.91</td>
<td>&lt;0.001</td>
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</tbody>
</table>
Figure 2.4. Mean proportion of times Japanese drills (*Ocinebrina inornata*) observed hiding (A) and per capita drill feeding rate (B) for drills in presence (n=12) and absence (n=12) of predation cue. Error bars indicate one SEM.
Table 2.2 GLM (binomial error distribution, log link) results for oyster survivorship as a function of predation cue for Atlantic and Japanese drills. Bold values indicate statistical significance at $\alpha \leq 0.05$.

<table>
<thead>
<tr>
<th>Species</th>
<th>Factor</th>
<th>Estimate</th>
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<th>z</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atlantic drill</td>
<td>Predation cue</td>
<td>1.092</td>
<td>0.500</td>
<td>-2.18</td>
<td>0.023</td>
</tr>
<tr>
<td>Japanese drill</td>
<td>Predation cue</td>
<td>-0.142</td>
<td>0.060</td>
<td>-2.35</td>
<td>0.019</td>
</tr>
</tbody>
</table>
Experiment 2: The effect of conspecific density on drill behavior. Analysis of the frequency of hiding and oyster survivorship yielded significant main effects and interaction terms for predation cue, drill density, and block. However none of the observed effects supported the hypothesis that increased conspecific density reduces the response to predation effluent for either species of drill.

For Atlantic drills, there was a significant three-way interaction of predation cue x density x block (Table 2.3)—based on Figure 2.5A, it appears the effect of density differed between blocks in the absence of crabs: density did not alter drill behavior in block B, but density did increase time drills spent hiding in block A. There was also a significant two-way interaction of predation cue and density, driven primarily by the difference in effect of density in the absence of predation cue. The main effect of predation cue was highly significant, and had an effect similar to the first experiment. Atlantic drills were observed hiding approximately 4 times as often in the presence of effluent from crabs eating conspecifics, regardless of block or conspecific density treatment. I was able to remove the three-way interaction of predation cue x density x block from the model of Japanese drill hiding behavior because it was not significant ($P = 0.232$). All remaining two-way interactions that included block were significant (Table 2.3). The only significant main effect on Japanese drill hiding was predation cue, which caused drills to increase hiding by a factor of two (Figure 2.6A).

For Atlantic drills, all interaction terms that involved block were removed from the model, because they were not significant predictors of oyster survivorship. The reduced model,
Table 2.3 GLM (binomial error distribution, logit link) results for the frequency of hiding as a function of predation cue, conspecific density, and time block for Japanese and Atlantic drills. For Japanese drills, the 3-way interaction term was removed from the model because it was not significant ($P = 0.232$). Bold values indicate statistical significance at $\alpha \leq 0.05$.

<table>
<thead>
<tr>
<th>Species</th>
<th></th>
<th>Estimate</th>
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<th>z</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atlantic drill</td>
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<td>-1.68</td>
<td>0.446</td>
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<td>0.441</td>
<td>0.44</td>
<td>0.660</td>
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<tr>
<td></td>
<td>Time block</td>
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<td>0.422</td>
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<td></td>
<td>Predation cue x density</td>
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<td>$0.018$</td>
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<td></td>
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<td>-0.75</td>
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<tr>
<td></td>
<td>Density x block</td>
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<td>0.602</td>
<td>-0.74</td>
<td>0.459</td>
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<td></td>
<td>Predation cue x density x block</td>
<td>2.64</td>
<td>1.073</td>
<td>2.46</td>
<td>$0.013$</td>
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<td>Japanese drill</td>
<td>Predation cue</td>
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<td>$&lt; 0.001$</td>
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<tr>
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<td>Predation cue x density</td>
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<td></td>
<td>Predation cue x block</td>
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<td>$&lt; 0.001$</td>
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<tr>
<td></td>
<td>Density x block</td>
<td>-0.679</td>
<td>0.321</td>
<td>-2.11</td>
<td>$0.035$</td>
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</table>
Figure 2.5. Mean proportion of times Atlantic drills (*Urosalpinx cinerea*) observed hiding (A) and per capita drill feeding rate (B) when subjected to the presence or absence of predation cue, and high or low conspecific drill densities. The experiment was conducted in two time blocks (gray bars = Block A; white bars = Block B). Error bars indicate one SEM.
Figure 2.6. Mean proportion of times Japanese drill (*Ocinebrina inornata*) observed hiding (A) and per capita drill feeding rate (B) when subjected to the presence or absence of predation cue, and high or low conspecific drill densities. The experiment was conducted in two time blocks (gray bars = Block A; white bars = Block B). No oyster mortality occurred any replicates in the predation cue, low density treatment in block A. Error bars indicate one SEM.
which included only the three main effects and the two-way interaction of predation cue, indicated that only the main effect of predation cue had a highly significant effect on oyster survivorship (Table 2.4). Similar to experiment 1, drills reduced their feeding rate in the predation cue treatments by approximately 50% compared to the controls (Figure 2.5B).

The GLM on oyster survivorship for the Japanese drill experiment (with the pseudo-mortality in the predation cue, low density treatment of block A) yielded a significant three-way interaction. The difference in the effect of density between blocks was much greater in the absence of predation cue than in the presence of predation cue. The main effect of predation cue greatly reduced mean feeding rate, and significantly increased mean oyster survivorship compared to treatments lacking predation cue (Table 2.4).

Discussion

Can non-native drills detect and respond to native crab predation cues? My study demonstrates that two species of invasive oyster drill recognize and respond to a predation threat from a relatively novel predator. Both Atlantic and Japanese drills hid more often and consumed fewer oysters when exposed to predation effluent in experiments 1 and 2. These behaviors are similar in direction and magnitude to other species of whelk in their native habitat (Trussell et al. 2006, Bourdeau 2009b, Bourdeau 2010), and are likely adaptive (Sih 1985). Snails stop eating and hide when they detect a threat of being consumed by predators, whereas snails leave refuges and forage when they detect little or no threat of being
Table 2.4. GLM (binomial error distribution, log link) results for oyster survivorship as a function of predation cue, conspecific density, and time block for Japanese and Atlantic drills. For Atlantic drills, the interaction terms for block were removed because they were not significant. For Japanese drills, one pseudo-mortality was added to one replicate of the predation cue, low density treatment in block A to permit the GLM to estimate parameters. Bold values indicate statistical significance at \( \alpha \leq 0.05 \).

<table>
<thead>
<tr>
<th>Species</th>
<th></th>
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<td></td>
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<td>0.623</td>
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<td>drill</td>
<td>Block</td>
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<td>-1.94</td>
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consumed by predators. This response in invasive drills contradicts the hypothesis that non-native species will have difficulty detecting and defending themselves from novel predators.

Both of the non-native species drills I tested displayed inducible defenses to native predators. It is unlikely that both species would display inducible defenses if inducible defenses rarely occur in invaded systems. Kimbro et al. (2009) also found that Atlantic drills in Tomales Bay, CA, reduced their feeding rates by approximately 85% in the presence of the native rock crab *Cancer antennarius* that fed on conspecific drills. Several other invasive prey species have also demonstrated inducible defenses in response to native predators, including deer (Flueck 2004), mink (Salo et al. 2008), larval anurans (Pearl et al. 2003), crayfish (Gherardi et al. 2002), freshwater snails (Carlsson et al. 2004), and cladoceran zooplankton (Engel and Tollrian 2009). These results also suggest that inducible defenses commonly occur in invaded systems.

There are two general hypotheses for when these inducible defenses evolved. It is possible that prey evolved an inducible defense in their native habitat, and then expressed the defense in the non-native habitat. Alternatively, non-native prey might have evolved these defenses during their invasion. Because the history of this invasion (~90 years) is probably too short to permit evolution of these defenses *de novo*, it seems most likely that drills evolved defenses in their native habitat, and have co-opted them to respond adaptively to a novel predation threat. It has been hypothesized that prey that use general predation cues (i.e., cues from injured conspecific prey) could demonstrate inducible defenses in response to an
unfamiliar predator (Payne et al. 2004, Sih et al. 2009). Investigating the source of cues that induce defenses in these drills would test this hypothesis.

Regardless of when and where inducible defenses evolved, they likely facilitate invasion. Non-native prey that can defend themselves from predators should have a greater chance of surviving and persisting in a novel habitat than non-native prey that cannot defend themselves. This is analogous to the enemy-release hypothesis (ERH, Keane and Crawley 2002), which states that an introduced species is able to dramatically increase in abundance and distribution in a new habitat because the new habitat lacks natural enemies (predators, parasites, and pathogens) to control introduced prey populations. Similar to the ERH, introduced prey that respond with appropriate induced defenses to native predators will not experience high levels of predation, and CEs will not play a large role in interaction dynamics. Because of this, it might even appear as if the introduced species lacks any native enemies. However, the dynamics of a system invaded by prey with inducible defenses will differ from an ERH scenario because NCEs will impact the invader populations, potentially reducing growth rates, and might also propagate through the community (see below).

One possible consequence of the presence of induced defenses in invasive prey is an increased ability to overcome, or persist in the face of, potential biotic resistance. Inducible defenses provide intermediate species in trophic chains with a refuge from consumptive effects of the top predator, and can theoretically stabilize the dynamics of a three species food chain (Lima 1998, Ramos-Jiliberto et al. 2008). In this system, however, even if drills persist in Washington oyster beds, crabs might still offer some level of biotic control for drill
populations. Red rock crabs are willing and efficient predators of both species of drill, and therefore will probably have at least small CEs on drill populations. Though inducible defenses demonstrated by both drill species will reduce the CEs, the magnitude of that change will depend on how effective the hiding defense is against predation. Moreover, the defenses themselves might also limit drill population growth by reducing drill growth rates and fecundity.

**Does this response transmit NCEs to oysters?** My results suggest that Atlantic and Japanese drills transmit a positive indirect non-consumptive effect of native crabs on oysters. When crabs were present, drills consumed fewer oysters. The effect of crabs on oysters was likely caused by drills hiding more often when they detected predation cue from crabs and therefore consuming fewer oysters. This conclusion is supported by the strong negative correlation I observed between hiding and oyster mortality.

While it has been suggested that dynamics of invaded systems are influenced primarily by consumptive effects (Preisser et al. 2005), our results demonstrate that non-consumptive effects occur in novel predator-prey systems. The effects of hiding and reduced feeding rates I observed for both Japanese and Atlantic drills were large, and likely biologically relevant. However, the net effect of crabs on drills will depend on other aspects of the predator-prey interaction not explored here, including crab and drill demographics, the effect of predator density on drill behavior, and the effectiveness and persistence of the drill behavioral changes.
In oyster beds, the presence of crabs might benefit oysters by causing an indirect NCE. However, the net effect of crabs on oysters (CEs and NCEs) in this system is harder to predict because crabs also prey directly on oysters. It is quite possible, in fact, that crabs prefer eating oysters to their drill predators, and/or can eat them more quickly than they can eat the drills, or than the drills can eat the oysters. While the indirect NCE of predation cue reduces oyster mortality by causing drills to hide, this positive effect could be swamped by negative direct consumptive effects of crabs consuming oysters. Knowing which prey crabs prefer is important for understanding the overall effects of crabs on oysters.

**Does density of drills alter the response of drills to predation cues?** The results of the experiments in which I manipulated conspecific drill density in the presence and absence of predation effluent do not support the hypothesis that increased drill density attenuates the drills’ response to predation cue. I predicted that, in the presence of crabs, drills in the high-density treatment should hide less and feed more than drills in the low-density treatment. This is because per capita risk of predation likely decreases as prey density increases (Jeschke and Tollrian 2000, Jeschke 2006). Additionally, I predicted that in the absence of crabs, drills should behave similarly in the high- and low-density treatments. This is because prey should forage at a maximum rate when there is no threat of predation (McCoy 2007, Relyea 2004). For the Atlantic and Japanese drills, these patterns were not observed—in the presence of predators, drills in high- and low-density treatments behaved similarly, and any
interactions between conspecific density and predation cue were due to differences between high- and low-density treatments when crabs were absent.

The effects of conspecific density on inducible defenses vary among species, but the causes are unclear. Trussell et al. (2003), studying another marine whelk (*Nucella lapillus*), did not observe that increased prey density altered responses to predators. However, in fish and amphibian systems, researchers have observed that inducible defenses can vary with prey density (Dill and Fraser 1984, Downes and Hoefer 2004, Relyea 2004). It is difficult to infer whether these studies support or refute the hypothesis that prey adjust their defenses based on information about conspecific prey densities because their experiments did not exclude the effects of competition among conspecific prey. With my experimental design, I excluded the potential influence of intraspecific competition among prey. The results of this study are therefore the first direct test of whether prey detect conspecific density and adjust defenses.

There are several hypotheses for why drills did not reduce their defenses to predation cue as drill density increased. First, it is possible that crabs do not have a saturating functional response, in which case per capita prey risk would not change with prey density. However, because a saturating functional response (type 2) has been observed in another cancrid crab (Wong and Barbeau 2006), it is more likely that the number of conspecific drills in the high-density treatment did not simulate a change in predation risk for the drill. A second possible reason why drills did not show a density-dependent response to predation cue could be that drills are not able to assess conspecific density, or that my design prevented them from doing so. My perforated bin system minimized direct foot-to-foot contact of the focal drill with the
density drills. So, if drills use contact rather than dissolved chemical or slime trail cues to assess density, they might not have been able to detect the other drills in my simulated high-density treatment.

Density-independent inducible defenses are one mechanism by which the community effects of an invasive species can change over the course of an invasion. Specifically, because drills do not reduce the magnitude of their defenses at increased drill densities, we would expect the strength of NCEs relative to CEs increase as the invasion progresses and drill densities increase. One reason that CEs and NCEs cause different types of community dynamics is that CEs affect only the organisms directly involved in the interaction, only the predator and the prey it consumed. On the other hand, when inducible defenses are elicited by chemical cues of predation, NCEs will be transmitted by any prey that can detect those cues. Therefore, at low prey densities, NCEs will only be transmitted by the few prey that are present, whereas at high densities, NCEs will be transmitted by a large number of prey, and will therefore increase in total strength if the magnitude of each individual’s defense is not reduced. In this system of density-independent drill defenses, as densities of introduced drills increase dramatically over the course of an invasion, the relative strength of NCEs to CEs will increase. In the early stages of the invasion, the magnitude of CEs will be large relative to NCEs, but as the invasion progresses, community dynamics will change, and will be more strongly influenced by NCEs.
Conclusions. Invasive Atlantic and Japanese drills can and do show appropriate defenses against a relatively novel, native predator, and inducible defenses might be more common in invaded systems than is currently supposed. Indeed, inducible defenses might increase the potential for successful invasion by defended prey. This also has implications for community dynamics. Defenses induced by a native crab transmit a positive indirect NCE to oysters. Moreover, neither Atlantic nor Japanese drills reduce their defense with increasing prey density, which suggests that the relative influence of NCEs compared to CEs can increase as the invasion progresses. Therefore, investigation of inducible defenses and NCEs is critical to understanding community dynamics in invaded habitats.

In conservation terms, induced defenses complicate our ability to forecast the success of a species introduction. Managers cannot assume that a native predator capable of consuming an invasive species will in fact control that invasion. This reinforces a growing theme in invasion biology: we must not only consider properties of the invaded system (i.e., capacity for resistance) and/or properties of the invader (i.e., inducible defenses) when forecasting invasions, we must also consider how those properties interact.
CHAPTER III: CUE SPECIFICITY FOR INDUCIBLE
DEFENSES OF AN INVASIVE MARINE SNAIL

Introduction

Inducible anti-predator defenses structure communities by changing the behavior, morphology, or life history of prey (Lima 1998, Agrawal 2001, Orrock et al. 2008, Schmitz et al. 2008). These changes can alter dynamics of prey populations (Miner et al. 2005, Fievet et al. 2008), which, in turn, can alter the strength and direction of interactions with other species in the community (Abrams 1995, Werner and Peacor 2003). Because the effects on community dynamics are transmitted by prey that are not killed, they are called non-consumptive effects (NCEs, also sometimes called trait-mediated indirect effects). For instance, Turner (1997) demonstrated that snails respond to a perceived predation threat (odors of crushed conspecifics) by increasing use of covered habitats and reducing the time spent grazing. As a result, the snails in his experiments grew less, and periphyton abundance increased significantly compared to the control. Inducible defenses can also transmit NCEs by altering the outcome of competition. van der Stap et al. (2008) observed that in the presence of a zooplankton predator, a less competitively dominant, but inducibly defended, rotifer became more abundant than an undefended, but competitively superior, rotifer. Further, the stable coexistence of the two rotifers was mediated by the presence or absence of inducible defenses in the basal algal resource (van der Stap et al. 2008).
Though it is accepted that organisms in aquatic habitats often use dissolved chemicals to obtain information about predators, and that chemical cues alone can induce defenses (Havel 1987, Dodson et al. 1994), little is known about whether chemical cues originate from predator or prey, not to mention the chemical identity of the cues. The source of these chemicals is likely to have significant impacts on the ecological and evolutionary consequences of the defenses they induce. Since inducible defenses can structure communities by causing NCEs, knowing the source of the cue will help predict when NCEs are likely to influence community dynamics. For example, if prey respond to cues from the predator, then presence of the predator alone would be sufficient to cause NCEs. In contrast, if prey mount defenses only when they detect cues of injured conspecifics, NCEs would be propagated only when injury or mortality is actually occurring, regardless of whether injury is caused by a predator or something else. Evolutionarily, reliance on either of these cues might carry costs if they cause prey to respond defensively when there is no risk of predation (Sih et al. 2009), such as when prey are injured by intraspecific competition or environmental disturbance, or predators are present, but are satiated or specialized on another prey species. Therefore, to better understand the evolution of inducible defenses and how they will affect community dynamics, it is important that we learn more about the cues that elicit inducible defenses.

Roughly two decades of research on cue-specific inducible defenses has begun to parse “predation cues” into relevant organismal sources (e.g., Appleton and Palmer 1988, Alexander and Covich 1991a, Bronmark and Pettersson 1994, Yamada et al. 1998, Slusarczyk 1999, Schoeppner and Relyea 2009a, Bourdeau 2009a). When predators attack
and consume prey, they release a mélange of chemicals, which can originate from the
 predator itself (kairomones), the injured prey (e.g., alarm cues, contents of injured tissues and
cells), and the interaction between the predator and prey (e.g., metabolic or waste products of
the predator, altered cues from digested prey, predator responses to prey presence). These
cue sources are often described as either general or specific in the information they provide
to prey (Payne et al. 2004, Sih et al. 2009). Cues from injured conspecifics are general in the
sense that they do not provide any information about the presence, identity, or condition of a
 predator, and so can only serve as a warning to the prey detecting these cues that there is
imminent threat of injury. It is common for prey to show a generalized behavioral defense,
such as hiding or reduced activity, to cues of injured conspecific prey (Chivers and Smith
1998). On the other hand, cues that depend on the identity of the predator are specific, and
can induce directional morphologic defenses appropriate for the predator’s attack strategy
(Turner et al. 2000, Relyea 2003, Bourdeau 2009b). Prey recognition of both of these
sources can be “generalizable” to a certain extent, as some prey, like tadpoles, respond when
they detect injured heterospecific prey (Schoeppner and Relyea 2005), and some respond to
novel predators that are similar or related to familiar predators (Ferrari et al. 2008).

Investigators have attempted to characterize how prey respond to predation cues with
experiments using treatments of single or multiple cue sources. Table 3.1 outlines the
treatment combinations used in 34 experiments outlined in 25 studies. One clear pattern that
emerges from the literature is that the consumptive predation treatment, when predators are
allowed to attack, consume, and digest prey, always elicits the strongest defense in the prey.
Table 3.1 Treatments used in 25 cue-specific inducible defense studies. Check marks in each column indicate treatments used, but studies might have used more than one of each treatment type (e.g., several treatments of different unfed predator species). Where multiple experiments appeared in a single publication, separate rows are used for each experiment. Response types measured include morphological (M), behavioral (B), and life history (LH).

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<sup>1</sup>Combined cues of unfed predator and injured conspecifics, without allowing predator to consume conspecifics

<sup>2</sup>Cues of predators allowed to consume conspecific prey

<sup>3</sup>Cues of predators allowed to consume heterospecific prey

<sup>4</sup>Experiments using additional treatments not covered in this summary

<sup>†</sup>Experiment used only treatments of conspecific and heterospecific-diet labeled predators, predators that had been previously maintained on conspecific and heterospecific prey, but were not actively feeding. No treatments of actively feeding predators were used

<sup>6</sup>Experiment also included additional treatments of conspecific and heterospecific-diet labeled predators, predators that had been previously maintained on conspecific and heterospecific prey, but were not actively feeding
There is more equivocal evidence for other trends, like the influence of predator diet (Jacobsen and Stabell 2004, Schoeppner and Relyea 2005, Bourdeau 2009a), predator identity (Marko and Palmer 1991, Aschaffenburg 2008) and attack strategy (Turner et al. 2000, Relyea 2003, Bourdeau 2009b), and the necessity of multiple cues (Slusarczyk 1999, Hagen et al. 2002, Schoeppner and Relyea 2005, Griffiths and Richardson 2006, Laforsch et al. 2006, Bourdeau 2009a), particularly where the costs of defenses are thought to be great, such as in morphological or life history responses (Slusarczyk 1999, Schoeppner and Relyea 2005).

However, experimental designs that rely on only a few treatments have limited the inferential power of the majority of previous studies on cue-specific inducible defenses. Several elegant studies recently published by Schoeppner and Relyea (Schoeppner and Relyea 2005, 2008, 2009a, 2009b) use more extensive treatment combinations that address shortcomings in prior designs. For instance, multiple cues, in combination, do not always have an additive effect on the magnitude of defense induced (e.g., Schoeppner and Relyea 2005, Bourdeau 2009a). Therefore, when an experiment compares a treatment of full predation cue (predators consuming injured conspecifics), and a predator-only cue, to a laboratory control, the effect of injured conspecifics cannot necessarily be inferred as the difference between the full predation cue and the predator only cue – they therefore must be directly tested. Similarly, Schoeppner & Relyea (2005, 2009a) are some of the only studies that fully disentangle the contribution of chemical cues of digestion from the full predation cue.
Ultimately, to better understand how dissolved chemical cues play a role in regulating community dynamics and evolution of induced defenses, we will need to fully characterize the cue. First, however, we need to precisely identify the source of the cue (Fraker et al. 2009). To do this we need improved treatment designs that are both economical, and effective. Here, I propose one such design.

I studied the behavioral defenses of the invasive Atlantic oyster drill, *Urosalpinx cinerea*, induced by a native crab predator, *Cancer productus*, in the Pacific Northwest. Evidence is mounting that inducible defenses can facilitate invasions (Engel and Tollrian 2009) and structure invaded habitats (Wikstrom et al. 2006, Pangle et al. 2007). Because novel predator-prey systems represent a challenge to risk-recognition systems in prey, an improved understanding of the various cue strategies prey use to identify risk (i.e., general versus specific) can inform the field of invasion biology (Sih et al. 2009). Previous research from two invaded habitats has demonstrated that Atlantic oyster drills increase avoidance behavior (Grason & Miner, unpublished data), and decrease feeding rate (Kimbro et al. 2009) in response to cues of native cancrid crabs eating live conspecifics. Identifying the cue sources that elicit these defenses will enable me to make predictions regarding when inducible defenses are likely to be important in novel species interactions.

**Materials and Methods**
I conducted an experiment to determine which cue sources cause the Atlantic oyster drill, *Urosalpinx cinerea*, to hide and reduce feeding. The experiment had six treatments, each consisting of one or more potential organismal cue sources (Table 3.2), which enabled me to test several relevant hypotheses (Table 3.3). A control, which lacked any predator or conspecific cues, controlled for the effects of the laboratory mesocosm set-up. I used two single-source cue treatments: an unfed crab to generate cues of predator kairomones, and cracked conspecific drills to create the alarm cues that are released by injured conspecifics. The combined effect of these two cues was tested in two ways: one treatment allowed the crab to feed on injured conspecific drills, and another treatment prevented the crab from smelling or consuming injured conspecific drills to test the effect of adding the two cues together without also adding crab digestive cues. For the sixth, and final treatment, I fed frozen fish to crabs to generate cues from a predator consuming a generic, heterospecific prey. Comparing specific combinations of treatments allowed me to determine which of the following cue sources caused a response: predator kairomones, conspecific alarm signals, general predator digestive chemicals, and digested conspecific prey (Table 3.2).

The Atlantic oyster drill, *U. cinerea*, is a marine whelk native to the northwest Atlantic Ocean, and has been a pest to the Pacific Northwest oyster industry since it was unintentionally introduced in the early 1920’s (Chapman and Banner 1949). I used native red rock crabs, *Cancer productus*, as the predator. *C. productus* is an abundant, large-clawed, generalist predator that eats Atlantic drills and occurs with the drills in oyster beds (Holsman et al. 2006).
<table>
<thead>
<tr>
<th>Treatment</th>
<th>Predator kairomones</th>
<th>Conspecific alarm cues</th>
<th>General digestive chemicals</th>
<th>Digested conspecific prey</th>
</tr>
</thead>
<tbody>
<tr>
<td>I  Control (C)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>II  Unfed predator (P)</td>
<td>✓</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>III Injured conspecifics (IC)</td>
<td>-</td>
<td>✓</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>IV  Additive combination* (P + IC)</td>
<td>✓</td>
<td>✓</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>V   Consumptive combination** (P X IC)</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>VI  Predator eating heterospecific prey (P X H)</td>
<td>✓</td>
<td>-</td>
<td>✓</td>
<td>-</td>
</tr>
</tbody>
</table>

*Additive combination separates predator from injured conspecifics, only adding two cues together.

**Consumptive combination treatment allows predator to consume injured conspecifics
Table 3.3. Hypotheses tested about which cue sources induce defenses in Atlantic drills, and the contrasts used to test predictions of hypotheses.

<table>
<thead>
<tr>
<th>Hypothesis</th>
<th>Predicted magnitude of defense</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Drills respond to novel crab predator</td>
<td>Unfed predator (P) &gt; control (C)</td>
</tr>
<tr>
<td>2. Drills respond to injured conspecifics</td>
<td>Injured conspecifics (IC) &gt; control (C)</td>
</tr>
<tr>
<td>3. Injured conspecifics are most influential cue source</td>
<td>Injured conspecifics (IC) = consumptive combination (P x IC)</td>
</tr>
<tr>
<td>4. Effects of injured conspecifics and predator are additive</td>
<td>Unfed predator (P) + injured conspecifics (IC) = additive combination (P + IC)</td>
</tr>
<tr>
<td>5. Digestive chemicals produced by crab are important</td>
<td>Predator eating heterospecific prey (P x H) &gt; unfed predator (P)</td>
</tr>
<tr>
<td>6. Predator digestion of conspecific prey is important</td>
<td>Consumptive combination (P x IC) &gt; additive combination (P + IC)</td>
</tr>
</tbody>
</table>
Drills were collected from a naturally recruiting Pacific oyster (*Crassostrea gigas*) reef in Long Island National Wildlife Refuge in Willapa Bay, WA, where the substrate is primarily muddy, soft sediment, and rock crabs are uncommon. Atlantic drills were maintained in closed 140 L aquaria on a diet of hatchery-reared juvenile Pacific oysters and mussels (*Mytilus* sp.) collected from pilings. Crabs were collected from beaches in and around Anacortes, WA, where Atlantic drills are not known to occur, and maintained on a diet of frozen fish and mussels prior to the experiment. Crabs were starved 24 hours prior to the experiment to allow their guts to clear, reducing the potential that previous diet would confound these treatments.

A coupled, flow-through bin set-up allowed drills to be treated with chemical cues only, isolating focal drills from visual or tactile cues, and predation (Figure 3.1). All cues were generated upstream of the focal drills. A single focal drill was randomly assigned to the downstream bin. I also stocked each downstream bin with 10 hatchery-reared juvenile Pacific oysters, which were single diploids, from Taylor Shellfish Farms (18.6 – 36.8 mm in length, mean 27.5mm). Drills were allowed to feed freely on the oysters (without replacement) during the experiment. A smaller plastic container was placed inside the downstream bin with the focal drill, but away from the oysters, to offer submerged refuge habitat for the drills. The drills could therefore not be actively feeding while they were hiding.
Figure 3.1 Bin set-up for cue specificity experiments. Coupled flow-through bins allowed me to apply treatments to individual drills. Solid arrows indicate the direction of water flow through the system. Cue sources were manipulated upstream of the focal drill as described in Methods. Dotted arrow indicates the mesh pouch containing injured drills, in appropriate treatments, located in the pipe between bins. The focal drill and 10 juvenile Pacific oysters offered as prey were isolated from tactile crab cues in the downstream bin. The crab, when present, was isolated from visual or chemical cues of the focal drills in the downstream bin. The drill was also offered a refuge in the downstream bin. Each treatment was replicated 8 times.
One of the six cue source treatments was randomly assigned to each bin system, and treatments were replicated eight times. In treatments that included a predator, a single *C. productus* was held in each upstream bin and hand-fed prey (in consumptive combination, P x IC; and predators eating heterospecific prey, P x H) to ensure feeding. For each bin that was assigned to a treatment with injured conspecifics (injured conspecifics, IC; additive combination, P + IC; and consumptive combination, P x IC), I cracked the shells of two drills with a hammer to damage the drill shell and tissue, but not crush the tissue. In the additive combination treatment (P + IC), an unfed crab was held in the upstream bin and damaged drills were placed in mesh pouches and secured in the pipe between the two bins of each replicate set-up. This ensured that the starved crabs were not exposed to the scent of injured prey and that the flow to the focal drills adequately circulated any cues released by injured conspecifics. To control for the distribution of these odors, I also used the mesh pouches in the pipes for the injured conspecifics treatment (IC). For treatments of predators eating heterospecific prey (P x H), crabs were offered 0.75-0.90 grams of macerated, frozen tilapia filet, to provide a similar mass of prey flesh as two drills. In the control treatment, the upstream bin was left entirely empty of crabs or drills.

The experiment was run for 16 days (August to September 2009) in the flow-through seawater system at Shannon Point Marine Center in Anacortes, WA. Every other day, I recorded the number of oysters consumed by each focal drill, and whether or not each drill was hiding. Oysters consumed by drills were easily identified by the presence of a funnel-shaped hole in one of the two valves. Any dead oysters lacking drill holes were excluded from analyses by adjusting the total number of oysters in the bin. Hiding was defined as a
drill located above the water line or underneath structure in the bin. On observation days, I fed crabs fresh food appropriate for their treatment, and removed shell debris and waste from the upstream bin. I also replaced mesh pouches of injured conspecifics with freshly injured conspecifics every other day. Five crabs, all in different treatments, molted or died during the experiment, and were replaced within 24 hours.

**Statistical Analysis.** Data were analyzed with a generalized linear model (GLM) with a binomial error structure, a log link function for oyster survivorship data and a logit link function for drill hiding data. The predictor variable was treatment, and modeled as a fixed factor. The response variable was either the number of oysters that survived (modeled as the odds: number survived/number consumed) or the number of hiding behaviors observed (modeled as the odds: number of days hiding/number of days not hiding). I performed *a priori* contrasts to test my hypotheses (Table 3.3).

I also performed a two-factor GLM to test whether cues from the unfed predator (P) and injured conspecific (IC) treatments were additive. The two factors in this model were predator and injured conspecifics, both of which were modeled as fixed factors and had two levels, present or absent. Only four of the six treatments were used in this test: predator present: Unfed predator (P) and additive combination (P + IC) treatments, predator absent: injured conspecifics (IC) and control (C) treatments; injured conspecifics present: injured conspecifics (IC) and additive combination (P + IC) treatments, injured conspecifics absent: unfed predator (P) and control (C) treatments. The interaction term between the two factors
was used to determine whether there was a non-additive relationship between the two cue sources. All analyses were performed with R (R Development Core Team, 2009).

**Results**

Oyster drills in the control treatment consumed juvenile oysters at an average rate of 0.19 oysters per drill per day (one oyster consumed every 5.27 days) over the course of the experiment. This is consistent with feeding rates previously observed for Atlantic drills preying on juvenile Pacific oysters (one oyster consumed per drill every 4.12 days, Buhle and Ruesink 2009).

Cues from an unfed predator increased drill hiding by a factor of three compared to the control (Figure 3.2). However, treatments that exposed drills to cues from a starved crab had only a marginally significant effect on oyster survivorship compared to the control (hypothesis 1—Table 3.4).

Cue from injured conspecifics had a strong effect on drill behavior. Treatments that included injured conspecific drills (IC) significantly increased drill hiding and oyster survivorship compared to the control (hypothesis 2—Table 3.4). Drills reduced their feeding by
Figure 3.2 Mean proportion of times Atlantic drills were observed hiding (A) and per capita drill feeding rate (B) when subjected to six cue treatments. No focal drill mortality occurred in this experiment. Error bars represent 1 SEM.
### Table 3.4

GLM (binomial error distribution, log link for oyster survivorship, logit link for hiding) results for contrasts conducted on oyster survivorship and hiding behavior. Bold values indicate statistical significance at $\alpha \leq 0.05$.

<table>
<thead>
<tr>
<th>Hypothesis</th>
<th>Estimate</th>
<th>SE</th>
<th>z</th>
<th>$P$</th>
<th>Estimate</th>
<th>SE</th>
<th>z</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Drills respond to novel crab predator</td>
<td>1.501</td>
<td>0.456</td>
<td>3.29</td>
<td><strong>0.001</strong></td>
<td>0.170</td>
<td>0.090</td>
<td>3.30</td>
<td>0.060</td>
</tr>
<tr>
<td>2. Drills respond to injured conspecifics</td>
<td>2.456</td>
<td>0.458</td>
<td>5.37</td>
<td><strong>&lt;0.001</strong></td>
<td>0.271</td>
<td>0.082</td>
<td>1.88</td>
<td><strong>&lt;0.001</strong></td>
</tr>
<tr>
<td>3. Injured conspecifics are most influential cue source</td>
<td>0.278</td>
<td>0.373</td>
<td>0.74</td>
<td>0.457</td>
<td>0.014</td>
<td>0.051</td>
<td>-0.27</td>
<td>0.786</td>
</tr>
<tr>
<td>4. Effects of injured conspecifics and predator are additive $^1$</td>
<td>-1.63</td>
<td>0.58</td>
<td>-2.80</td>
<td><strong>0.005</strong></td>
<td>-0.184</td>
<td>0.104</td>
<td>-1.77</td>
<td>0.077</td>
</tr>
<tr>
<td>5. Digestive chemicals produced by crab are important</td>
<td>0.382</td>
<td>0.358</td>
<td>1.07</td>
<td>0.286</td>
<td>0.003</td>
<td>0.073</td>
<td>-0.37</td>
<td>0.971</td>
</tr>
<tr>
<td>6. Predator digestion of conspecific prey is important</td>
<td>-0.41</td>
<td>0.371</td>
<td>-1.10</td>
<td>0.270</td>
<td>&lt;0.001</td>
<td>0.053</td>
<td>&lt;0.01</td>
<td>1.00</td>
</tr>
</tbody>
</table>

$^1$ Additivity was tested as the interaction term in a two-factor GLM (structure as above) of effect of the presence and absence of predator and presence and absence of injured conspecifics (comparing Control, P+, IC+, P + IC).
approximately 67% compared to the control (Figure 3.2). The magnitude of this effect is similar to the effect observed in previous research in this (Grason and Miner, unpublished data) and other, similar systems (Trussell et al. 2006, Kimbro et al. 2009, Bourdeau 2010). Additionally, the cue from injured conspecifics elicited the same magnitude of defenses as the consumptive predation treatment (hypothesis 3—Table 3.4).

The effects of cues from the crab and the injured conspecifics alone do not contribute additively to the behavioral response of drills. The magnitude of response predicted by adding the effect of the unfed predator (P) treatment (compared to the control) to the effect of the injured conspecifics (IC) treatment (compared to the control) does not predict the effect of the additive combination treatment (P + IC) (Figure 3.2). The interaction term of a two way GLM was highly significant for hiding (hypothesis 4—Table 3.4), indicating that two cues have a non-additive relationship. The interaction term is marginally significant for feeding rates, but the response pattern is nearly identical to that of hiding, indicating that these cue sources do not have an additive effect on drill behavior.

Digestion does not increase the magnitude of drill defenses. Drills hid slightly more often when exposed to cue from crabs allowed to feed on injured conspecifics (consumptive combination treatment, P x IC) compared to the additive combination treatment (P + IC) (Figure 3.2). However, these treatments did not differ significantly in either behavioral metric (hypothesis 5—Table 3.4). Additionally, responses to the unfed predator did not increase when the predator was fed a heterospecific generic prey item (Figure 3.2). When
crabs ate frozen fish, oyster survivorship and drill hiding were equivalent to when crabs were starved (Hypothesis 6—Table 3.4).

**Discussion**

The treatments I employed in this study enabled me to clearly identify the role of predation cue sources in eliciting changes in avoidance and feeding behavior in Atlantic drills. Three important conclusions can be drawn from this study. First, Atlantic drills recognize a novel, native predator as a potential predation risk, and respond accordingly. Second, cues from injured conspecific drills elicit the greatest magnitude of response, a response equivalent to that of the full predation cue of predators consuming and digesting injured conspecifics. Finally, digestive cues, both general to the crab and specific to conspecific prey, are not necessary to elicit a full defense.

Drills significantly increase hiding behavior, and slightly reduce feeding rates when exposed to the cues of unfed predator (P), supporting my first hypothesis that drills recognize a novel crab predator. The observed responses to the predator have two important implications: (1) Atlantic drills have the ability to recognize and respond appropriately to a relatively novel predator by itself; and (2) the presence of the crab alone might not provide sufficient evidence of risk to the drills to induce a full behavioral response.
The first implication, that drills have the ability to recognize predation risk by a novel predator, certainly warrants further study. The drills’ response to the crab alone could either be the result of a pre-existing recognition of the crab or an example of rapid evolution. First, however, researchers must determine whether native populations of Atlantic drills have inducible defenses as I am not aware of any studies that have done so. If the drills were able to recognize and respond the unfamiliar red rock crab when they were introduced approximately 90 years ago, there are two possible mechanisms: (1) drills evolved a generalized predator recognition system (i.e., predators are recognized at the genus or higher taxonomic level) in response to co-evolved predators that enables them to respond to an unfamiliar but similar predator and; (2) associative learning enables drills to defend against novel predators (Payne et al. 2004).

Further exploration of defenses induced in several populations of Atlantic drills by a range of predators, including both red rock crabs and crabs from the native range of Atlantic drills, is necessary to distinguish between these two mechanisms. Several species of cancrid crab co-occur with native drill populations, but the strength of predation in those systems is not well studied (Kimbro et al. 2009). Nonetheless, it is possible that drills can recognize kairomones released by the red rock crab because the crabs are similar to a co-evolved predator. However, a population of non-native Atlantic drills in California with a similar invasion history (first reported in Tomales Bay, CA in 1935) does not respond to a congener of the red rock crab, Cancer antennarius (Carlton 1979, Kimbro et al. 2009), so it does not appear that the invasive California population of drills recognizes congeneric predators, which makes the first mechanism unlikely.
I cannot yet rule out a possible influence of associative learning in the drills, the second mechanism. Some prey learn to behave defensively in response to a novel predator only after experiencing the combination of predator cues along with cues from injured conspecifics (Brown and Dreier 2002, Kristensen and Closs 2004, Aizaki and Yusa 2010). However, the drills in this experiment were collected from an area where rock crabs do not occur so it is unlikely that they had the opportunity to learn to recognize this predator. Rearing first generation offspring in a common laboratory environment could eliminate learning as source of predator recognition in drills (Payne et al. 2004).

Alternatively, rapid evolution of defenses in native prey as a response to invasive predators has been observed (Freeman and Byers 2006, Strauss et al. 2006), and presumably this ability extends to invasive prey as well. I am not aware of any case where defenses have evolved de novo in invaded systems, but in several cases, it appears prey have adapted their recognition systems, co-opting a defense they evolved in response to native predators to respond to a novel predator (Freeman and Byers 2006). I believe that this is the most likely explanation for why invasive Atlantic drills can recognize red rock crabs. More study of defenses in native and the several invasive populations of the Atlantic oyster drill in response to multiple predators will determine the plastic or adaptive nature of specific predator recognition for this species.

The second implication of my results, that predator cues alone are not sufficient to elicit the maximum behavioral response, is consistent with other of studies in which the treatments
adequately isolate the effect of predator kairomones (Jacobsen and Stabell 2004, Schoeppner and Relyea 2005, 2009a). However, the fact that the drills respond, even at an intermediate level, to the predator itself, is somewhat unusual. Often, even in co-evolved systems, isolated unfed predator cues do not elicit any behavioral response from prey (Alexander and Covich 1991a, Griffiths and Richardson 2006, Smee and Weissburg 2006, Schoeppner and Relyea 2009a, 2009b). Evolutionarily, a smaller response to only one source of information could be adaptive, since over-responding can be costly if, for instance, the crab being detected is not large enough to consume drills.

My second major conclusion, that cue from injured conspecific drills was the most influential cue, was supported by testing hypotheses 2, 3, and 6. Drills do respond to injured conspecifics (hypothesis 2), and the magnitude of this response is equivalent to the full consumptive cue (hypothesis 3). This result is somewhat surprising because cue from injured conspecifics rarely induces the maximal or full suite of behavioral responses (Slusarczyk 1999, Schoeppner and Relyea 2005, Laforsch et al. 2006, but for an exception see Smee and Weissburg 2006), but clearly demonstrates why it is important to test all possible cue sources directly.

The finding that drills respond strongly to cues of injured conspecifics has important implications for invasion biology. As long as drills are being consumed, predator identity is potentially irrelevant to the magnitude of defense. This supports the theory that prey can use general risk recognition strategies to respond to novel predators (Payne et al. 2004, Sih et al. 2009). Moreover, inducible defenses provide intermediate species in trophic chains with a
refuge from consumptive effects of the predator, and can theoretically stabilize the dynamics of a three species food chain (Lima 1998, Ramos-Jiliberto et al. 2008). Therefore, if defenses do reduce drill mortality, any predator intended as biotic control or resistance against drills would likely be less effective at reducing or eradicating invasive drill populations.

Novel communities exert qualitatively different selective forces than native communities on cue responses of inducible defenses, which might offer an explanation for why prey use divergent information strategies (general vs. specific). It is often supposed that general cues will cause prey to overestimate risk more often then specific cues (e.g., Sih et al. 2009), and therefore be more costly to rely on than specific cues. However, responding to general cues could be advantageous for invasive prey, and even be selected for if prey experience widely variable predation regimes, and serial introductions (Payne et al. 2004). Indeed, a general cue strategy could facilitate invasion by allowing prey to overcome resistance by novel native predators, and prey that respond strongly to cues from injured conspecifics might be more likely to become invasive (Payne et al. 2004).

Understanding the evolutionary implications of a strong response to injured conspecifics requires further investigation, because we need an improved understanding of the precise nature of this cue. Drills might be detecting the contents of damaged cells, or hormones released (actively or passively) by injured conspecifics. Though pheromones actively released by injured conspecifics have been implicated in the defenses induced in larval anurans (Fraker et al. 2009), how selection would act to favor a purely altruistic signaling
response in attacked or injured prey (non-colonial) remains an open question (Havel 1987). We might, therefore, predict that drills are “eavesdropping” on their neighbors and are informed about predation risk by detecting contents of lysed cells or damaged tissues (Danchin et al. 2004). Honing in on how drills identify injured conspecifics will strengthen our understanding of the selective forces behind chemical signaling.

The responses to cues from the unfed predator (P) and cues from injured conspecifics (IC) underestimated the response to the additive combination of cues (P + IC), and I therefore rejected the hypothesis that the effects of injured conspecifics and predators are additive (hypothesis 4). The non-additivity in this case is driven primarily by the fact that drills respond just as strongly to the full consumptive cue as they do to injured conspecifics alone. Further study is needed to determine whether this trend would hold true for inducible morphological defenses in the Atlantic drill, which are thought to be more costly than behavioral defenses, and which might be irreversible (Bourdeau 2009a). Indeed, many signaling systems, particularly those where the associated costs are hypothesized to be great, require multiple sources of information to elicit a response (Crowl and Covich 1990, Bourdeau 2009a, Schoepner and Relyea 2009a). In this sense, chemical sensory systems can be a kind of “combination lock” that requires increased information to access high-stakes responses.

My third major conclusion is that digestive chemicals do not increase the magnitude of inducible defenses in Atlantic drills. Hypothesis 5, that general crab digestive cues increase the magnitude of the defense, was rejected because drills responded similarly to cues of
predators eating heterospecific prey (P X H) and cues of unfed predator (P). Therefore, any general digestive chemicals created by the crab are not further informing drills to predation risk. This result contrasts with other studies where cue from predators fed heterospecific prey elicits a larger magnitude of defense than cue from unfed predators (Griffiths and Richardson 2006, Smee and Weissburg 2006, Schoeppler and Relyea 2009b). However, in these experiments the heterospecific prey are most often fed to predators live, or slightly damaged, and therefore could also be a source of alarm cues. I deliberately chose to feed crabs with frozen fish filet to reduce, as much as possible, any cues that would originate from the heterospecific prey itself during consumption. Incidentally, this finding might also provide tentative validation for other crab-whelk studies where researchers have inferred that crabs fed frozen fish are equivalent to an unfed crab (Appleton and Palmer 1988, Palmer 1990, Trussell and Nicklin 2002, Bourdeau 2009a), though whether or not fish were fed whole to predators might be important since club cells, located in the skin are believed to be the source of fish alarm cues (Stabell and Lwin 1997).

It also appears that digestion and digestive byproducts of conspecific prey do not increase the magnitude of defense, allowing me to reject hypothesis 6. Drills responded equally to the full consumptive combination (P x IC) and the additive combination (P + IC) of predator and injured conspecifics. Other research has shown strong support for the effect of “predator labeling” when prey respond strongly to predators that have been maintained on diets of conspecifics outside of the experimental arenas (Bronmark and Pettersson 1994, Jacobsen and Stabell 2004, Schoeppler and Relyea 2009a). It is possible that drills in my experiment do recognize specific signals of digested conspecific prey, and that this response is swamped
by cues of injured conspecifics. To disentangle this factor, a treatment could be added where
the predator consumes conspecifics outside of the experimental arena, and either the predator
or its waste products are then added to the upstream cue bin. However, I have clearly
demonstrated that it is not necessary for rock crabs to consume and digest conspecifics to
induce the largest magnitude of defense observed.

To my knowledge the additive combination treatment (P + IC) has only been used in two
other studies. Jacobsen and Stabell (2004) compared the avoidance response of an
herbivorous marine snail, *Tegula funebralis*, to cue of injured (homogenized) conspecifics in
the presence and absence of cue from an unfed crab. The added cue of the unfed crab
significantly increased crawl-out (escape) behavior, but the authors believed that their set-up,
which bathed the unfed crab in the effluent of conspecifics, might have elicited the release of
additional chemical cues (such as urine) from the crab in anticipation of predation.
Schoeppner and Relyea (2005, 2009a) also appear to have generated this additive cue
treatment in a way that allowed the unfed predator to react to the scent of injured prey.
Though Schoeppner and Relyea (2005, 2009a) did directly compare this treatment to a full
consumptive predation cue, their results were mixed with regard to behavioral and
morphological responses. In contrast, because of the flow-through design of my experiment,
it is highly unlikely the predators sensed or responded to the injured conspecifics in this
treatment. Considerations of this kind are potentially important for disentangling synergistic
effects of predator and prey cues or the possibility of additional cues resulting from an
interaction of predator and prey.
Much more information is needed to determine how response specificity will transmit NCEs in the community. The magnitudes of defenses observed here might increase with cue concentration as has been observed in other systems (Relyea 2004, Laforsch et al. 2006, Schoeppner and Relyea 2008, Fraker et al. 2009). The chemical structure of the cues will influence their distribution and persistence in variable aquatic environments. Since NCEs are propagated by any prey detecting and responding to the cues, we need to improve our understanding of prey response curves and the chemical nature of cues to determine how many prey will detect the cue, and what the magnitude of their response will be, before we can accurately model NCEs in the community (Harvell 1990, Abrams 1995, Bolker et al. 2003).

Conclusions. The combination of treatments employed here offers a template for future work on cue-specific inducible defenses. Several of the unexpected trends I observed emphasize the importance of directly testing the contribution of each cue (e.g. injured conspecifics), and combination thereof (additive cue treatment versus consumptive) rather than inferring it. Rigorous inference is necessary when testing hypotheses about the adaptive value of inducible defenses because these hypotheses are founded on assumptions of what information, and how much of it, is required to elicit various types of defenses. Moreover, the breadth of studies published on this subject demonstrate that prey are probably divergent in their information strategies, and that no one strategy is universally adaptive, even in co-evolved predator-prey relationships. Therefore, in systems that are not well known, or where
novel predator-prey relationships are being examined, the treatments proposed here should be considered the minimum to rule out any potential unanticipated response patterns.

While characterization of the chemicals and systems involved in the production and recognition of these cues is the ultimate goal of this research, the process of parsing predation cues has to begin with identifying the broad organismal source of the information through relatively inexpensive ecological experiments such as this one. Additional treatments and statistical contrasts can be used, as needed, to further parse cue sources. This design, while still limited to relatively few treatments, will increase the resolution of preliminary experiments and help advance the fields of ecology and evolution in chemical signaling in predator-prey relationships.
CHAPTER IV: PREFERENCE ALTERS CONSUMPTIVE EFFECTS OF PREDATORS: TOP DOWN EFFECTS OF A NATIVE CRAB ON A SYSTEM OF NATIVE AND INTRODUCED PREY

Introduction

Predation is a major force structuring un-invaded (Paine 1974, Hixon and Beets 1993) and invaded (Gherardi and Acquistapace 2007, Salo et al. 2007) communities. Directly, predators can limit (Fitzner et al. 1994, Gurevitch et al. 2000), regulate (Korpimaki and Krebs 1996), or extirpate (Kavanagh 1988) prey populations through consumption. These effects can, in turn, be propagated indirectly through the community in many ways, including trophic cascades, indirect facilitation, and apparent competition (Wootton 1994a). For instance, predators can enhance community diversity by consuming competitively dominant prey, thereby reducing interspecific competition and facilitating the persistence of competitively inferior prey (Paine 1966). Additionally, predators can alter prey populations without consuming them, by inducing anti-predator defenses, including changes in behavior, morphology, or life history (Harvell 1990, Kats and Dill 1998, Lima 1998). The effects of defenses induced by predators can also be transmitted through the community, in what have been termed non-consumptive or trait mediated indirect effects (Dill et al. 2003, Abrams 2007, Peckarsky et al. 2008). In all of these examples, the net effects of predators depend not only on the number of prey they consume, but also on their selectivity.

The preference of predators for different prey can alter the community dynamics. Preference
(sensu Singer 2000) for one prey species over another can modify the effects and interaction strength of predators. When offered a choice, by definition, the predator will consume proportionally more of the preferred prey than expected based on the predator’s ability to consume that prey in the absence of choice (Underwood et al. 2004). This usage of “preference” is distinct from “electivity”, which refers only to the difference between the proportion of prey items in a predator’s diet relative to the proportion of those same items available in the predator’s habitat (Singer 2000). Under this definition, preference is a property of the predator, whereas electivity is a property of the interaction between predator and prey, and might be attributable to factors like search and handling time (Singer 2000). Therefore, where prey preference exists, the number of prey consumed by the predator in a system with two or more species of prey cannot be predicted by measuring the number of each prey consumed by the predator when offered only one species at a time. As in other, multi-species, systems the outcomes of pair-wise species interactions might not predict dynamics in the larger community when predators express preference among prey (Wootton 1994b).

Prey preference might be particularly important in invaded communities, because preference could be affected by the novelty of prey. Three possible outcomes could occur: native predators might prefer either an invasive or a native prey, or not express a preference at all. Invasive prey populations can often constitute a large new food resource for native predators (Pratt and Grason 2007), in which case predators might benefit from preying on the invasive (Barber et al. 2008). Preference for the invasive prey would be adaptive if there are benefits to the predator of specializing on the invasive. In this scenario, we might expect that native
predators could offer a measure of biotic resistance against invasive prey species (Symondson et al. 2002, Gruner 2005). By consuming populations of invasive prey, a native predator could also attenuate the negative effects of interspecific competition on the native prey (Juliano et al. 2010).

Alternatively, a native predator’s response to novel prey might lag due to lack of experience, or necessary machinery to identify, subdue and digest unfamiliar prey (Carlsson et al. 2009). We might, therefore, expect that native predators prefer familiar prey, even if they are capable of preying on novel invasive prey. In this scenario, it is possible that the native predator would actually facilitate the invasion by releasing the invasive prey from competition with native prey, or via apparent competition (Juliano et al. 2010).

The third possible outcome is that native predators express no preference for either prey, in which case the consumptive effects of predators would be predicted by electivity and relative prey densities. Therefore, in modeling the effects of a native predator in an invaded system, it is necessary to determine both the feeding rates on, and preferences for, possible prey types encountered by the predator.

To test which of these hypotheses is supported, I investigated a commercially important food web with a native predator, mediated by invasive intermediate prey. In Washington State, two species of oyster drill (marine whelks) are invasive in commercial and natural oyster beds. Despite control and eradication efforts in commercial oyster beds, drills persist and present an ongoing problem for oyster culture (Bill Dewey, Taylor Shellfish Farms, Inc.,
Pers. Comm.). In addition, these invasive drills might be inhibiting the recovery of the threatened native Olympia oyster (Cook et al. 2000, Buhle and Ruesink 2009). The drills’ habitat overlaps with that of several native decapod crabs, including the red rock crab, *Cancer productus* (Holsman et al. 2006). The red rock crab is a large-clawed generalist predator that preys on native whelks and can strongly influence the structure of intertidal habitats (Yamada and Boulding 1996). It is therefore possible that the crab could help reduce oyster mortality by limiting drill populations. However, crabs also prey directly on oysters. If crabs prefer to eat oysters, the negative effects of oyster predation might counteract, and even swamp, the indirect positive effect of predation on drills. Therefore, to estimate the net effect of the crabs on oysters, I investigated crab feeding rates and preferences on the drills, and on two species of oyster.

**System.** Both Atlantic (*Urosalpinx cinerea*) and Japanese (*Ocinebrina inornata*, synonyms include *Ceratostoma inornatum*, *Ocinebrellus inornatus*, and *Tritonalia japonica*) oyster drills were unintentionally introduced to the Pacific Northwest in the early 1920’s (Chapman and Banner 1949). During that time, shellfish growers were importing non-native species of oyster to replace the collapsing stocks of the native Olympia oyster (*Ostrea lurida*), including the European flat oyster (*Ostrea edulis*), the Eastern oyster (*Crassostrea virginica*), and the Pacific oyster (*Crassostrea gigas*). Both drills arrived as hitchhikers on cultch of these species, the Atlantic drill with the Eastern oyster, and the Japanese drill with the Pacific oyster. Interestingly, native whelks are not considered a pest in oyster beds (Chapman and Banner 1949), so I did not include them in my study.
While at least five species of oyster are currently grown and harvested in Washington, I was particularly interested in the effects of crabs on Pacific and Olympia oysters. Pacific oysters are the most widely introduced, and commercially valuable oyster species worldwide (Ruesink et al. 2005). Their rapid growth and high yield makes them a favorite for oyster growers (White et al. 2009), and these factors have allowed them to establish naturally recruiting wild populations in bays and inland waters of Puget Sound and coastal Washington (Ruesink et al. 2005). In contrast, Olympia oysters, the only native oyster species in Washington, have not sustained a harvestable wild population since the early 1900’s (White et al. 2009). Conservation and restoration efforts currently aim to increase the population of naturally recruiting Olympia oysters in Washington (Trimble et al. 2009). Due to their thin shells, juveniles of both oyster species are most vulnerable to drill predation (Buhle and Ruesink 2009). Both drills consume small Pacific and Olympia oysters at similar rates of about 0.25-0.30 oysters per drill per day. Additionally, despite divergent evolutionary histories with these oyster prey, both drills prefer (sensu lato) Pacific to Olympia oysters of similar size (Buhle and Ruesink 2009).

Rock crabs are known to reside in mature oyster beds that also include both species of drill (Holsman et al. 2006). Therefore, in habitats outside of management by shellfish growers, it is likely that crabs and drills would coexist with these two species of oyster and it has been hypothesized that crab predation might limit distribution of drills (Buhle & Ruesink 2009).
The ability of crabs to limit drill distribution depends, in part, on the effects of crab predation on drills, which is not yet known. I hypothesized that crabs are able to consume both species of oyster much more quickly than either species of drill, and that, given a choice they preferentially consume oysters over drills. Determining crab feeding rates on and preferences among oysters and drills will inform predictions about community dynamics in habitats where all three species occur.

**Materials and Methods**

In two experiments conducted at Shannon Point Marine Center, in Anacortes, WA, I estimated rock crab feeding rates and preferences for two oysters (Pacific and Olympia oysters) and their invasive drill predators (Atlantic and Japanese drills). An initial experiment attempted to determine whether crabs consumed the two oyster species at different rates. Because oyster species were consumed at similar rates, a follow up experiment explored crab feeding on both drills and Pacific oysters only.

**Animal Collection and Husbandry.** Red rock crabs, *C. productus*, were collected by hand and trap from beaches and docks around Anacortes, WA, where neither oysters nor invasive drills were present. While in the lab, crabs were maintained in flow-through aquaria, on a diet of mussels (*Mytilus* sp.) or frozen tilapia. Crabs were not starved prior to the feeding experiments.
Atlantic drills, *U. cinerea*, were collected from naturally recruiting Pacific oyster reefs in the southeastern corner of Willapa Bay, WA. Japanese drills, *O. inornata*, were collected from commercial Pacific oyster beds owned by Taylor Shellfish Farms in West Samish Bay, WA. It did not appear that rock crabs were present at either drill-collection site. Drill species were maintained in separate, closed 140 L aquaria, and allowed to feed *ad libitum* on mussels and juvenile Pacific oysters.

I obtained juveniles of both species of oyster from Taylor Shellfish Farm. Pacific (diploids) and Olympia oysters were obtained at a size at which they could typically be out-planted by commercial or recreational growers (Pacific = 2.7 ± 0.4 cm, Olympia = 2.5 ± 0.2 cm). Oysters were held in sea-tables and had access to a limited amount of plankton that came in with the natural seawater. I supplemented this diet with commercial shellfish diet (Shellfish Diet 1800-Reed Mariculture) at least once a week.

**Preference Experiments.** For the purpose of this study, preference was defined as a deviation of feeding behavior (proportion of prey consumed of one species) in the presence of choice compared to feeding behavior without choice (Underwood et al. 2004). Therefore each experiment included treatments in which crabs were offered one prey species only, and one treatment where all prey species were offered simultaneously in equal abundance (Figure 4.1 & 4.2). This design has the advantage of providing researchers with several relevant estimates of feeding rate and clearly differentiates between preference and electivity.
Figure 4.1. Schematic of treatments for experiment 1 - oyster preference experiment. One treatment for each species of oyster (Pacific or Olympia) offered only that species as prey, and a third treatment offered both species in equal abundance. A single crab was placed in each bin and allowed to feed freely on respective prey for 24 hours without replacement. Pacific oysters are marked with a “P”, and Olympia oysters are marked with an “O”. 

<table>
<thead>
<tr>
<th>Treatment</th>
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<tr>
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<td>Pacific oysters only</td>
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<td>No choice Olympia oysters only</td>
<td>Olympia oysters only</td>
<td>n=12</td>
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<td>Choice</td>
<td>Both oysters</td>
<td>n=12</td>
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Figure 4.2. Schematic of treatments for experiment 2 – drill/oyster preference experiment. One treatment for each species of prey (either Pacific oysters, Japanese drills, or Atlantic drills) offered only that species as prey, and a fourth treatment offered both species in equal abundance. A single crab was placed in each bin and allowed to feed freely on respective prey for 24 hours without replacement.
Two experiments were conducted to estimate preference and feeding rates among the prey items. I first estimated *C. productus* feeding rates on and preferences for Pacific (*C. gigas*) and Olympia (*O. lurida*) oysters (September, 2009). Then, I estimated *C. productus* feeding rates on and preferences for Pacific oysters and two species of drill (*U. cinerea* and *O. inornata*) (October, 2009). Oysters in both experiments were marked with enamel to facilitate correct species identification. Separate groups of crabs were used in each of the experiments and I assumed crabs had no experience with either species of drill or oyster because those organisms were not found in the areas where crabs were collected. In the first experiment, crabs were randomly assigned to one of three treatments (*n*=12): (1) 25 Pacific oysters only, (2) 25 Olympia oysters only, or (3) 25 Pacific oysters and 25 Olympia oysters (Figure 4.1). In the second experiment, crabs were randomly assigned to one of four treatments (*n*=14): (1) 25 Pacific oysters only, (2) 25 Japanese Drills only, (3) 25 Atlantic Drills only, or (4) 25 Pacific oysters, 25 Japanese drills, and 25 Atlantic drills (Figure 4.2).

One day prior to each experiment, individual crabs were placed in separate flow-through bins with 10 individuals of their assigned prey species to allow feeding behavior to stabilize. At the start of the experiment, all waste, uneaten food, and shell material was removed from the bins, and crabs were provided with 25 individuals of each prey species appropriate for their treatment. The number of surviving prey of each species was recorded at 12 and 24 hours without replacement. In only one replicate did a crab consume all available prey of one species. In the second experiment, I periodically checked bins to ensure that drills were not feeding on oysters. However, individual drills take a minimum of 3 to 5 days to consume a
single oyster, and therefore the 24-hour feeding experiments would have been too short to allow any oyster consumption by drills.

**Analysis.** To determine whether crabs preferred a species of prey, I used the new method described below. Currently there is debate about how best to statistically test for preference, and the proposed methods all have benefits and drawbacks (Manly 1993, Underwood et al. 2004, Underwood and Clarke 2005, 2007, Taplin 2007). With this method, I tried to address common criticisms about testing for preferences with a test that was easy to interpret.

If the proportion of each prey item consumed was different when the predators were offered a choice, versus when they were only given a single species, the interaction term of a two-factor ANOVA, with prey species and presence or absence of choice as fixed factors, would be significant. However, use of the F-statistic distribution results in a high probability of type 1 error. This is likely because of the way I calculated the proportion of individuals consumed when only one prey species was offered (see below). For each experiment, I estimated my own null F distribution to determine the F value above which $P < 0.05$.

The proportion of prey consumed without choice was calculated by combining data from replicates in treatments without choice, and the proportion of prey consumed with choice was calculated from the single treatment where a choice of prey was offered. Because I wanted to use the variation among replicates to test for statistical differences, I randomly paired bins in the no-choice treatments to calculate proportion of each prey consumed out of a total...
number of prey of all species consumed. For example, in the first experiment, I paired each bin in which only Pacific oysters were offered with a bin in which only Olympia oysters were offered. For each randomly chosen pair, the proportion of Pacific oysters consumed was calculated as the number of Pacific oysters consumed in the Pacific oyster-only bin divided by the total number of Pacific and Olympia oysters consumed for the pair of bins. So if, in this random pair of bins, 7 Pacific oysters were eaten in the Pacific oyster only bin, and 3 Olympia oysters were eaten. The proportion of Pacific oysters eaten in the absence of choice was 0.7. The inverse operation—the number of Olympia oysters consumed divided by the total number of both oyster species consumed—was conducted on the same pair of bins to calculate the proportion of Olympia oysters consumed out of the total number of prey consumed in the absence of choice. These data represent the proportion of prey consumed when no choice was offered.

The proportion of prey consumed when crabs were offered a choice was calculated separately for each bin in the choice treatment. For each species of prey, I divided the number of individuals consumed of that species by the total number of prey of all species consumed in that bin. The data represent the proportion of prey consumed with choice.

Distributions of $P$ values of the interaction term were generated by randomly pairing bins in the no-choice treatments 1000 times, and comparing the estimated proportion of each prey species consumed in the absence of choice to the observed proportion of each prey species consumed in the presence of choice. A distribution of $P$ values of the two-way interaction
between species and choice, calculated from the adjusted F statistic, enabled me to determine whether the proportion of each species consumed was different in the two treatments.

**Results**

**Oyster preference experiment.** There was no evidence that crabs preferred one species of oyster to another. Crabs consumed a similar number of juvenile Pacific as juvenile Olympia oysters when they were offered only one species and denied a choice (Figure 4.3). When offered a choice of both species, crabs consumed slightly more Pacific than Olympia oysters. The proportion of each oyster species consumed by crabs was similar between crabs offered a choice and those denied a choice. All of the $P$ values of the interaction between prey species and choice generated in the random pairing of bins were greater than 0.05 (Figure 4.4A), suggesting that it is very unlikely that the interaction term is significant. Crabs, therefore, consume the same proportion of Pacific and Olympia oysters in the presence and absence of choice.

**Pacific oysters and drills preference experiment.** There was evidence that crabs preferred oysters more than drills. When offered only one species of prey, crabs did not appear to consume prey at very different rates (Figure 4.5). Approximately 7 juvenile Pacific oysters,
Figure 4.3 Number (mean ± SE) of oysters consumed by crabs over 24 hours in experiment 1 - oyster preference experiment. Gray bars are Pacific oysters, and white bars are Olympia oysters. In the treatment labeled “Choice” both species were offered simultaneously in equal abundance.
Figure 4.4. Frequency distributions of $P$ values of interaction terms generated by randomly pairing bins in the no-choice treatments of experiment 1, oyster preference (A), and experiment 2, drill and oyster preference (B). Note the difference in x-axis scales.
**Figure 4.5.** Number (mean ± SE) of prey consumed by crabs over 24 hours in experiment 2 - drill/oyster preference experiment. Black bars are Pacific oysters, gray bars are Japanese drills, and white bars are Atlantic drills. In the treatment labeled “Choice” all three species of prey were offered simultaneously to the crab in equal abundance.
7 Japanese drills, or 9 Atlantic drills were consumed per crab per day in the absence of choice. In treatments in which crabs were allowed to choose from among the three prey, oysters were disproportionately preyed on compared to drills. Crabs consumed nearly 6 times as many oysters as either species of drill.

Pacific oysters constituted a significantly greater proportion of the crab diet in the presence of choice than when crabs were denied a choice. Approximately 99.9% of the $P$ values of the interaction between factors prey species and choice generated by randomly pairing bins were less than 0.05, indicating that crabs consumed a significantly different proportion of prey species in the presence and absence of choice (Figure 4.4B).

**Discussion**

In the first experiment, crabs did not prefer either species of oyster and consumed both species at similar rates. Red rock crabs did not consume Pacific and Olympia oysters differently when the predators were offered a choice versus when they were presented with only a single species. This indicates that the crabs do not have a preference between juvenile oysters of similar size and will consume both at similar rates in the presence and the absence of the other oyster.

In contrast, crabs in the second experiment strongly preferred Pacific oysters to either species of oyster drill. In the absence of choice, crabs consume Atlantic drills slightly faster than
either Japanese drills or Pacific oysters. But when offered a choice, crabs consumed many more oysters than drills. This difference is not a product of different handling times for each prey species, as all were consumed at relatively similar rates when offered in single species treatments. Instead, this result suggests that crabs will preferentially prey on juvenile oysters if they are available, and will, for the most part, ignore drills unless there is no other food offered.

It is important to note that there are two ways that this preference could be explained here. Preference could either be a property of the crabs when they came into the lab, or could have been altered by experimental acclimation. Because crabs were acclimated to their treatments, and allowed to feed on 10 of each of their assigned prey, for 24 hours prior to the start of the experiment, I cannot rule out the possibility that the preference observed was a result of conditioning. However, the acclimation period was quite short, and I do not believe this significantly impacted crab preferences. It would be straightforward to test this assumption by comparing crab preference between crabs that had, and had not experienced a 24-hour acclimation period.

Combining my results with published estimates of drill predation rates on oysters enables me to make a few rough estimates about interaction strengths in this system when all four species are present. The net consumptive effects of crabs on oysters (the sum of the direct negative effect and the positive indirect effect) are strongly negative for both species. Crabs do consume a small number of drills, even when oysters are available. However, crabs consume oysters more quickly than drills do. Based on my data, on average, one crab
removes approximately 5.8 oysters and 2 drills (one of each species) per day from an oyster bed. Drills consume at most 0.2 to 0.3 oysters per day (Buhle and Ruesink 2009). In the presence of choice, therefore, the direct negative effect of crabs on oysters is approximately -5.8, while the positive indirect effect is +0.4 to +0.6 oysters. The net effect of crabs in the system is still strongly negative: -5.2 to -5.4 oysters removed per crab per day. Therefore, despite the fact that they can be highly efficient and motivated predators on drills, *C. productus* is unlikely to have a strong indirect positive effect on oysters by consuming drills.

It is likely that crabs will affect both oysters similarly, since they do not have a preference between species. However, it is important to note that I used only juvenile oysters. At maturity, Olympia oysters are significantly smaller than Pacific oysters, and this could strongly influence handling times. If the same experiment were run with adult oysters, it is possible that crabs would take longer to consume Pacific oysters, and prefer smaller Olympia oysters.

Revisiting my original three hypotheses, these results suggest that predator preference is not related to prey novelty in this system. Rock crabs do not have a preference for either native (Olympia oyster) or invasive prey (Japanese and Atlantic drills, and Pacific oysters). In the first experiment, where one species of oyster was a native and the other an invasive, the predator expressed no preference. In the second experiment, all three potential prey species were invasive, and it is likely that the preference for Pacific oysters results from the relatively greater energy yield per unit effort required to obtain the food from oysters as opposed to well-armored drills. The invasive prey species in this study are functionally similar to prey
items that are native to Washington; the introduced Pacific oyster is similar to the native Olympia oyster, and the invasive drills are roughly equivalent to native whelks, such as *Nucella lamellosa*. Therefore, these prey might not be a completely “novel” type for the red rock crab.

The most important factor, then, in determining whether crabs will offer biotic resistance against the drills is not novelty, *per se*, but the presence or absence of oysters. This conclusion has several implications for oyster growers and conservation biologists. First, rock crabs are not likely to effectively reduce drill densities in oyster beds through consumption. The feeding rates and preferences observed here indicate that crabs are more likely to negatively impact oysters than drills in commercial beds. Second, in addition to acting as a vector, oysters might have facilitated the invasion and population growth of the drills after introduction. Had the drills been introduced into habitats without oysters, crabs would probably have caused a greater predation effect than they currently do. However, having been introduced along with oysters, it is likely that the drills have experienced a degree of enemy release, because crabs preferentially prey on oysters when they have a choice. Even in the event where oysters facilitated a population increase in crab populations, the preference for oysters over drills is so strong that that the oysters would have to sustain an extremely large population of crabs to have a noticeable negative effect on drills.

Third, crabs might help limit the range of invasive drills to oyster beds. Where oysters are rare, it is likely that crabs will consume more drills, and crabs could thereby provide greater biotic resistance against drill incursion into these habitats.
Notwithstanding the evidence presented here that crabs are unlikely to reduce drill densities in oyster beds, other researchers have noticed that at least in Willapa Bay, WA, there is an overall negative correlation between abundance of crabs and oyster drills (Holsman et al. 2006). This might be explained by the fact that crabs do still prey on drills at low levels, even where oysters are present. Additionally, both drill species increase hiding and decrease feeding the presence of red rock crabs eating conspecifics (Grason & Miner, unpublished data). These inducible defenses, along with emigration and predation, might together explain lower drill densities where red rock crabs are present.

The dynamics in this crab-drill-oyster system demonstrate that estimating predator preference is critical to an understanding of community dynamics. If I had neglected to explore the possibility of predator preference, I would have overestimated the potential for crabs to provide biotic resistance against the invasive drills. It is easy to imagine how management recommendations might have subsequently recommended stocking oyster beds with additional crabs to combat drills. Due to crab preference for oysters over drills, this would have had dramatically negative effects on oyster populations. Preference is therefore an example of how species interactions can be context-dependent and should be incorporated into investigations of predator-community dynamics. Further, in invaded systems, an improved understanding of how novelty affects native predator preference will facilitate better predictions about the spread and impacts of invasives, as well as more effective management practices.
CHAPTER V: DISCUSSION

My research demonstrates that native red rock crabs, *Cancer productus*, can alter the dynamics among invasive oyster drills and native and introduced oysters. Five general conclusions are well supported by my data. (1) Both species of invasive drill behave defensively when they detect predation cues from a novel, native predator. Drills increase the time spent using refuge habitat, which is an adaptive anti-predator behavior in many systems (Kats and Dill 1998). (2) Drills exposed to predation cues consume fewer oysters, which causes a positive indirect NCE of crabs on oysters. (3) Both species of drill have a density-independent response to predation effluent; drills do not use conspecific density to decide whether the probability of being eaten is offset by cost of hiding. Drills display the same level of defense regardless of density. This is one of the only studies that avoid confounding density-dependent risk assessment with intraspecific competition in the experimental design. (4) The cue of injured conspecifics is sufficient to induce the maximum defense in Atlantic drills, but Atlantic drills do have the ability to detect the novel predator itself. The behavioral response to injured conspecifics is equivalent to the full predation cue of predators attacking, consuming, and digesting conspecific drills. (5) Crabs consume both species of oyster, and both species of drill at similar rates (~7-9 prey items day$^{-1}$), but strongly prefer to feed on Pacific oysters over either species of drill.

All of these results inform our understanding of the dynamics of crab-drill-oyster populations. Rock crabs affect drills and oysters through both consumptive and non-consumptive effects. Feeding rate and preference experiments suggest that the negative
direct CEs of crabs on oysters are likely stronger than the positive indirect CEs. Additionally, experiments on defensive behavior of drills in response to predation effluent suggest that it is likely that positive indirect NCEs between crabs and oysters are large and alter oyster survivorship.

While I have shown that crabs affect these dynamics through both predation and intimidation, it is also important to know something about the relative importance of CEs and NCEs, which is largely dependent on drill density in this system. Because I have demonstrated that drill defenses are induced by dissolved chemical cues, the NCEs of crabs transmitted by drill anti-predator behavior have the potential to operate on a much larger spatial scale than CEs (Preisser et al. 2005, Sih et al. 2009). While a predation event only exerts a CE on the drill that is consumed, a NCE is transmitted to any drill that can smell that predation event (or, in the case of at least the Atlantic drills, any drill that can smell a crab). However, where prey use conspecific density to assess risk, defenses become less important at high prey densities, attenuating the overall NCE of the predator (Grand and Dill 1999). The density-independent response to predation effluent shown by both invasive oyster drills in my research suggests that at high prey densities, relative to CEs, NCEs will become increasingly important in structuring the community. Because CEs and NCEs can have qualitatively different, opposing, and potentially destabilizing effects on the community (Peckarsky et al. 2008, Sih et al. 2009), understanding their relative influence and context dependence is crucial for understanding dynamics on this system.
Relevance

My research can inform the developing framework for understanding the role of plasticity, anti-predator behavior, and NCEs in invaded systems (Sih et al. 2009). Much theoretical speculation exists on whether plasticity influences the success of invasions (Agrawal 2001, Miner et al. 2005, Peacor et al. 2006), but there is relatively little empirical research exploring plasticity in invading marine invertebrates (Edgell and Rochette 2009). However, the fact that not just one, but both species of invasive drill I tested respond defensively to novel predation effluent strongly suggests that this type of plasticity could be much more prevalent in invasive animals, particularly invasive snails, than currently assumed. This result argues that we should re-examine well known invaded communities, because inducible defenses and NCEs might indeed underlie many invasions (Peckarsky et al. 2008). Because these systems are already well characterized, investigators can use them to examine the role of NCEs in invasions (Sih et al. 2009).

The strong response of Atlantic drills to injured conspecifics indicates that these drills use a general cue that is not predator species-specific to detect and respond to predation risk. This finding supports the theory that generalized cue responses enable prey to mount appropriate defenses against novel predators (Payne et al. 2004, Sih et al. 2009). Though general risk responses such as this are believed to incur fitness costs related to overestimating risk, invaded systems offer a habitat where the benefits of increased survivorship potentially outweigh those costs, and where natural selection favors general responses. Thus, it is very possible that this risk-recognition strategy lends itself to invasion success, and could be an
informative trait in characterizing potential invaders. Additionally, this strategy could help explain observed exceptions to trends in what facilitates or prevents a successful invasion.

The magnitude of NCEs observed in these laboratory experiments warrants study on the extent to which these effects translate to the field. Abrams (2007) and other authors have commented on the appropriateness of using laboratory studies to estimate NCEs that are likely to exist in the field (Miner et al. 2005). For example, drills in the present study were subjected to a relatively constant stream of predation cue for 16 or 38 days. Cue concentration and dispersal in the field is likely much noisier, due to tidal cycles, flow dynamics, and temporal and spatial variation in predator and prey densities. Therefore this set-up might lead to an over-estimate of the typical defensive response that drills display in the field. While laboratory experiments are helpful in discovering and understanding the mechanisms of defenses, whenever possible, models of community dynamics should be built on effect sizes observed in the field.

Future Research

Both species of oyster drill offer rich opportunities for testing theory about the evolution and ecology of inducible defenses. Further study on the evolution of these behaviors is predicated, however, on validating the assumption that they have a heritable component. Anti-predator behavior can have a genetic basis (Abjornsson et al. 2004, Bleakley et al. 2006, Wright et al. 2006), but can also be learned by association (Marcellini and Jenssen 1991,
Drills in this study were collected from sites where *C. productus* was not abundant, so it is less likely that the defenses demonstrated in these experiments were learned through prior experience. Studies of laboratory-raised drills and their offspring should be conducted to verify whether this behavior is also heritable in drills.

The overarching question then becomes: how did Japanese and Atlantic drills come to have appropriate defenses to a novel predator? There are at least three possible explanations: (1) these defenses evolved after introduction, and have resulted from rapid evolution in recognition and response systems; (2) drills originally evolved the defense in their native habitats, as a response to co-occurring native predators, but have adapted their risk recognition system since introduction to include cues from a novel predator; or (3) drills originally evolved the defense in their native habitats, as a response to co-occurring native predators, and the risk recognition systems they possessed were sufficiently flexible to account for the current response, no evolution was necessary.

Of these, the first is least likely, since the time scale of these invasions, approximately 90 years (135-180 generations), is probably too short to permit the *de novo* development of the machinery necessary for recognition and response. Further, similar defenses are exhibited by other muricid gastropods (Appleton and Palmer 1988, Palmer 1990, Trussell et al. 2006, Bourdeau 2010), and parsimony suggests that it is less probable that similar plasticity evolved multiple times in a single taxonomic family. Determining whether native populations of either species of drill demonstrate similar behavioral defenses to predators...
from the drills’ native habitat would provide a direct test of explanation 1. This explanation
would be supported if drills from a native population did not respond to any predation cues of
predators from the drills’ native habitat.

Distinguishing between the second and third explanations is more complex. If native
populations of drills do demonstrate inducible defenses, it would be informative to compare
the specificity of the defense to that of invasive drill populations. That is, do both
populations of drills respond similarly to injured conspecifics, and a range of potential
familiar and unfamiliar predators? For instance, Atlantic drills from the invasive population
do recognize and respond to Cancer productus by itself, a predator that is not present in
native Atlantic drills ranges. If drills from the native population do not recognize C.
productus per se, then it provides evidence that the recognition system has adapted since
these drills were introduced to the Pacific Northwest (explanation 2). Indeed, it appears that
rapid evolution of recognition systems can occur. Freeman & Byers (2006) demonstrated
that native mussels, Mytilus edulis, from populations with ~15 years of exposure to an
invasive crab predator, Hemigrapsus sanguineus, grew thicker shells in the presence of crab
 cues than mussels from populations that had never been exposed to the crab. It is common
for M. edulis to produce a thicker shell when they detect cues from native predators including
whelks (Smith and Jennings 2000), and another invasive crab with a 50-100 year history with
the mussels (Leonard et al. 1999). Over the 15 years of shared history, it is possible that M.
edulis has adapted to recognize H. sanguineus and co-opted the pre-adapted shell thickening
response to generate an appropriate defense to the novel predator.
Alternatively, it is possible that the predator recognition system of drills were already sufficiently broad to permit invasive drills to recognize *C. productus* when they were introduced to Washington waters. In native ranges, both drills have co-evolved with species of *Cancer* that are capable of exerting significant predation pressure on the drills. If drills from the native habitat also respond defensively to *C. productus*, which is completely unfamiliar, this would provide support for the third explanation.

For at least the Atlantic drills, one piece of information is already available that might help answer this question. Kimbro et al. (2009) demonstrated that Atlantic drills that have invaded Tomales Bay, CA reduce their feeding when exposed to the full predation cue (*Cancer antennarius* eating conspecifics), but not to the crab alone. Atlantic drills have a similar history of invasion in Tomales Bay, with the first sighting recorded in 1935, but the drills might have invaded earlier, as introductions of the Eastern oyster began in 1875 (Carlton 1979). Because *C. antennarius* is a very similar congener of *C. productus*, it appears that there are differences in the predator recognition ability of the two invasive populations of drills, a conclusion that tentatively provides support for explanation 2. Common laboratory experiments with both invasive populations of drills and both species of crab could determine whether recognition is truly divergent between the two invasive populations of drills, and whether that divergence is at the genus level, or the species level.

Explanations 2 and 3 are not mutually exclusive. It is possible that drills came equipped with a sufficiently flexible recognition system to enable them to defend against a novel predation cue, but later evolved the ability to recognize the specific predator itself. This idea is
supported by the fact that Atlantic drills demonstrate the largest response to cues from injured conspecifics, which are a generalized risk cue. Also, these drills recognize the novel predator, and there is some indication that predator specific recognition might be a novel adaptation (as described in previous paragraph). If native populations of drills also respond strongly to injured conspecifics, then when Atlantic drills were introduced, they probably already had the necessary flexibility to avoid predation, regardless of predator identity. However, following introduction, predation by *C. productus* might have selected for the recognition of the predator itself. In invasives, then, the pre-adaptive general risk assessment strategy might facilitate invasion, and allow the population to survive long enough to evolve a more adaptive, predator-specific recognition strategy that enables drill populations to grow and expand their ranges.

This crab-drill system can also be used to test other theories about how various selective forces might favor either general or specific risk recognition strategies. Sih et al. (2009) hypothesize that selection should favor general risk responses when prey have a low likelihood of escaping once they have been detected by predators. In this situation, the costs of over-estimating risk are outweighed by the certainty of death if the predator smells the prey before the prey detects the predator. Certainly, snails, which have little chance of “out-running” a crab, and whose shells might not provide sufficient protection in the event of an encounter, fall into this category. I have demonstrated that Atlantic drills do respond in a way consistent with this hypothesis. An alternative explanation for this outcome is that the exposure to a novel predator, rather than the inability to escape, has selected for the general risk response in prey. In stable predator-prey systems, where prey only experience
significant predation by a single species of predator, we might expect that prey evolve to respond to only those threats that indicate predator presence, because this strategy would reduce the probability that prey would over-estimate risk as compared to prey that respond to injured conspecifics. One way to distinguish between these hypotheses would be to compare risk assessment strategies for invasive snails to those not known to invade. Under Sih et al.’s (2009) reasoning, Japanese drills should also respond primarily to injured conspecifics. Additionally, *Nucella lamellosa* and *Nucella lapillus*, two muricid gastropods not known to be invasive, respond behaviorally and morphologically to sympatric predators. If Sih et al.’s (2009) theory of predator escape is universally important, these snails should also respond strongly to information from injured conspecifics. Alternatively, if snails that are invasive respond to injured conspecifics, but snails that are not invasive rely on cues from the predator, this would suggest that the stability of the predation regime experienced by snails (i.e., relatively few invading predators) might be more important than the escape tactics of the prey in selecting for risk assessment strategy.

**Washington Drills: Prognosis and Recommendations**

The inducible defenses employed by Japanese and Atlantic drills in response to native predation cue increase the likelihood that, without greater human intervention, the drills will persist in Washington oyster beds. Hiding grants drills a refuge from the crabs that reduces the ability of crabs to control drill populations, despite the fact that crabs are willing and capable predators of the drills. At the population level, hiding behavior can dampen
predator-prey oscillations (Ives and Dobson 1987, Ruxton 1995), and stabilize tri-trophic interactions (Ramos-Jiliberto et al. 2008).

Other researchers have noted that in Willapa Bay, WA, drill abundance appears to be inversely correlated with *Cancer* crab density (Kirstin Holsman, Pers. Comm.). While this trend might result from physical gradients in habitats, it could also be explained by emigration from or predation in areas where crabs occur. It is not yet clear for which *Cancer* species this correlation is strongest. However, I suspect that the other local *Cancer* species, *Cancer magister*, the Dungeness crab, and *Cancer gracilis*, the graceful crab, do not significantly influence drill distribution. As compared to *C. productus*, the other species of *Cancer* have relatively small claws. Based on personal observation, even large *C. magister* are not efficient predators of either drill. In feeding trials in which different size ranges of *C. magister* were presented with three size classes of each species of drill, very little predation occurred (Grason, unpublished data). Only a few large Atlantic drills were consumed by small crabs, apparently by extracting them through the aperture of the shell. No size class of *C. magister* appeared capable of crushing either species of drill. Conversely, even very small (70 mm carapace width) *C. productus* could crush large, well-armored, Japanese drills. If *C. magister* is unable to injure and consume many drills, it is likely that both CEs and NCEs on drills will be minimal. Drills might therefore find refuge in areas with low densities of *C. productus*.

Despite the fact that I have demonstrated that oyster survivorship can be improved in certain contexts by adding a crab to these drill-oyster systems, I certainly cannot recommend this as
a conservation strategy until models can be further developed. Application of cues from injured conspecific drills could provide at least an immediate, if temporary, benefit to oyster growers. Bed managers already allocate significant resources to drill removal. In 2009, Taylor Shellfish Farms spent a half million dollars on drill removal and prevention (Danny Lomsdalen, Pers. Comm.). An alternative strategy is to crush the collected drills and redistribute them throughout the beds. This is still a labor-intensive practice, but could reduce oyster-loss to drills during the early summer periods of highest drill densities. An improved understanding of the chemistry of drill defenses could facilitate the development of a more-easily applied, and persistent spray based on the proximal alarm cues from injured conspecifics. Alarm pheromones have also been adapted to increase the efficacy of pest control products in bed bugs, *Cimex lectularius*. Researchers have found that adding bed-bug alarm pheromones to desiccant pesticides increases the movement of bed bugs and their contact with the desiccants (Benoit et al. 2009). Alternatively, if the defense is heritable, and there are naïve populations available, it has been suggested that managers could “breed” naïveté into an invasive population to facilitate biotic resistance by natives (Sih et al. 2009). Much more research needs to address the costs and efficacy of any of these strategies before any of them can be recommended for use in the field.

**Conclusions**

Barring a major increase in human intervention, Atlantic and Japanese oyster drills are likely to persist in Puget Sound. Their success as invasives might be attributed to several factors
discussed in my research, including the presence of inducible defenses, the general cue strategy of Atlantic drills, and the simultaneous co-introduction of a prey preferred by a native predator. However, it is still possible that the negative effects of drills on commercial and native oysters might be mitigated, and their spread limited, by consumptive and non-consumptive effects of the native generalist red rock crab, *C. productus*.

My research suggests that inducible defenses are probably more common in invaded systems than is currently thought, and that NCEs are therefore likely to be influential in structuring many invaded communities. Additionally the importance of NCEs in dynamics of invaded communities might change over time. These findings add complexity to our understanding of novel species interactions and community assembly. Invasion biologists need to consider not only how characters of the invader (i.e., plasticity) or of the novel habitat (i.e., resistance) contribute to invasion success, but also how these factors interact and change over time.


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