Mollusca Non-Grata: The influence of top-down control and residence time on the abundance,

distribution, and behavior of non-native marine snails in Washington State

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ABSTRACT

Mollusca Non-Grata: The influence of top-down control and residence time on the abundance, distribution, and behavior of non-native marine snails in Washington State

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Chair of the Supervisory Committee:
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Invasive species can cause complex, unpredictable changes in ecological community dynamics because they do not share a long evolutionary history with resident species, meaning interactions could be much stronger or weaker than expected. For instance, invasive species often face a suite of both novel potential predators, and novel potential prey, but might not have the ability to recognize or respond appropriately (i.e., to increase fitness). The success or failure of recognition and response in novel predator-prey systems influences the probability of invasions success and the ecological dynamics that follow. Invasive species that fail to respond adaptively to novel, native predators, might persist in only a limited portion of their potential non-native range at low abundances. Conversely, invasive prey with effective defenses could reduce the efficacy of biotic resistance by native predators. The ability of native predators to recognize and overcome such defenses in invasive prey also influences the strength of biotic resistance.

Through a combination of field and laboratory studies, I explored how native predatory crabs influence the abundance, distribution, and behavior of four species of non-native marine snail,

and consider possible outcomes in conservation of native species. All four species of snail are successful invaders in Washington State nearshore systems, despite the fact that this region has a relatively high richness of large native predatory crabs that could confer biotic resistance against these species.

In a field experiment, I explored the potential for an invasive snail to interact with novel native species as both predator and prey at a native oyster restoration site. In this system, invasive marine whelks, *Ocenebra inornata* (Japanese oyster drills) prey on native oysters (*Ostrea lurida*) and might be inhibiting recovery of this rare ecosystem engineer. In the laboratory, native cancrid crabs prey both on oyster drills and on oysters, but prefer to eat oysters. Thus this tri-trophic system includes intra-guild predation (IGP), and crabs might exert top-down control on oyster survival via several pathways: 1) crabs could reduce oyster survival via direct consumption; 2) crabs could increase oyster survival by reducing drill abundance through predation; and 3) crabs could increase oyster survival by reducing drill feeding rates through intimidation. I explored the separate and combined effects of crabs and drills on oyster survival using cages to control access of top predators (native cancrid crabs), and the intermediate (or intraguild) prey (oyster drills) to the resource (oysters). Though crabs were predicted to have a strong negative effect on oysters via direct predation, in fact, the presence of oyster drills had the strongest impact on oyster survival. Drills consumed up to 80% of oysters in experimental cages per month and accounted for an average of 70% of total mortality when they were present. Contrary to my prediction, crabs almost never attacked oysters directly, and consumed drills primarily during only one out of four months. Crabs also did not appear to reduce individual drill feeding rates (i.e. an intimidation effect) or initiate a strong indirect positive effect on oyster

survival. This experiment demonstrated that the role of the invasive predator in IGP as well as the strength of the interaction between the native and invasive species combine to influence the dynamics of the system. In addition, these observations underscore the importance of considering non-native predators as obstacles to the recovery of threatened species, as well the value of experimentally identifying, *in situ*, which of the possible interactions in an invaded food web are ecologically important. This work was published in The Journal of Experimental Marine Biology and Ecology in 2016, Volume 479, pages 1-8.

In a second field study, I used a combination of field surveys and laboratory experiments to assess the role of top-down control by both native and non-native species in influencing regional and local abundance and distribution of the invasive snail, *Batillaria attramentaria*. Two Washington populations of this species have substantially different invasion histories (~10 years versus >80 years) and exhibit markedly different densities and tidal ranges. The less-dense, vertically-restricted population was recently introduced, and thus has had less opportunity to fill the fundamental niche at that site. I investigated three possible explanations: 1) residence time, 2) infection by a co-evolved, castrating, parasite, and 3) biotic resistance by native predators. However, I only found strong support for biotic resistance from native predators; the younger population experienced much greater effects of native cancrid crabs than the older, high-density population, particularly below the minimum tidal elevation of observed snail distribution where crabs are found in the greatest densities. This is the first study documenting effects of predators on this invasive snail, which is widespread along coastlines of the northeast Pacific, whereas previous studies have suggested that the primary restriction on population growth rate was likely to be castration by the co-evolved parasite. Further, this study supports the general belief that,

while novel predators can reduce the impacts or population growth rates of invasive species, such biotic resistance is not likely to preclude persistence at a given site. These observations also affirm the suggestion that residence time could be less important in predicting indicators of invasion success at the local, than at the regional or global scale.

Lastly, I addressed the question of how novelty in predator-prey interactions could constrain the risk recognition ability of the prey. Though prey use a variety of information sources to assess predation risk, non-native prey might fail to recognize risk from a novel predator, with which they share only a short co-history. It has been theorized that non-native prey could compensate via generalized risk assessment, i.e., relying on general alarm signals from injured conspecific prey rather than cues from predators themselves. I tested the influence of shared predator-prey history on information use by comparing responses among three native and four non-native prey species to chemical cues from a native predator and cues from injured conspecific prey. Non-native prey demonstrated information generalism: (1) responding stronger to alarm cues released by injured conspecific prey than to the predators, and (2) responding similarly to alarm cues as to cues from predators consuming injured conspecific prey. By contrast, native prey required multiple sources of information, with increased information content, to elicit the greatest defense. The influence of other sources of chemical information on risk assessment was not predicted by co-history with the predator: only one non-native snail responded to the predator itself; digestion was only important for two native species; the identity of injured prey was generally important in risk assessments; but predator and prey cues always contributed additively to prey response. These results suggest that information generalism, though hypothesized to be costly in co-evolved interactions, might play a role in facilitating biological invasions, either as a driver of, or

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response to, introduction to novel habitats. The impact of generalized risk assessment, relative to other patterns of information use, on community dynamics remains an open and inviting question. Nevertheless, understanding how prey use information to assess predation risk is critical to precisely characterizing the selective forces operating on predator-prey arms races. Biological invasions afford excellent opportunities to investigate these questions because selection can be strong in novel interactions and community perturbations are often readily apparent.

Together, these studies address ways in which novelty can influence predator-prey interactions with implications for predicting and managing biological invasions. Biotic resistance by novel native predators can be an important factor in reducing the impacts of invasive species, by limiting their range or abundance. However, I have also observed support for several mechanisms by explaining why biotic resistance by native predators is unlikely to completely preclude establishment and survival of invasive populations. Biotic resistance by predators varies over space and time, permitting prey to persist via spatial and temporal refuges. Moreover, even where they do co-occur, native predators might not necessarily have a novelty advantage over naïve prey; I have observed that some invasive prey are able to circumvent an inability to recognize a novel threat from a native predator via reliance on generalized alarm cues. Management strategies that take these factors into account could complement biotic resistance by targeting invasive species refuges in control and removal efforts. An improved understanding of the generalities of novel predator-prey interactions could offer novel approaches to conservation, as well as insight into the role of evolution in species interactions.

Clifford. Each graduate student blazes their own trail, and often that journey can feel pretty lonely and isolating, but knowing that all of us are out there has been key.

My committee demonstrated unwavering support of, and faith in, my work and abilities. I am thankful for their helpful feedback and suggestions, the excellent examples they stand as of scientific professionals and all around good people. I appreciate their willingness to tailor their leadership and advisement to my interests and goals – it is a large part of my success in this program. In particular, I would like to confer the status of honorary committee member on Benjamin Miner, who continued his advisement through these 6 years, well beyond his obligation, yet he never shied away from offering me time, friendly reviews, and use of his students, or from saying exactly what I needed to hear at exactly the right time. My advisor Jennifer Ruesink was with me every step of the way, and she is an exemplary advisor on all levels, diving into the minutiae of gluing snails to fishing line and providing clarity to writing, thinking, and logic when none could be found in my own brain. Both Ben and Jen stand as role models for exactly the scientists and humans that I would like to be.

To be real, my family and partner Matt Flora-Tostado deserve the lion's share of the credit. My family instilled in me pride in doing hard work, and pursuit of knowledge, as well as a healthy dose of humility and humor. All of them have demonstrated unflagging compassion for me during my time as a graduate student, from laundry to coffee/cocktails,

I hope I have done all of your contributions justice in the pages that follow, but I understand if you would rather just have your money back…

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CHAPTER 1

COMPARING THE INFLUENCE OF NATIVE AND INVASIVE INTRAGUILD PREDATORS ON A RARE NATIVE OYSTER[1]

1.1 Abstract

Invasive species can cause complex, unpredictable changes in community dynamics because they do not share an evolutionary history with native species, meaning interactions could be much stronger or weaker than expected. A field experiment tested hypotheses generated from previous laboratory experiments about interactions in an invaded tri-trophic intertidal food chain that is also characterized by asymmetric intra-guild predation. Cages controlled access of a top predator (native cancrid crabs) and an intermediate (or intraguild) prey (invasive oyster drills, *Ocenebra inornata*) to a resource (native oysters, *Ostrea lurida*) in order to explore the separate and combined effects of these predators on a native ecosystem engineer of conservation concern. Though crabs were predicted to have a strong negative effect on oysters via direct predation, the presence of oyster drills had the strongest impact on oyster survival. Drills consumed up to 80% of oysters in experimental cages per month and accounted for an average of 70% of total mortality when they were present. Contrary to the hypothesis, crabs almost never attacked oysters directly, and consumed drills primarily during only one out of four months. Crabs also did not appear to reduce individual drill feeding rates (i.e. an intimidation effect) or initiate a strong indirect positive effect on oyster survival. This experiment demonstrates that the role of

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the invasive predator in IGP as well as the strength of the interaction between the native and invasive species combine to influence the dynamics of the system. In addition, these observations underscore the importance of considering non-native predators as obstacles to the recovery of threatened species, as well the value of experimentally identifying, in situ, which of the possible interactions in an invaded food web are ecologically important.

1.2 Introduction

Introductions of nonnative species can cause significant ecological disruption to resident populations, communities, and ecosystems (Mack et al., 2000). The extent to which an introduced species impacts a community depends in part on the type and strength of interactions with resident species. Yet these interactions can be difficult to predict due to the relatively short shared evolutionary history among the species involved (Payne et al., 2004; Sih et al., 2010). For instance, if a native organism fails to recognize a nonnative predator as a threat, or to respond effectively, the predator could have a strong negative effect on the prey population, potentially leading to further cascading indirect effects in the community (Fritts and Rodda, 1998; Kimbro et al., 2009). Because organisms in ecological communities never interact with only one other species, indirect interactions can have major consequences for community structure (Wootton, 1994a), with many well-described examples from invaded communities (White et al., 2006). In tri-trophic food chains, for example, the top predator interacts directly with intermediate prey via consumption, and indirectly with the resource (prey of the prey) by changing the abundance of the intermediate prey (a consumptive indirect effect) (Menge, 1995; Strauss, 1991; Wootton, 1994a).

In a food chain characterized by intraguild predation (IGP), the top predator both preys on and competes with the intermediate prey by directly consuming the resource (Polis et al., 1989). IGP could theoretically slow the population growth of an invader by reducing the availability of shared prey. In the case of asymmetric IGP, in which only one competitor is a predator of the other, the strength of biotic resistance to the invader will depend on the invader's position in the interaction web. Models of IGP in invaded systems predict accelerated invasion rates when the invader is the intraguild predator (Hall, 2011). This is because the invader can consume either native species, while the native intraguild prey is wholly reliant on their shared resource, a scenario favoring population growth of the invader. On the other hand, if the invader is the intraguild prey, predation and competition from the native intraguild predator could both function to inhibit invader success. The latter of these two scenarios (native intraguild predator, invasive intraguild prey) is less well studied. Understanding the IGP dynamics of an invaded system is critical to management, because in some cases removal of a top invasive predator, by releasing population control on an invasive intraguild prey, has been counterproductive to conservation goals (Bergstrom et al., 2009; Courchamp et al., 1999).

The present field study manipulated interactions among a native intraguild predator, an invasive intraguild prey, and a shared native resource species to investigate how these interactions combine to impact the ecologically and economically valuable resource. In the Pacific Northwest USA, invasive Japanese oyster drills (*Ocenebra inornata* Récluz) cause damage that is both ecological, because they consume rare native oysters (*Ostrea lurida* Carpenter), and economic, because they are a pest for the shellfish industry (White et al., 2009). This species of drill was unintentionally introduced in the early 20th century when juvenile Pacific oysters (*Crassostrea*

gigas Thunberg), along with shell used as larval settlement substrate, were imported from Asia to supplement the failing native oyster industry (Chapman and Banner, 1949). Subsequent dispersal of drills has been primarily human-mediated because these intertidal whelks develop in benthic egg capsules and emerge as crawl-away juveniles, limiting their natural dispersal rates (Chapman and Banner, 1949). Management of drills by shellfish growers consists of manual removal of egg capsules and adult snails, but these efforts are time-consuming and costly (Buhle et al., 2005), and achieve only limited success at reducing drill populations. Oyster growers suffer losses from drill predation and occasionally abandon beds due to drill infestation. Reclaimed oyster beds are often sites for *O. lurida* restoration, where predation on juvenile oysters by remnant populations of drills could be inhibiting restoration efforts (Buhle and Ruesink, 2009; Wasson et al., 2015). Native cancrid crabs (e.g., Cancer (*Metacarcinus) magister, Cancer productus, C. gracilis*) co-occur with drills in oyster beds (Holsman et al., 2006) and these crab species can be strong interactors in intertidal communities through predation on molluscs (Yamada and Boulding, 1996).

This crab-drill-oyster system therefore generates the potential for complex trophic dynamics (Figure 1.1). Crabs could influence oyster populations via predation on oysters (direct consumptive effects, pathway 3), predation on drills (indirect consumptive effects/trophic cascade, pathways 1 and 2), and by reduction of the per capita effect of drills on oysters (non-consumptive indirect effects, pathway 4) (Abrams, 2007; Lima, 1998; Werner and Peacor, 2003). Previous research in similar systems suggests that the combined and separate effects of crabs and non-native drills are likely context dependent (Kimbro et al., 2009; Wasson et al., 2015). Some of the potential interactions with these species have been studied independently in laboratory

experiments, allowing us to hypothesize dynamics that might be observed in natural

communities. Laboratory mesocosm studies demonstrated that *O. inornata* eats 50% fewer

oysters and hides more often when exposed to chemical cues from *C. productus* attacking,

consuming, and digesting conspecific drills (Grason and Miner, 2012a). When given a choice,

however, crabs preferentially consume juvenile oysters over drills (Grason and Miner, 2012b).

Based on both sets of experiments, we predicted that any positive indirect effect of crabs (both

consumptive and non-consumptive) on oysters would be swamped by the negative direct effect

of crab predation.
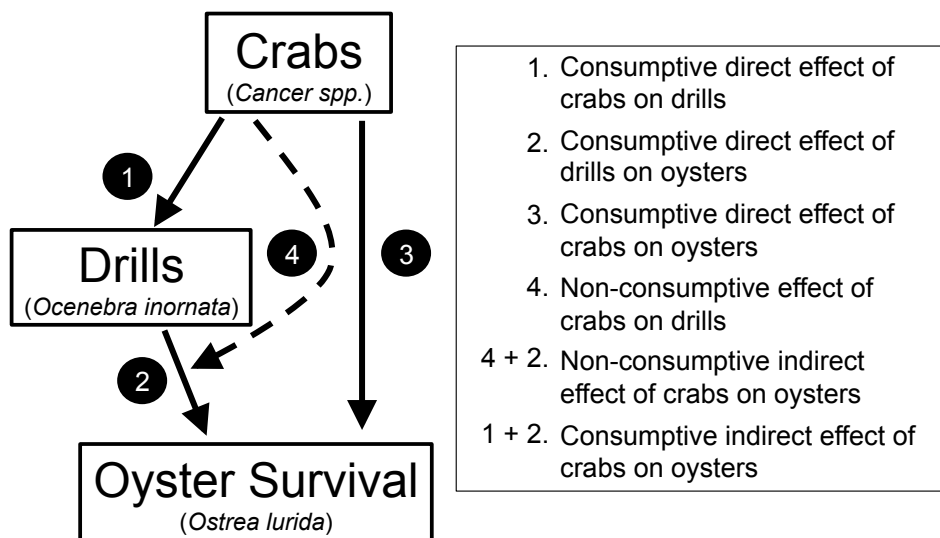
Figure 1.1 Diagram of potential interaction pathways in the three-species trophic web investigated here. Arrows point from the initiator species to either the receiving species (solid lines), in the case of consumptive effects, or to the interaction arrow between two species (dashed line), in the case of non-consumptive effects whereby crabs modify the rate at which oyster drills feed on oysters.

To examine the trophic dynamics of this system in the field, crab and drill access to oysters was

manipulated in a four-month caging experiment in Liberty Bay, Washington, an inlet of Puget

Sound. Based on forensic observations of shell damage, oyster mortality was attributable to

respective predator types. This allowed quantification of the direct effects of each predator type

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as well as the indirect effects when both predators were present. Lastly, drill feeding rates were measured to distinguish whether any indirect effects were due to changes in drill density, per capita effect, or both.

1.3 Materials and Methods

To determine the separate and combined effects of predators on oyster mortality, the presence and absence of crabs and oyster drills were manipulated over four months (April – August 2011) using enclosure and exclosure cages. The cages were deployed on the mudflats at Scandia on the west side of Liberty Bay, WA (47.72204°N, 122.65412°W) between -0.3 and -0.6 m MLLW. Ongoing native oyster restoration efforts at this site are conducted by a local nongovernmental organization (the Puget Sound Restoration Fund), and oyster recruitment substrate consisting of *C. gigas* shell material was most recently deposited in 2005. The benthic community now includes *O. lurida* that have recruited onto the shell as well as *O. inornata* and large, mature *C. gigas*.

To estimate the effects of both crabs and drills on *O. lurida*, oyster mortality was recorded monthly in a factorial caging experiment consisting of four treatments: no predators, drills only, crabs only, and both drills and crabs (n = 5 cages per treatment). Predator manipulation cages (56 cm L × 56 cm W × 25 cm H) that controlled the access of each predator type to oysters were constructed from plastic mesh (10 mm hole size on top and bottom, 4 mm on sides). In treatments that exposed oysters to crab predation, cages allowed crabs to enter via holes (23 cm L × 10 cm H) on two of the four side panels. The edges of the holes were lined with copper flashing to prevent snails from exiting or entering the cages. This method has previously been shown to be effective in controlling movement of *O. inornata* (Buhle and Ruesink, 2009). In

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treatments that included drill predation, three *O. inornata* (30-40 mm shell length, 12-24 mm at the widest point) collected from the surrounding area were enclosed in each cage and replaced on each monthly sampling occasion. This yielded a density of approximately 9.6 drills m^{-2}, which is within the range of observed field densities (Buhle, 2007; Buhle and Ruesink, 2009). Thus the cages allowed crabs to come and go, while drills were confined or excluded. This is an ecologically relevant design because crabs are highly mobile and forage intertidally only when the tide is in, while drills are restricted in their movement, and remain on or near prey they are actively consuming, even when exposed at low tides.

In all cages, 10 stakes were inserted into the substrate with a single juvenile *O. lurida* (mean shell length ± SD = 36.1 ± 4.0mm) epoxied and fastened to each stake so that the hinge of the oyster shell was flush with the sediment surface and the valves of the oyster were oriented perpendicular to the ground. Staking oysters prevented fouling and enabled tracking of individual oysters. This arrangement also simulated that of naturally-recruited Olympia oysters at this and other sites in Puget Sound where they typically occur individually or in small clusters. No other substrate (e.g., shell material or mud) was included in the cages, such that drills were not provided with any additional prey or refuges. Additionally, for the last three months of the study (May – August), a no-cage treatment provided a control for cage effects. In these plots, 10 oysters were staked across a portion of the tideflat covering the same area as the base of the cage, and secured to a central rebar stake with nylon cord.

Several observations suggest that cage treatments manipulated predators as intended. First, drills were effectively kept from entering and leaving the cages. Drills entered nominal exclusion

cages on only three of 40 potential occasions, with two of those cases occurring in cages that had openings for crabs. This suggests that cages controlled the movement of drills effectively even when they permitted crab entry. In addition, drills were never observed climbing the walls of the cages and there was no evidence that drills were "rafting" in or out on floating debris. It is therefore most likely that drills that disappeared from cages were consumed by crabs. Given tidal flushing during the long time interval between observations and the fact that crabs could remove drills from the cages and consume them elsewhere, one would not expect to find much physical evidence of crab predation on drills (i.e., shell fragments). Nevertheless, damaged, empty drill shells were observed on two occasions. This direct evidence of shell crushing also supported the inference that crabs entered the cages on the high tide, despite the fact that we could not quantify crab abundance or visitation to cages. Moreover, previous trapping and observations in the area indicate an extremely high abundance of the graceful crab, *Cancer (Metacarcinus) gracilis* on mudflats in Liberty Bay (B. Allen, unpublished data). In addition, red rock crabs, *Cancer productus*, are abundant in the channels adjacent to the Scandia flat and also likely visit these oyster beds. Both species are known to consume snails, the former by extracting or chipping at the aperture of the shell and the later by crushing.

Cages were deployed on 20 April 2011, and drill and oyster mortality were surveyed approximately monthly for four full tidal cycles. On observation days (18 May, 14 June, 13 July, and 11 August) oyster mortality was assessed and all oysters were replaced with new individuals. Drills remaining in the cages were counted and all drills in drill-enclosure treatments were replaced with locally-collected individuals to bring the total number back up to three. Dead oysters were brought back to the lab to determine whether they had been consumed by predators

or had died of other known or unknown causes. The two predator types have very different, easily distinguishable, methods of shell entry. The whelk, *O. inornata*, drills very small (>3 mm) diameter holes in one valve of the shell while crabs, depending on size, either chip or crush small oysters. Oysters that died but showed no evidence of damage were excluded from analyses because hypotheses were related to mortality due only to predation. A small proportion (1%) of dead oysters had only a single valve remaining, precluding a determination of cause of death. These were counted as uncategorized mortalities and excluded from analyses. Thus estimates of predator effects are likely conservative because some of the latter group of oysters were likely killed by either crabs or drills. As mentioned above, very occasionally drills or drilled oysters were found in cages designed to exclude drills. These instances were rare (drills found in 5% of no-drill cages; drilled oysters found in 7.5% of no-drill cages constituting 3.7% of total caged oysters drilled) and do not affect the qualitative results, so they were not removed from analyses.

1.3.1 Analyses

Trophic interactions in the cages were analyzed by modeling predation on oysters, drill feeding rates, and drill survival using generalized linear mixed-effects models (GLMMs; Bolker et al. 2009). Because the predation regime in the no-cage control plots could not be quantified (i.e. the number of drills present in the area was unknown), that treatment was excluded from all statistical analyses.

Both oyster survival (the number of surviving oysters conditioned on the number remaining at the end of the sampling interval, omitting non-predation related mortalities) and drilling rates (total number of oysters drilled during each monthly interval) were analyzed as a function of the number of drills (average of initial and final counts), crab presence, time (linear and quadratic

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terms of study day as described below), and the two-way interactions of these factors using GLMMs. For oyster survival, a binomial error structure (logit link) was used, where "successes" were oysters remaining alive and "failures" were known predation mortalities, thus excluding oysters whose cause of death could not be determined. The model of drilling rates used a Poisson error structure and log link. The second-order polynomial of elapsed time in days was chosen as a parsimonious model for change over time in oyster survival, and assessed using residual plots. The linear and quadratic terms were treated *en bloc* as a "time effect"; that is, they were always either included or excluded together in candidate models, and their interactions with treatment effects were similarly grouped together. All models included a normally-distributed cage-level random intercept to account for potential heterogeneity among cages.

Oyster survival was predicted to decrease when either crabs or drills were allowed into cages, but it was also predicted that any positive indirect effects of crabs on oyster survival would be masked by direct crab predation on oysters, resulting in a crabs × number of drills interaction. The number of oysters drilled per month was a minimum estimate of drill feeding rates because some uncategorized dead oysters with only one valve remaining could have been drilled (less than 1% of cases). The total number of drilled oysters was predicted to increase with drill abundance (a positive main effect of drills), but a crabs × number of drills interaction would indicate a change in the per capita drill feeding rate in the presence of crabs (a non-consumptive effect of crabs on drills). Note that "per capita effect", as used here, refers to the regression coefficient of drill abundance, but this parameter does not correspond directly to "per capita interaction strength" as defined in standard population-dynamic models (Bender et al., 1984; Wootton, 1994b). The effect of prey abundance on drill feeding rates (i.e., the drill functional

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response; Buhle and Ruesink 2009) was not considered because there was little variation in oyster density within or among cages; experimental oysters were replaced monthly and were never fully depleted.

Drill survival was modeled as the number of live drills remaining at the end of each month given the initial number (three) using a binomial error distribution and logit link, where the potential predictors were crab presence (allowed or excluded), time (linear and quadratic terms for day of study as described above), and their two-way interaction. The main effect of crabs represents their direct predatory impact on drills. Drill survival was only analyzed in the treatments that included a known number of drills (i.e., cages where drills were enclosed, with or without access by crabs).

Information-theoretic model selection (Burnham and Anderson, 2002) was used to evaluate the importance of the predictors in each analysis. In both cases a set of candidate GLMMs was constructed by taking all subsets of fixed effects in the global model; interactions were allowed only if the corresponding main effects were included, and all models included a random intercept. As explained above, linear and quadratic terms of time were grouped together, as were their interactions with each of the other factors. The small-sample version of Akaike's information criterion (AIC_c) was calculated for models and the corresponding Akaike weights were used to construct a 95% confidence set of models (Burnham and Anderson, 2002). The parameter importance for each main effect or interaction term was calculated by summing model weights across all models in the 95% confidence set that included that term. All analyses were conducted

in R (R Development Core Team, 2013) using the lme4 (Bates et al. 2013) and MuMIn (Barton 2013) packages.

1.4 Results

Native crabs and non-native oyster drills differed in their impacts on native oysters, and the strength of those top-down effects varied throughout the 4 months of the experiment. Up to 80% of the oysters in cages were consumed by predators in any given month, but nearly all of the total predator-related mortality occurred in treatments that included drills (Figure 1.2). Forensic evidence from oyster shells in cages clearly identified Japanese drills as the most important predator of oysters, accounting for an average of 70% and up to 100% of total oyster mortality in drill-enclosure treatments. There was strong support for the influence of the number of drills and day/time on predator-related oyster mortality (Table 1.1, parameter importance = 1.00 for both), and these parameters appeared in all of the top candidate models of oyster predation. By contrast, crab predation on oysters was rare. Only two crushed shells, where a partial remnant valve was found still attached to the epoxy, were found over the entire four-month study: once in August in a cage that allowed both predators, and once in July in a cage that allowed only crabs. Crab predation constituted 0.8% of total oyster mortality and 2% of total predation on oysters. Only weak support for the effect of crabs (parameter importance = 0.66) on predation-related oyster mortality was found, and very little support for any of the two-way interactions (Table 1.1). To put the effects of predators in context, on average, 13% of oysters in the study were found dead, but had no clear sign of predation attempts, and were presumed to have died from heat stress or other causes.
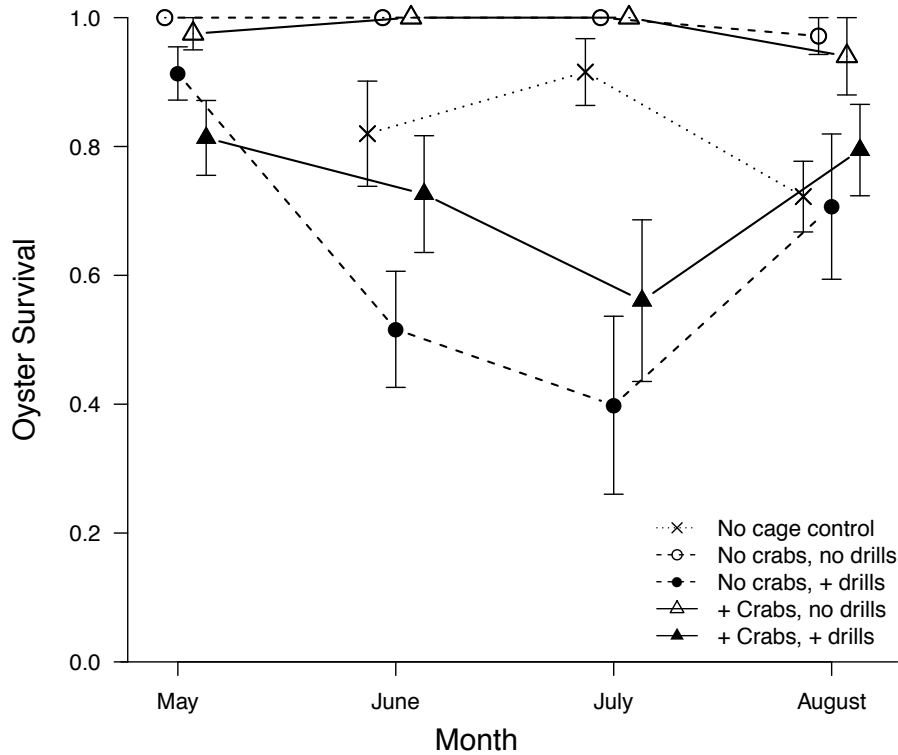
Figure 1.2 Average proportion of oysters surviving predation (not consumed by either crabs or drills) each month for all treatments (location on the x axis has been jittered for visibility). Filled symbols indicate treatments where drills were included in cages. Triangles and solid lines indicate treatments where crabs were allowed to enter cages. Circles and solid lines show treatments were crabs were excluded. The "X" symbols represent the no-cage control treatment. Error bars = 1 SEM.

Consistent with the analysis of oyster survival, models of drill feeding rate indicated that the strength of the drills' impact varied throughout the spring and summer (Figure 1.3, Table 1.2). The number of oysters drilled per day increased from May through July and declined slightly in August, and time effects appeared in all candidate models (parameter importance = 1.0). The observations provided only weak support (parameter importance = 0.43) for a main effect of crabs on drill feeding rate (Table 1.2; Figure 1.4). Despite slightly lower mean per capita drill feeding rates in the presence of crabs during June and July, there was even weaker support (parameter importance 0.12) for the crabs × drills interaction term, meaning nonconsumptive effects of crab presence on drill behavior were not detectible (Figure 1.4).

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Table 1.1 Generalized linear mixed-effects models (binomial error, logit link) of oyster survival within 95% confidence set of candidate models. "Y" indicates inclusion of categorical factor in candidate model. Model-averaged coefficients are generated from 95% confidence set of candidate models

Model	Intercept	Crab	Day (Linear)	Day (Quadratic)	Avg. No. Drills	Crab X Day (L)	Crab X Day (Q)	Crab X No. Drills	No. Drills X Day (L)	No. Drills X Day (Q)	df	Δ AICc	Model Weight
1	4.835		-0.686	-0.401	-1.638				0.132	0.401	7	0	0.223
2	3.330	Y	-0.450	1.075	-1.220	Y	Y				8	0.49	0.174
3	4.144	Y	-0.450	1.103	-1.537	Y	Y	Y			9	1.02	0.134
4	3.571		-0.372	0.636	-1.172						5	1.39	0.111
5	4.383	Y	-0.739	0.088	-1.605	Y	Y		0.102	0.362	10	1.46	0.107
6	5.165	Y	-0.865	0.155	-1.895	Y	Y	Y	0.142	0.335	11	2.46	0.065
7	4.836	Y	-0.686	-0.401	-1.639				0.132	0.401	8	2.48	0.064
8	5.810	Y	-0.688	-0.427	-2.015				0.142	0.416	9	2.65	0.059
9	3.600	Y	-0.373	0.635	-1.175						6	3.72	0.035
Averaged parameter importance		0.66	1.00	1.00	1.00	0.49	0.49	0.27	0.53	0.53			
Model-averaged coefficient (SE)	4.314 (1.125)	<0.001 (1.059)	-0.582 (0.391)	-0.315 (0.787)	-1.516 (0.391)	0.118 (0.282)	-0.826 (0.323)	0.526 (0.399)	0.128 (0.163)	0.386 (0.218)			
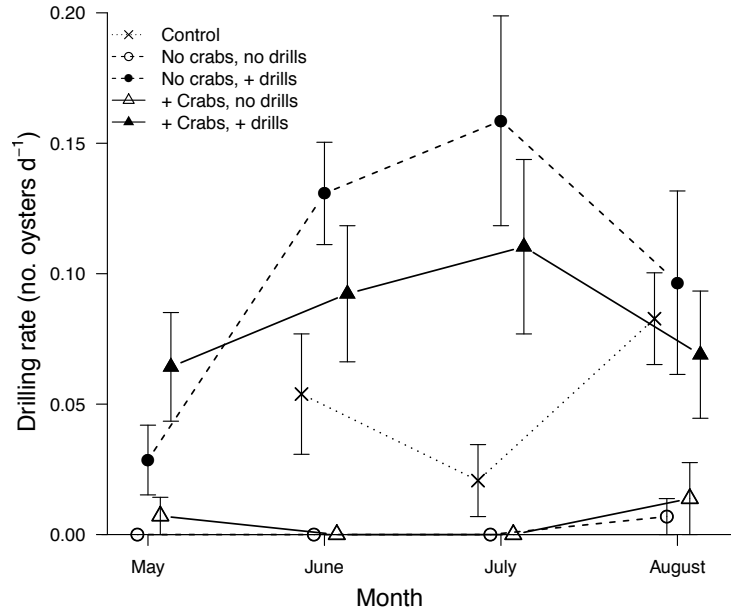
Figure 1.3. Average daily drilling rates as the total number of oysters killed by drills per day observed monthly for all treatments (location on the x axis has been jittered for visibility). Filled symbols indicate treatments where drills were included in cages. Triangles and solid lines indicate treatments where crabs were allowed to enter cages. Circles and solid lines show treatments were crabs were excluded. The "X" symbols represent the no-cage control treatment. Error bars = 1 SEM.
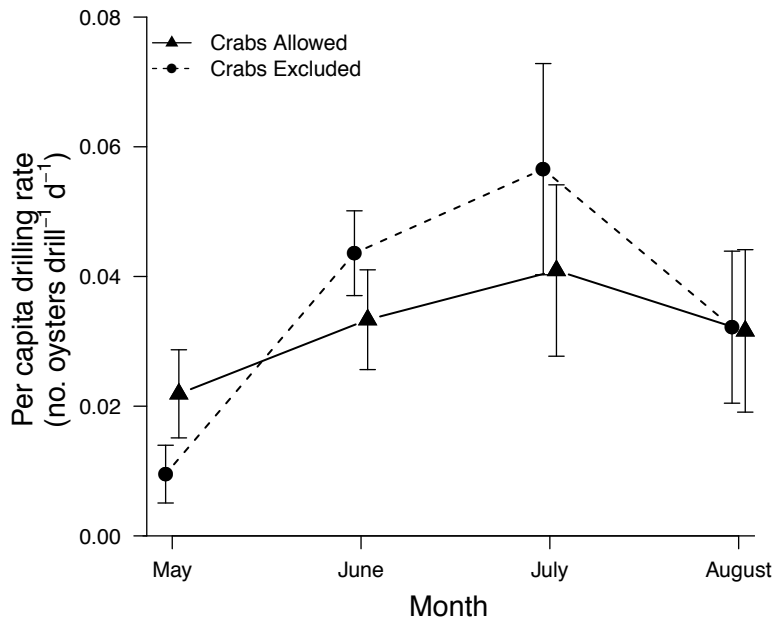
Figure 1.4 Average monthly *per capita* drilling rates in number of oysters drilled per drill per day for treatments in which drills were manipulated (location on the x axis has been jittered for visibility). The number of drills is the average of the initial (3) and final numbers.

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Table 1.2 Generalized linear mixed-effect models (Poisson error, log link) of drill feeding rate (total number oysters drilled per month) within 95% confidence set of candidate models. Drill abundance was modeled as the average of the initial and final number of drills. "Y" indicates inclusion of categorical factor in candidate model. Model-averaged coefficients are generated from 95% confidence set of candidate models.

Model	Intercept	Crab	Day (Linear)	Day (Quadratic)	Avg. No. Drills	Crab X Day (L)	Crab X Day (Q)	Crab X No. Drills	No. Drills X Day (L)	No. Drills X Day (Q)	df	Δ AICc	Model Weight
1	-1.656		0.279	-0.309	1.002						5	0.00	0.405
2	-2.423		0.575	-0.296	1.275				-0.112	-0.224	7	2.04	0.146
3	-1.649	Y	0.282	-0.307	1.007						6	2.28	0.130
4	-1.559	Y	0.382	-0.569	1.025	Y	Y				8	2.67	0.107
5	-2.223	Y	0.272	-0.314	1.195			Y			7	3.62	0.066
6	-2.054	Y	0.383	-0.572	1.199	Y	Y	Y			9	4.32	0.047
7	-2.441	Y	0.573	-0.297	1.277				-0.111	-0.224	8	4.50	0.043
8	-2.996	Y	0.662	-0.004	1.257	Y	Y		-0.098	-0.201	10	5.90	0.021
Averaged parameter importance		0.43	1.00	1.00	1.00	0.18	0.18	0.12	0.22	0.22			
Model-averaged coefficient (SE)	-1.872 (0.655)	0.107 (0.669)	0.362 (0.260)	-0.226 (0.398)	1.087 (0.223)	-0.128 (0.231)	0.520 (0.257)	-0.301 (0.316)	-0.111 (0.151)	-0.222 (0.193)			

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While there was very little evidence of crab predation on oysters, there was strong evidence

(parameter importance = 0.76) that crabs reduced drill survival (Table 1.3, Figure 1.5). Support

for the effect of crabs was primarily driven by low drill survival in crab-permeable cages in

August. Crabs had a smaller effect on drill survival during the other three months; however,

there was no support for the crab × time interaction (0.07) and relatively little evidence of the

influence of time on change in drill survival over the course of the study (0.42).

Table 1.3. Generalized linear mixed-effect models (binomial error, logit link) of drill survival within 95% confidence set of candidate models. "Y" indicates inclusion of categorical factor in candidate model. Model-averaged coefficients are generated from 95% confidence set of candidate models.

| Model	Intercept	Crab	Day (Linear)	Day (Quadratic)	Crab X Day (L)	Crab X Day (Q)	df	Δ AICc	Model Weight
1	3.814	Y					3	0.00	0.425
2	4.304	Y	-0.617	-0.263			5	0.94	0.265
3	2.694						2	2.06	0.152
4	3.063		-0.589	-0.236			4	3.08	0.091
5	3.957	Y	0.544	0.135	Y	Y	7	3.68	0.067
Averaged parameter importance		0.76	0.42	0.42	0.07	0.07			
Model-averaged coefficient (SE)	3.898 (1.167)	- 2.282 (1.179)	-0.427 (0.632)	-0.194 (0.552)	-1.471 (0.992)	-0.468 (1.073)			

Similar to the cages, drill predation accounted for 100% of the total predation-related oyster

mortality in the uncaged control plots (Figures 1.2 & 1.3); no crushed shells were ever found for

tethered oysters. The effects of ambient predation regimes were explored by qualitatively

comparing oyster mortality in the uncaged control plots to oyster mortality in the cages. Oyster

survival in the uncaged treatment exceeded that in both of the drill-enclosure treatments in June

and July; during these same months, drilling rate was lower outside the cages than inside the

cages. Conversely, during August, drilling rates in the uncaged control increased to levels similar

to the drill-enclosure treatments (Figure 1.3), and oyster survival rates were also comparable

among the three treatments that included or allowed drills. No evidence that oysters in the

uncaged control treatment had been consumed by crabs could be found.
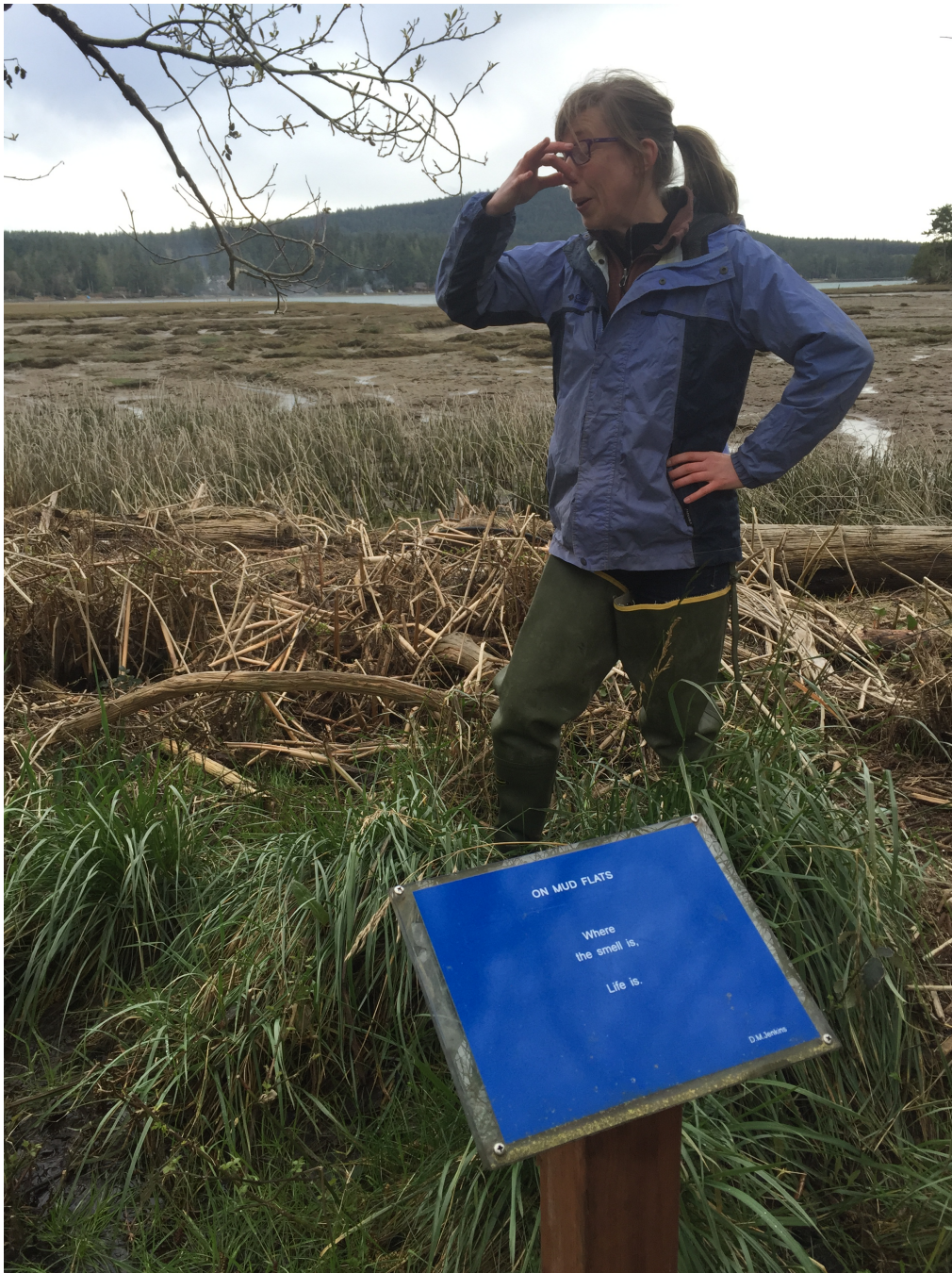
Page 18 Mudflats, such as Westcott Bay, pictured here, are home to oysters, oyster drills and other mollusca non-grata. The diversity and intense sensory experience they provide has even inspired poetry.
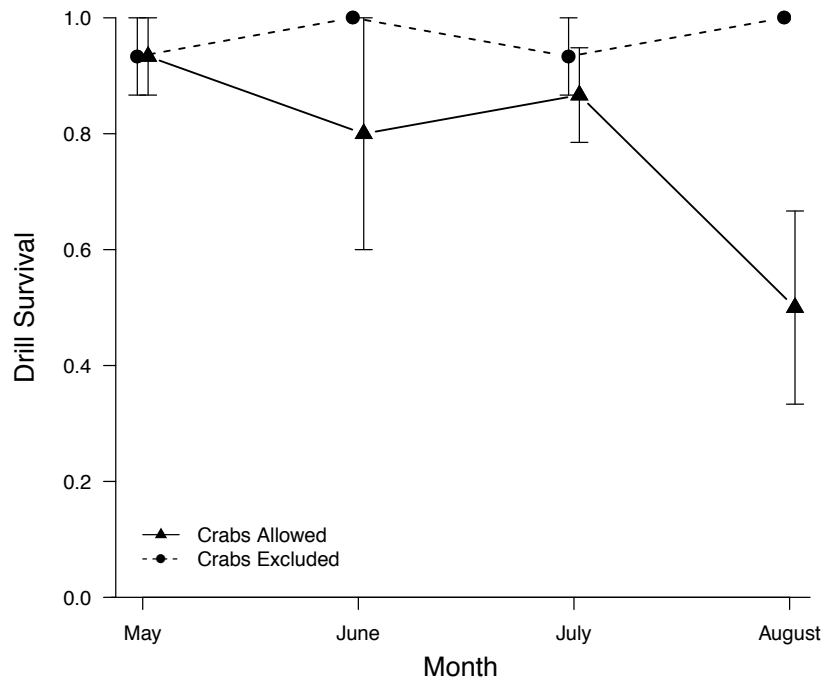
Figure 1.5 Average proportion of initial (3) drills surviving at the end of each month (location on the x axis has been jittered for visibility). Only treatments where drills were manipulated are shown. Triangles/solid lines indicate treatments allowing crabs, and circles/dashed lines represent treatments excluding crabs. Error bars = 1 SEM.

1.5 Discussion

The top-down direct effect of invasive oyster drills (pathway 2 in Figure 1.1) was the most important driver of juvenile native oyster survival in this study, accounting for an average of 70% of total mortality (including non-predator related mortality) in drill-enclosure treatments. Per capita drill feeding rates showed a hump-shaped trend over the course of the experiment, peaking in early-mid summer (Figure 1.2). This pattern could reflect elevated metabolic rates and consumption as water temperatures rise during the early summer but become stressful in August, when mid-day lower-low tides coincide with elevated air temperatures to increase the extreme high temperatures experienced by intertidal organisms. Overall, the range of feeding rates observed throughout the summer was consistent with previous observations of *O. inornata*

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feeding on juvenile oysters both in the laboratory and in the field (Buhle and Ruesink, 2009; Grason and Miner, 2012a).

Conversely, and contrary to predictions, crabs did not exert strong top-down effects on oysters. Crabs were predicted to have a large negative effect on oysters (pathway 3 in Figure 1.1) given the prey preferences observed in laboratory studies (Grason and Miner, 2012b). Yet, physical evidence of crab predation on oysters was nearly absent, and chipping or crushing of oyster shells was observed on only two occasions. Even assuming that all oysters of indeterminate fate (3%) had been consumed by crabs, the magnitude of crab effects would still be negligible to compared to that of drills.

Moreover, while it was expected that crabs could have an indirect positive effect on oyster survival by reducing drill abundance (a consumptive indirect effect; pathway 1 in Figure 1.1) or drill feeding rates (a non-consumptive indirect effect; pathway 1.4), there was not strong statistical evidence that allowing crabs into the cages reduced the effect of drills on oysters. Crab interactions with drills were evidently too weak to transmit important effects on oyster survival during most of the months we observed. Allowing crabs into cages did reduce drill survival in August, leading to a predicted 50% reduction in oyster mortality in those cages, but this coincided with a reduction in per capita drill feeding rates that was independent of crabs, ultimately diminishing the indirect benefit of crabs to oysters. This simultaneous change in crab and drill behavior, crabs increasing feeding rates at the same time that drills reduced their feeding rates (independent of crabs), is an example of field-setting complexities that could not have been anticipated from laboratory work.

There was also little support for the hypothesis that drills reduce their per capita feeding rates in the presence of crabs (Figure 1.4). Reductions in feeding rates were small and highly variable, both within and between months. Given previous laboratory experiments, this is not likely due to evolutionary naïveté (Grason and Miner, 2012a). Other researchers have observed that behavioral changes seen in mesocosms do not necessarily translate to the field for a variety of reasons (Chalcraft et al., 2005, Winkler and Van Buskirk, 2012). Predation on drills occurred less often in this field study (0.024 drills consumed per cage per day) than in experimental treatments used in laboratory experiments (e.g., 1 drill per replicate per day; Grason and Miner 2012a), and crabs left the cages during or after any predation events. Presumably, drills were therefore exposed to chemical cues indicating predation risk for much shorter time periods in the present field study. This, combined with greater dilution of chemical cues, could reduce the perception of risk under natural conditions.

The relatively small and variable role of crabs observed in this study suggests the possibility of spatial and temporal variability in the influence of predators. Future research could augment these findings with extended observation periods, and assessment of the correlation between the abundance and species composition of crabs on the tideflat with the influence of crabs on drill and oyster mortality. Given that previous research used to generate predictions was conducted with *Cancer productus* it is possible that use of the tideflat by the two crab species observed in this study varied temporally.

Differences in top-down effects on oysters between enclosures and uncaged control plots could be due to differences in either ambient predator densities relative to caged enclosures or predator behavior. Approximately half of the oyster mortality observed in uncaged plots was due to drilling (as opposed to non-predator related sources), which was lower in June and July than that simulated by manipulations in the cages, but more similar in August. It is possible that the cages afforded drills some protection from desiccation and heat stress, and drills outside the cages reduced their feeding rates or migrated lower in the intertidal during June and July. Similar to the caging experiment, crabs did not exert a strong top-down control on oyster survival in the absence of cages. Comparing the uncaged plots to the caged plots suggests that it is unlikely that crabs present on the tideflat were deterred by the cages, in which case oyster mortality would have been greater in the uncaged control than in any of the enclosure treatments. Moreover, there was no evidence of oyster consumption by crabs (e.g., crushed shells) outside of the cages. Possible indirect effects of alternative prey on the uncaged oysters cannot be excluded, however. The most abundant potential alternative prey for both drills and crabs is barnacles, which have been observed to reduce the per capita effects of drills on oysters on short time scales (Buhle, 2007). On longer time scales, however, barnacles could have a negative effect on oysters via apparent competition (Menge, 1995).

1.5.1 Conclusions

This study underscores the strong negative impact that invasive oyster drills can have on oyster restoration efforts on the west coast of North America, particularly in Washington State where the majority of surveyed native oyster populations is exposed to drill predation (Wasson et al., 2015). Negative effects of drills might not be attenuated by biotic resistance even in habitats where potential native predators of drills are present and abundant. Another invasive oyster drill

species, *Urosalpinx cinerea*, is a significant cause of *O. lurida* mortality in California (Kimbro et al., 2009). Indeed, ambient juvenile oyster survival was very low at the study site, and as the summer progressed nearly all naturally-recruited oyster shells showed evidence of drill predation (E. Grason, pers. obs.). Because drill control techniques (i.e., hand-removal) are resource-intensive and rarely achieve eradication, a crucial component of native oyster restoration should be selecting sites that are not already invaded by drills, and subsequently monitoring and protecting them from drill introduction. Alternatively or additionally, managers could explore methods of mitigating drill impacts by shifting the size structure of the population or manipulating availability or location of alternative prey.

Overall, these experimental manipulations of a tri-trophic interaction in the field demonstrated strong support for only a few of the theoretically possible direct, indirect, consumptive and non-consumptive effects. Nonnative oyster drills clearly reduced native oyster survival (Figures 1.2, 1.3, 1.4), and there were weaker indications that native crabs reduced drill survival (Figure 1.4). The scant support for direct consumptive effects of crabs on oysters was surprising, given previous evidence that crabs (*Cancer productus*) preferred juvenile oysters over drills (Grason and Miner, 2012b). Additionally, given that crabs consumed half of the drills in the cages during August, a reduction in the rate at which oysters were drilled was expected during at least that month. There was little support for a consumptive indirect effect, however, as drilling rates were already low at that point in the summer. Furthermore, while there was a trend toward a reduction in feeding by drills in the presence of crabs, this was temporally inconsistent (Figure 1.3) and the intimidation effect was not large enough to be important in models of drill feeding rates (Table

1.2). These results reflect the difficulty of inferring ecological dynamics in nature based on estimates derived from laboratory experiments (Skelly, 2002).

These results also emphasize that the behavior of invaded food webs with asymmetric IGP depends not only on whether the intraguild predator or the intraguild prey is the invader, but also on the strength of the interactions involved. While complex interactions can be important drivers of community organization (Werner and Peacor, 2003), despite the potential complexity of the tri-trophic interaction studied here, dynamics are dominated by a single strong feeding interaction between a nonnative predator and native prey.

CHAPTER 2

INVASIVE SUCCESS DIFFERS BETWEEN INTRODUCED POPULATIONS DUE TO TOP-DOWN CONTROL BY NATIVE PREDATORS

2.1 Abstract

Ecological and historical factors influence the probability that a known invader will experience success in new locations. Using field and laboratory studies, we investigated three possible explanations for differences between two populations of the intertidal snail, *Batillaria attramentaria*: residence time, infection by a co-evolved, castrating, parasite, and top-down control by native predators. The populations have substantially different invasion histories (~10 years versus >80 years) and exhibit markedly different densities and tidal ranges. The less-dense, vertically-restricted population was recently introduced, and thus has had less opportunity to fill the fundamental niche at that site. However, we only found support for top-down control from native predators; the younger population experienced much greater effects of native cancrid crabs than the older, high-density population, particularly below the minimum tidal elevation of observed snail distribution where crabs were found in the greatest densities. This the first study documenting effects of predators on this invasive snail, which is widespread along coastlines of the northeast Pacific, whereas previous studies have suggested that the primary restriction on population growth rate was likely to be parasitic castration. Further, this study supports the general belief that, while novel predators can reduce the impacts or population growth rates of invasive species, such top-down control is not likely to preclude persistence at a given site.

Lastly, residence time could be less important in predicting indicators of invasion success at the local, than at the regional or global scale.

2.2 Introduction

Many risk assessments for invasive species rely on the observation that similar abiotic conditions between native and introduced ranges, and the species' history of impact in prior invasions elsewhere should increase the probability of invasion (Moyle and Light 1996; Kolar and Lodge 2001; Peterson 2003; Thuiller et al. 2005). Exceptions to this rule, in which an introduction proves less successful than expected based on these factors, provide opportunities to examine the ecological and historical contexts that impede prediction. Invasion success can be defined both qualitatively, i.e., does the species progress through the introduction, establishment, spread, and impact stages of invasion, and quantitatively, i.e. how do species vital rates and interaction strengths compare at each of those stages. Two competing, but non-mutually exclusive, explanations for relatively poor performance of an otherwise successful invader are short residence time and natural enemies. Regarding the former, a recently-introduced population might fill only part of its total potential range if it is dispersal-limited (Pyšek and Jarošik 2005; Wilson et al. 2007) and might be present in small numbers if few propagules were initially introduced (Lockwood et al. 2005; Colautti et al. 2006; Simberloff 2009). Regarding the latter, biotic interactions with either co-evolved or local predators, pathogens, or competitors can limit the spread and population growth of non-native species (Levine et al. 2004; Colautti et al. 2006; Suwa and Louda 2012; Zenni and Nuñez 2013). Disentangling the role that each of these factors plays in influencing the trajectory of an invasion is key to forecasting the impact of the invasion, as well as helping to determine the probability of other invasions by the same species. Here we explore the influence of natural enemies and residence time on the vertical range and abundance

of a non-native marine snail, comparing the importance of each factor between two populations that differ in invasion history and relative success.

The intertidal snail, *Batillaria attramentaria* (hereafter: *Batillaria*), is native to the northwestern Pacific Ocean but has established populations along shorelines in the northeastern Pacific from Monterey, California to Boundary Bay, Canada (36.8°N to 49.0°N; Byers 1999). Negative impacts have been demonstrated where *Batillaria* overlaps with a similar native snail in the southern part of its invaded range (Byers 1999), but in the northern part, some facilitative effects occur (Wonham et al. 2005). Whether positive or negative, these strong interactions emerge in part due to *Batillaria*'s high population densities ($>3,000$ m^{-2}) for an organism with a shell length up to 4 cm (Byers and Goldwasser 2001). The initial introduction appears to have accompanied imports of Pacific oysters (*Crassostrea gigas*) in the first part of the 20th century (Wonham and Carlton 2005). Subsequent regional spread of *Batillaria* has primarily been human-mediated, because this species lacks a pelagic larval stage, and crawl-away juveniles hatch from benthic egg capsules (Yamada and Sankurathri 1977).

This history and ecology of this species sets up a scenario in which local populations can have quite different initial introduction dates and distinct dynamics. In this study, we compare two populations from Washington State (Figure 2.1) to discern whether differences in abundance and range are best explained by historical context or ecological interactions. *Batillaria* has likely been present in Padilla Bay since the 1930s (Padilla Bay National Estuarine Research Reserve 2014) but was not observed in Willapa Bay until 2004 (JLR, pers. obs.) and was not listed as present there in a compilation of introduced species published about that time (Wonham and
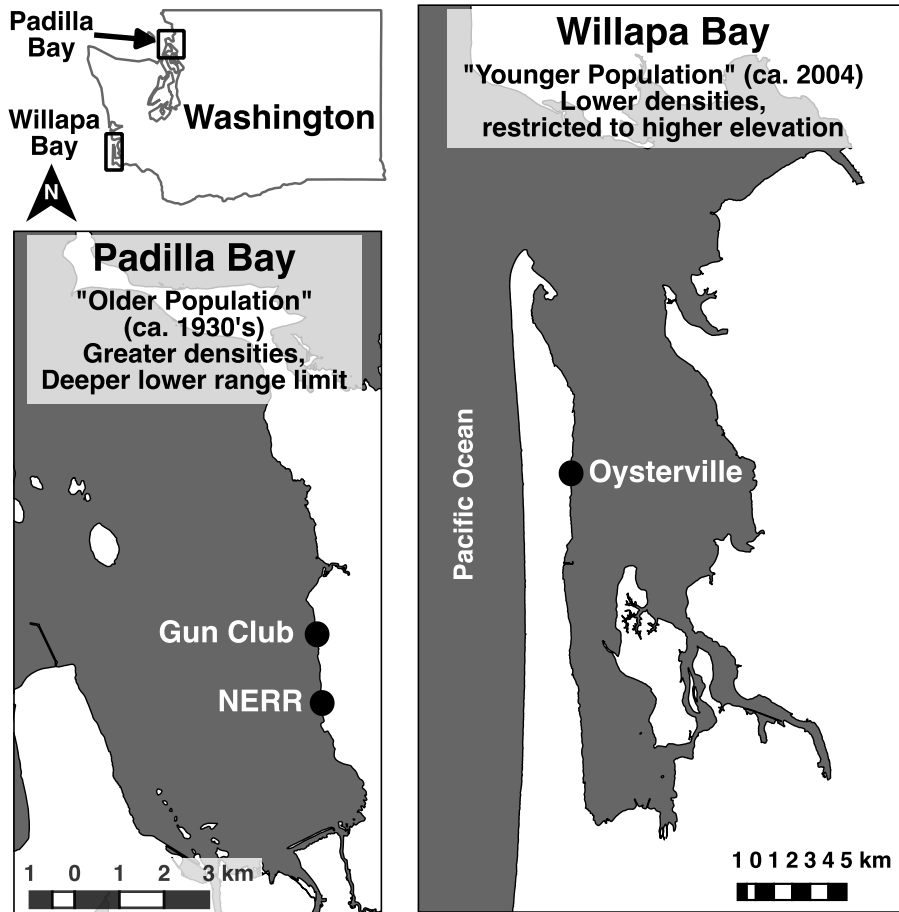
Figure 2.1 Map of study sites. In Padilla Bay, surveying was conducted at the Padilla Bay National Estuarine Research Reserve (NERR) interpretive center, and the tethering study occurred adjacent to the Sullivan-Minor Gun Club. In Willapa Bay, both surveying and tethering were conducted at Oysterville, the only location in the bay at which *Batillaria* has been reported.

Carlton 2005). The current population in Willapa Bay most assuredly results from a secondary

introduction via transport of material from another shellfish growing location in Washington, as

oyster imports from the western Pacific ceased by 1977 (White et al. 2009). To date, *Batillaria*

has only been observed around a single location on the west side of Willapa Bay.

Initial observations suggest that the Willapa Bay population is less successful than the Padilla

Bay population: restricted to higher tidal elevations, and lower densities. A short residence time

could explain a relatively small population of snails simply because of the time it takes

populations to grow and disperse. Note that range in this study refers to vertical range, although other uses of range have been applied in analyses where residence time impacts invasion dynamics (Pyšek and Jarošik 2005; Wilson et al. 2007; Byers et al.).

In addition to residence time, spread, and abundance of *Batillaria* could be influenced by either co-evolved or novel natural enemies. Low mortality rates shape both the dynamics and impacts of *Batillaria*, but the species appears to be less sensitive to variability in reproduction (Byers and Goldwasser 2001). Reproduction is influenced in part by co-evolved parasitic trematodes, which infect *Batillaria* as the obligate first intermediate host, ultimately castrating the snails by appropriating gonad tissue for their own reproduction (Torchin et al. 2005; Miura et al. 2006). Therefore, the population growth rate will largely depend on the proportion of adult snails infected with trematodes. Infection not only castrates the snail, but causes the snail to grow as much as 50% larger and to migrate deeper into the intertidal zone (Torchin et al. 2005; Miura et al. 2006, E.W. Grason Unpublished Data). The top-down effects of predators on *Batillaria* are not well understood, in that no publications have documented predation on this species, but it seems likely that generalist crabs and molluscivorous fish opportunistically consume snails as they forage on the incoming tides.

Using a combination of survey and experimental data, we explored three possible influences on the abundance and distribution of *Batillaria*, by comparing the vertical range, density, parasite prevalence, and local predator effects between the Padilla Bay (older population, greater vertical range and density) and the Willapa Bay (younger population, restricted vertical range and reduced density) populations. Our hypotheses regarding mechanisms for differential invasion

success between sites emphasize residence time and enemy release, because abiotic conditions appear similar and are considered less likely than biotic conditions to determine lower limits of intertidal organisms (Connell 1961):

1. Density and vertical range in the Willapa Bay population are not limited by species interactions, but rather reflect early phases of population growth and limited residence time. If this were the case, the younger population in Willapa Bay would exhibit exponential growth dynamics and the lower limit of vertical distribution would become deeper over time for the younger, but not the older, invasion.

2. Infection rates by the co-evolved, non-native, trematode differ between the two populations. Parasitic castration, movement, and somatic growth of parasitized snails would generate two opposing predictions. First, based on the ecology of infection, we would expect increased parasite prevalence to be associated with a lower vertical range limit, which corresponds to current observations of distribution in Padilla Bay. However, we would also expect that increased infection rates would lead to decreased population growth rate, and relatively lower densities, as has been observed in Willapa Bay.

3. Native predators exert different effects on each population across their vertical ranges, and preclude survival in deeper habitats. This hypothesis would be supported if we observed that snails from Willapa Bay experience greater predation rates than those in Padilla Bay, particularly at or below their currently-observed lower limit of distribution.

Elucidating the mechanisms that control abundance and distribution of this invasive snail will provide insight into the conditions which favor invasion for *Batillaria* and the extent to which these factors are context dependent.

2.3 Materials and Methods

We used a combination of surveying, tethering studies, trapping, and laboratory predation studies to explore support for the hypothesized mechanisms determining the difference in density and distribution of the two populations of *Batillaria attramentaria*.

2.3.1 Population Surveys

Batillaria density and parasite infection status were surveyed along vertical transects in each bay in 2007, 2008, and 2011 (Figure 2.1). In Willapa Bay, all surveys were conducted at Oysterville, and surveys of the older population were conducted near the Padilla Bay National Estuarine Research Reserve interpretive center. Parasite data for Padilla Bay was supplemented with observations from a transect approximately 1.3 km to the north, adjacent to the Sullivan Minor Gun Club. Along transects, we sampled between 3 and 25 evenly-spaced (in the horizontal direction) positions, estimating abundance by counting snails in 1 - 5 quadrats. In 2011, we collected a subsample of the first 20 snails gathered at each position, measured shell length, and brought snails back to the lab to assess parasite infection status (as in Torchin et al. 2005). *Cercaria batillariae* is believed to be the only species to infect *Batillaria* in the invaded range (Torchin et al. 2005), which is in concordance with our observations.

In Willapa Bay, tidal elevation of each sample was determined via geographic position. Coordinates were recorded on a handheld Global Positioning System (GPS) receiver, and elevation (m above MLLW) was subsequently extracted from a digital elevation model of

Willapa Bay (ONRC 2008). We were not able to obtain high-resolution elevation data for the

intertidal zone in Padilla Bay, so we measured elevation directly, using Real Time Kinematic

(RTK) and Post-Processed Kinematic GPS. We derived tidal elevations from GPS measurements

using VDatum software (www.vdatum.noaa.gov). Absolute tidal elevation is one of several

possible descriptors of the elevation of each sample. While this metric is straightforward to

compare across sites, it does not always reflect ecologically relevant parameters that could differ

between sites for the same absolute elevation. For instance, tidal inundation time, which directly

influences thermal regimes experienced by snails and the amount of time sub-tidal predators can

access tide flats, varied markedly between the observed vertical ranges of snails at the two sites

(Figure 2.2). We therefore also defined elevation as position normalized to the local observed

distribution of *Batillaria*, considering both the lower limit (0) and the upper limit (1) (relative

elevation). From a practical perspective, this also allowed us to directly compare ranges at the

two sites in statistical analyses, despite limited overlap in absolute elevation.

2.3.1.1 Analysis

We tested the first hypothesis (i.e., residence time limits population size and spread) by

examining changes in snail density over time in both populations, as well as the variation in the

lower limit at the site of the younger population in Willapa Bay. Density (snails m^{-2}) was

modeled as a function of year (continuous variable) and site (fixed factor: in a two-way

generalized linear model (GLM) with a log link function and poisson error distribution). Because

we were interested in modeling density within the observed range of distribution, we omitted

observations of zero snails, which would bias model estimates based on sampling scheme.
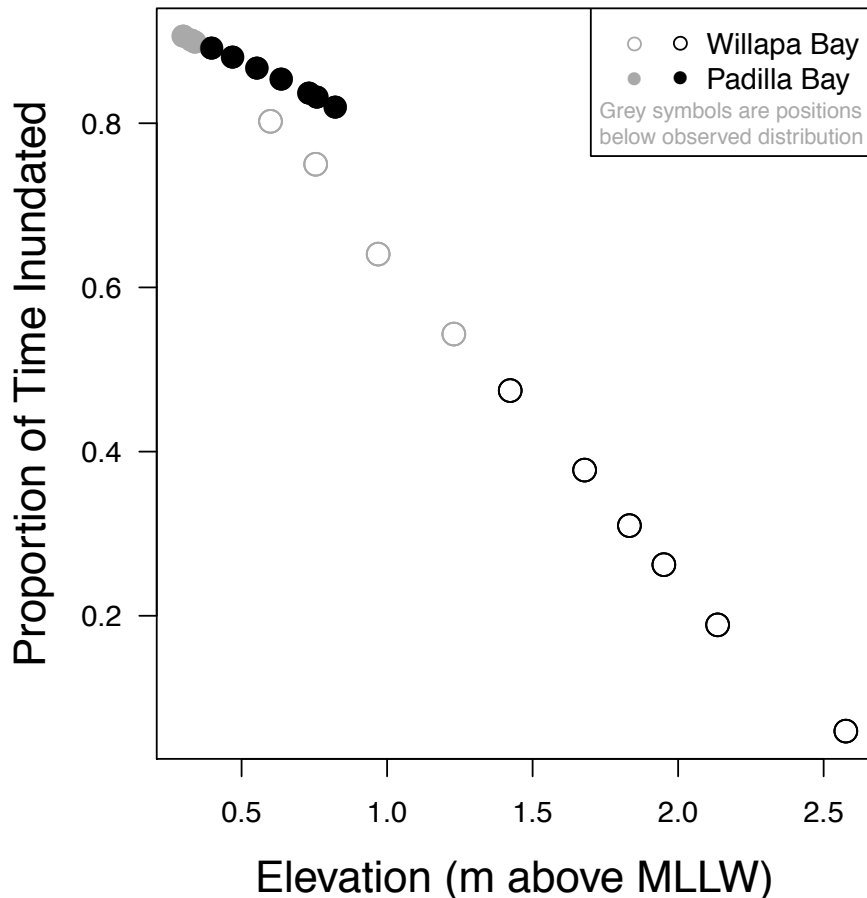
Figure 2.2 Elevation, in meters above mean lower-low water (MLLW) and proportional inundation times for the positions at which the tethering studies were conducted. Filled symbols represent tethering positions at Padilla Bay, while open symbols represent tethering positions at Willapa Bay. In both cases, the grey symbols represent the tethering positions that were below the lower limit of observed snail distribution at that site. There are three such positions (overlapping points) in Padilla Bay and four in Willapa Bay, WA.

We further explored whether the lower range limit became deeper over the same time period for the younger population (Willapa Bay) only. This observation would support the prediction of dispersal limitation in the first hypothesis. Because observations were taken at slightly different depth ranges each year, snail density at this site was modeled separately for each year as a function of elevation for each of the three years. In these models, absolute elevation (meters above MLLW) was included as both a linear and a quadratic term, as we expected densities to be lower at the upper and lower limits of distribution (Whittaker 1967). We established 95%

confidence intervals around each of the modeled relationships to determine whether the depth of the predicted lower limit differed between years.

We tested the second hypothesis (i.e., parasitic infection prevalence influences depth range and population growth) by determining whether the probability of being infected differed between the snail populations at the two sites during only the most recent year of sampling, 2011. Because snails migrate deeper when they become infected, snails found deeper are more likely to be infected. As a result, estimates of prevalence must account for the depth component of sampling, which was standardized between populations as relative elevation (described above). We then modeled the probability that a given snail was infected with a binomial GLM (logit link) with site and relative elevation (ranging from $0 - 1$) as predictors. Parasite prevalence did not fluctuate appreciably during the observation period (P.S. McDonald, unpublished data), and we present only the final year of data because it had the highest spatial resolution.

2.3.2 Predation Studies

We assessed the abundance of predators at each site with baited traps and evaluated predation on *Batillaria* using field-based tethering and a laboratory predation experiment. Field activities related to the predation study were done in the same locations as the aforementioned survey work (Figure 2.1). The laboratory experiment was conducted in flow-through seawater aquaria at Shannon Point Marine Center (Anacortes, Washington).

2.3.2.1 Trapping Surveys

To compare the communities of probable predators of *Batillaria*, we trapped fish and crabs across an elevation gradient at both embayments, at a subset of positions used in the tethering study (below). At four positions spanning the elevation range across which snails were tethered

(lowest, fourth, seventh, and highest positions), we deployed five rectangular Fukui fish traps, baited with approximately 200 g of frozen mackerel (e.g., Holsman et al. 2006). For logistical reasons (i.e. avoiding terrestrial predators, and emersing trap catches on the higher-low tide), traps set for one full tidal cycle in Padilla Bay (ca. 20h), but only from higher-low to lower-low tide in Willapa Bay (ca. 11h). Both trap deployments included an overnight high tide, when predators are most attracted to baited traps, and trap catches have been adjusted for the soak time and are therefore expressed as rates. At the end of each soak, crabs and fish in each trap were identified to species and counted. To estimate the size of crabs in the traps, we measured the carapace width of the first 10 crabs removed from each trap.

2.3.2.2 Tethering Surveys

To test whether the effects of native predators of *Batillaria* differed between sites, we conducted tethering studies in both embayments during consecutive years (Padilla Bay in 2012; Willapa Bay 2013). Tethered *Batillaria* of three size classes were deployed across a vertical transect for several weeks and observed for evidence of predation or predation attempts. At each of 10 positions along the depth transects, 10 individuals of each size class of snail were tethered to a single 1 m length of rebar with 20-30 cm of monofilament line and cyanoacrylate gel. Because we were interested in whether subtidal predators influence the lower range limit as well as the overall density of *Batillaria*, the lowest tethering positions were situated beyond the deepest observation of snails (Padilla Bay: 0.40 m MLLW; Willapa Bay: 1.35 m MLLW, Figure 2.2). We arrayed snails along the rebar in an order that haphazardly mixed individuals of different size classes, and laid the rebar flat in the mud, perpendicular to the elevation gradient. The density of tethered snails at each rebar position was 810 snails m^{-2} (300 snails in an area of approximately 0.4 m^2), which is within natural observed densities at the higher elevations. We used only snails

collected locally from a similar tidal elevation for each experiment. Very small snails were not available in Willapa Bay. As a result, size classes differed slightly between the two bays in the study (older: small: 11-16 mm, medium: 20-25 mm, large: 29-35 mm; younger: small: <20mm, medium: 22-25 mm, large: >28 mm).

Tethered snails were deployed on the same tidal cycle in successive years (older: 23 May, 2012; younger: 24 May, 2013) and retrieved after 57 (19 July) or 47 days (10 July) for the older and younger populations, respectively. For the duration of the study periods, we recorded observations of snail damage, death, and disappearance approximately every two weeks. Missing and damaged shells, either with or without living snails in them, were interpreted as evidence of attempted predation. We believe this is a safe assumption as mechanical damage due to storms or waves was unlikely at either of these low-energy beaches.

To determine whether predators differentially affected the two populations, we compared the proportion of snails from each size class that were damaged by predators at the end of 6 weeks. Though the study lasted another 10 d for the older population, there was no appreciable mortality after that time, and including those observations would not qualitatively alter our conclusions. To standardize elevations assayed between the two populations, we used relative elevation (as described above) as a continuous predictor, and restricted observations to the range for which relative elevations was similar. This removed the deepest three, and the highest two tethering positions for Willapa Bay where it was possible to tether snails outside the observed elevation limit at both the upper and lower ends. By contrast, in Padilla Bay, the upper limit coincided with terrestrial features, and the lower limit was rarely out of the water (Figure 2.2). We modeled the

proportion of snails that were damaged by predators using a GLM with binomial error distribution and a logit link function with relative elevation (as above), size class (small, medium, large), and site (Padilla or Willapa Bay) as predictors.

2.3.2.3 Laboratory Experiment

We evaluated size-selective predation of *Batillaria* by an abundant predator, Dungeness crab (*Cancer* [*Metacarcinus*] *magister*), using a laboratory experiment. Prior to the start of the experiment, crabs (109-120 mm CW) were fed crushed *Batillaria* ad libitum for at least 24 h, and individuals demonstrating normal feeding behavior were then starved for an additional 48 h prior to the experiment. Experimental enclosures were plastic baskets (22.1 cm × 35.4 cm × 13 cm) fitted with mesh screen (0.33 mm) side walls and lid and placed in a flow-through table receiving a constant supply of sea water. A small amount of clean sand was added to each enclosure and ten *Batillaria* from each of three size classes (small: 12-15 mm, medium: 20-24 mm, large: 30-34 mm) were distributed haphazardly on the substrate surface (n = 30 snails). *Batillaria* were allowed to acclimate for 1 h before one crab was added to each of seven enclosures; an additional enclosure received no crab and functioned as a partial control for natural mortality. The experiment was conducted for 72 h following the addition of the crabs. Enclosures were checked daily to ensure snails of each size class were available, but crabs never consumed all of the snails available for a single size class, so no replacement was necessary. At the end of the experiment, all *Batillaria* were removed and checked for obvious signs of crab predation, and the sand within each enclosure was sieved to collect all shell fragments.

We tested whether crabs demonstrated size-selective predation using a binomial generalized linear mixed effects model with the number of snails killed or damaged of each size class as the

response variable conditioned on the number of snails initially available. The model also included a randomly varying intercept for each individual replicate trial, to account for between–crab variation. No background mortality occurred in the partial control, and we therefore omitted this treatment from the analysis. Two trials where crabs did not consume any snails (i.e., no predation-related mortality) were excluded from the analysis, as was a trial in which the position of the enclosure preferentially allowed small snails to crawl out of the water confounding the treatments, yielding 4 replicate trials.

2.4 Results

2.4.1 Population Surveys

The present survey confirmed previous observations that the lower intertidal limit of *Batillaria* differs substantially between two sites. *Batillaria* in Padilla Bay were observed as deep as 0.3 m above MLLW (Figure 2.3), which is similar to observations made throughout that bay (E.W. Grason, unpublished data). However, *Batillaria* in Willapa Bay were restricted to a higher elevation, and only occurred as deep as 1.4 m above MLLW in Willapa Bay (Figure 2.3).

We did not have sufficient evidence to determine whether snails in Willapa Bay were found deeper in later years, which would support the hypothesis that residence time is limiting range. In 2011, snails were found at least 0.5 m deeper than the previous two surveys (Figure 2.3), primarily because we were able to sample on lower tides and deeper snails were more observable. By modeling snail density as a function of elevation for each of the three years, we attempted to determine whether the predicted lower limit (the deepest elevation at which density intercepted the x axis) was truly deeper in 2011 than in previous years (Figure 2.4) in Willapa Bay. However, because the number of samples was relatively low for 2007 and 2008, and did not extend as deep
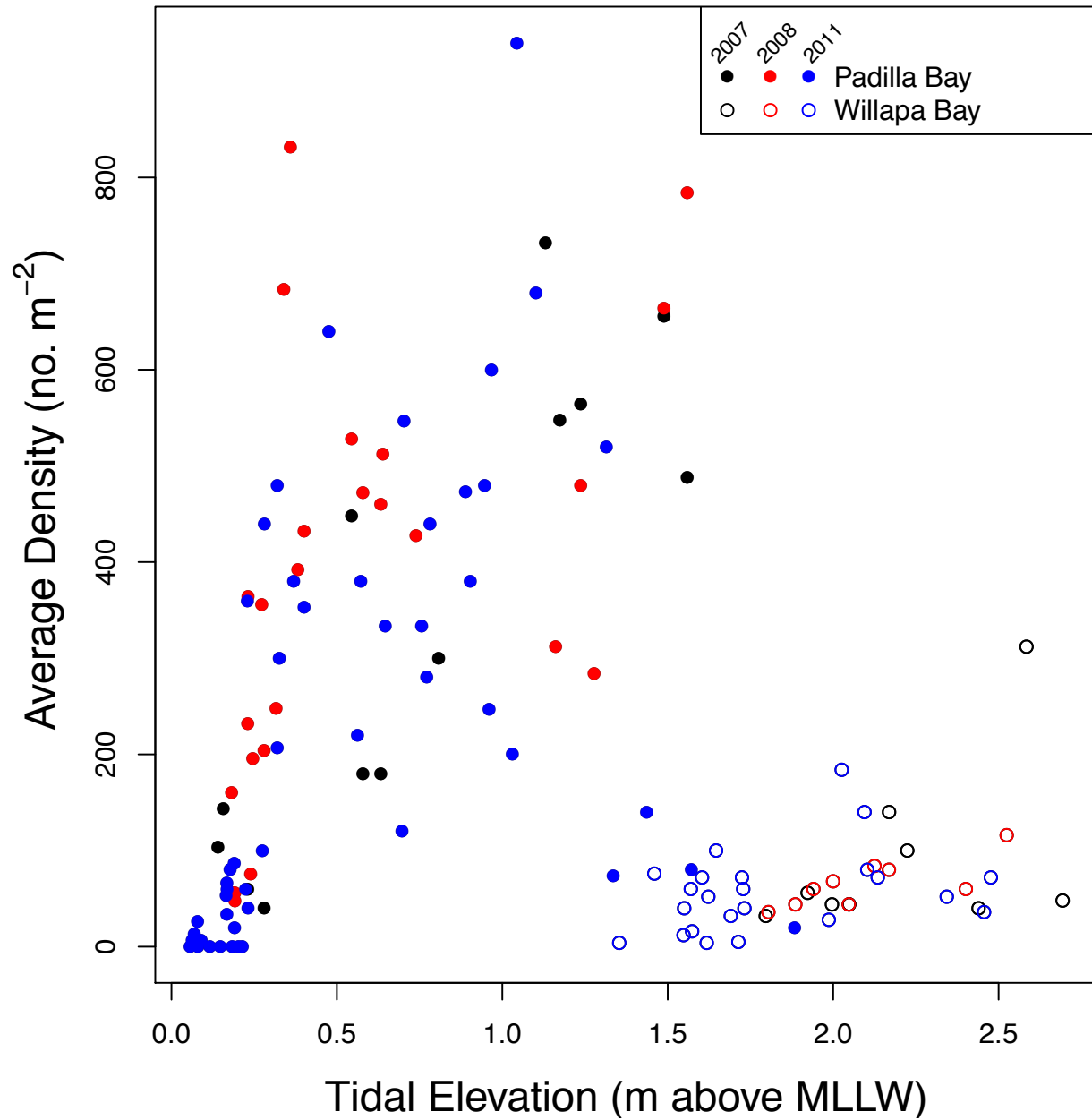
Figure 2.3 *Batillaria attramentaria* density across tidal elevation gradients in three survey years (2007, black; 2008, red; 2011, blue) at Padilla Bay (filled symbols) and Willapa Bay (open symbols), WA.
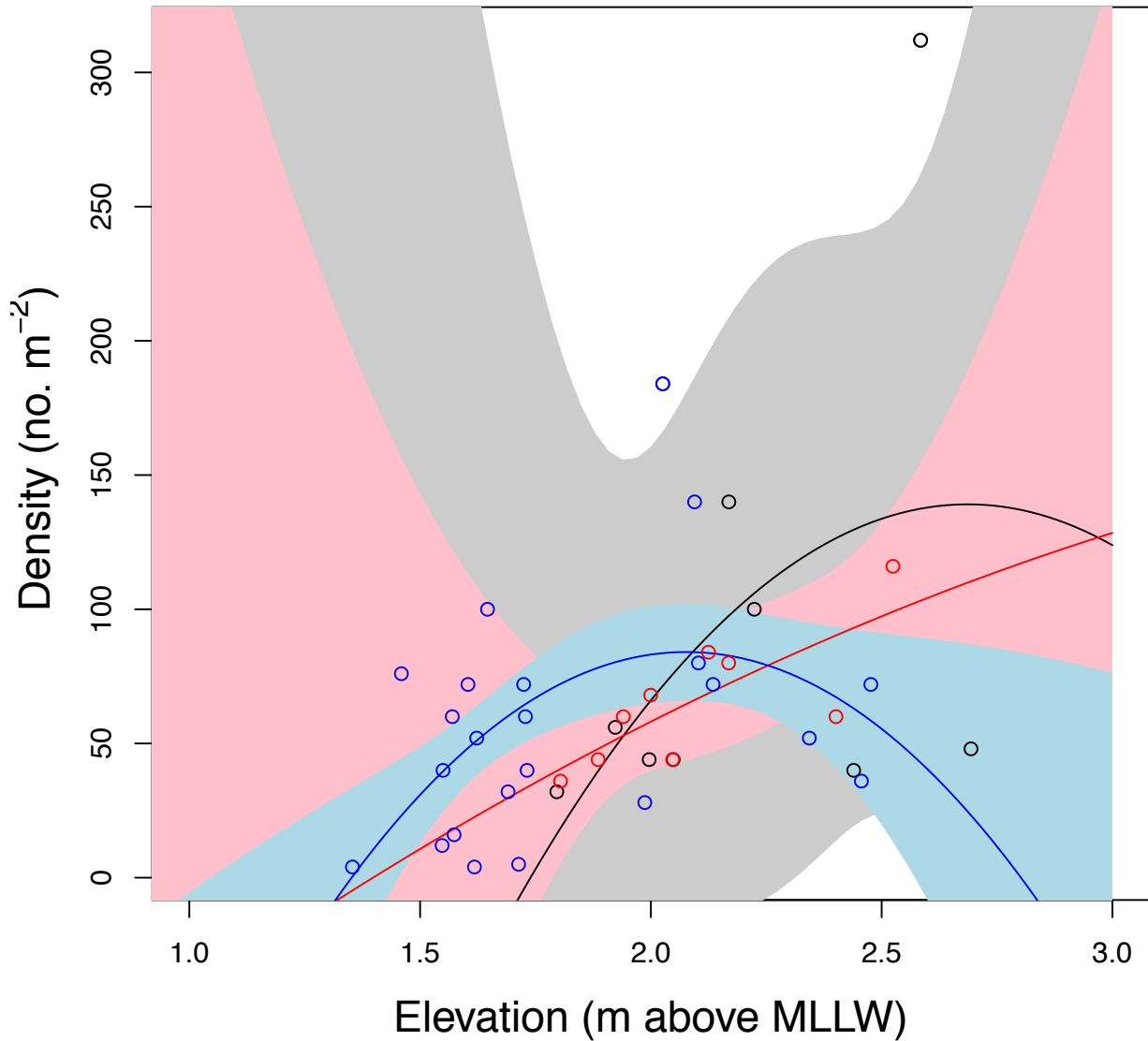
Figure 2.4 *Batillaria attramentaria* density across tidal elevation gradients at Willapa Bay, WA, for three years (2007 black/grey; 2008 red/pink; 2011 blue/light blue). Lines are predicted relationship between density and elevation (including a quadratic term) modeled separately for each year, with 95% confidence intervals in shaded portions.

as in 2011, these models have large confidence intervals that included the current lower limit. We are thus unable to rule out the possibility that the lower limit was similar in all three years.

Averaged across all years, *Batillaria* in Padilla Bay achieved three-fold greater density than at Willapa Bay, the site of the younger population (Figures 2.3 & 2.5, Table 2.1), but snail density decreased in Padilla Bay by an estimated 45% over the five-year sampling period (Table 2.1), a trend driven largely by the final sampling year (Figure 2.5). However, a concurrent decrease was not observed for the younger population (Figure 2.5, Table 2.1), which remained relatively stable over the course of the study.

Totaled over the entire transect, infection prevalence was similar between both populations; 60% of *Batillaria* in Padilla Bay, and 57% in Willapa Bay, was infected with parasitic trematodes. However, the effect of site on the probability of infection differed based on the relative elevation, as indicated by the significant interaction between site and elevation (Figure 2.6, Table 2.2). At the lowest end of the ranges, the probability of infection did not differ between the two sites (Table 2.2), and nearly all snails were infected. However, at the high end of the range, snails from Willapa Bay were more likely to be infected than those from Padilla Bay.

2.4.2 Predation Studies

2.4.2.1 Trapping Surveys

There were striking differences in the communities of potential predators of *Batillaria* captured in the trapping survey (Figure 2.7). Cancrid crabs (primarily *Metacarcinus magister*, but also *Cancer productus*, mean carapace width = 66.1 mm, standard deviation 10.2 mm, n = 97) were trapped only at Willapa Bay, and only at the two deepest trapping positions (corresponding with
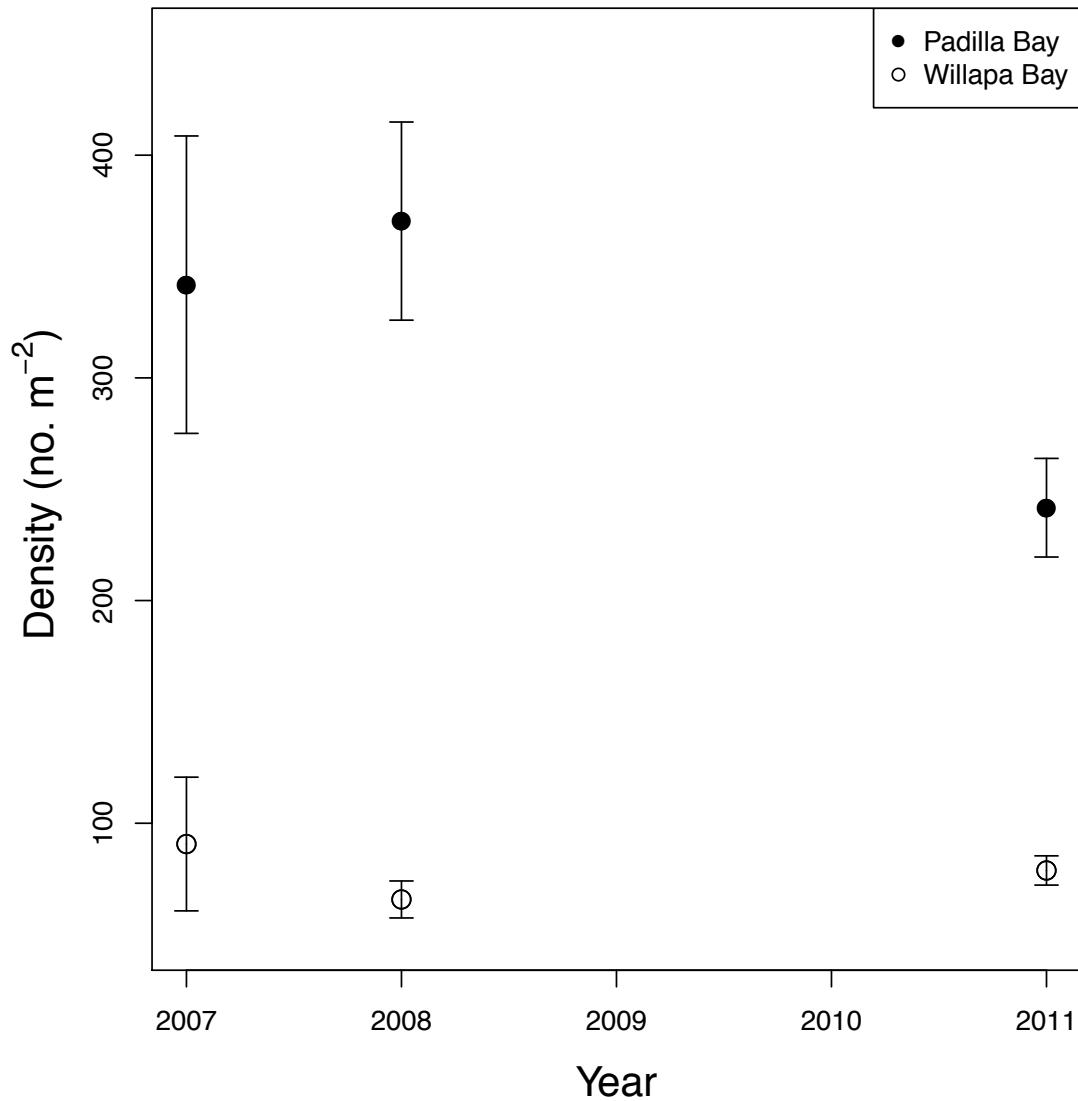
Figure 2.5. Average density (±1 SEM) of *Batillaria attramentaria* measured across vertical transects in 2007, 2008 and 2011 at Padilla Bay (filled symbols) and Willapa Bay (open symbols), WA.

Table 2.1. Generalized linear model (poisson error, log link) of Batillaria density across three years (2007, 2008, and 2011) and two introduction sites (older and younger populations).

Factor	Estimate	SEM	Z	p
Site	-1.563	0.032	-49.57	**< 0.001**
Year	-0.113	0.003	-34.78	**< 0.001**
Site x Year	0.107	0.009	12.13	**< 0.001**
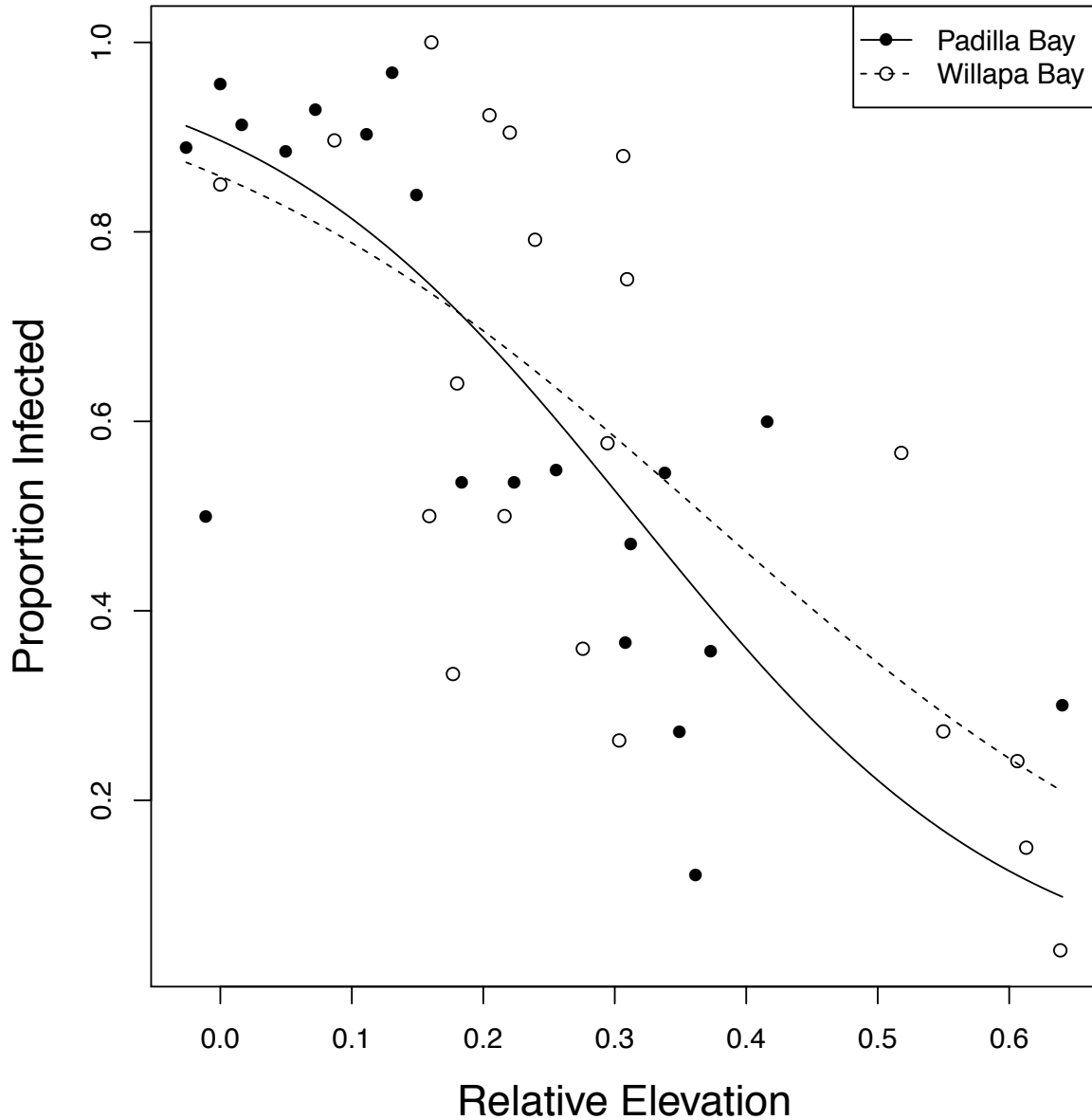
Figure 2.6 Proportion of *Batillaria attramentaria* infected with trematode parasites as a function of tidal elevation relative to local observed distribution (0 = lower limit, 1 = upper limit). Filled symbols, and solid line represent observations and predicted relationship, respectively, at the site of the older population, Padilla Bay, WA. Open symbols and the dashed line indicate observations and the predicted relationship, respectively, at the site of the younger population, Willapa Bay, WA.

Table 2.2 Generalized linear model (binomial error, logit link) of probability of infection by trematodes in *Batillaria* surveyed in 2011, across relative elevation and two introduction sites (older and younger populations).

Factor	Estimate	SEM	*Z*	*p*
Site	-0.355	0.305	-1.163	0.245
Relative elevation	-6.835	0.745	-9.181	**< 0.001**
Site x relative elevation	1.947	0.957	2.034	**0.042**
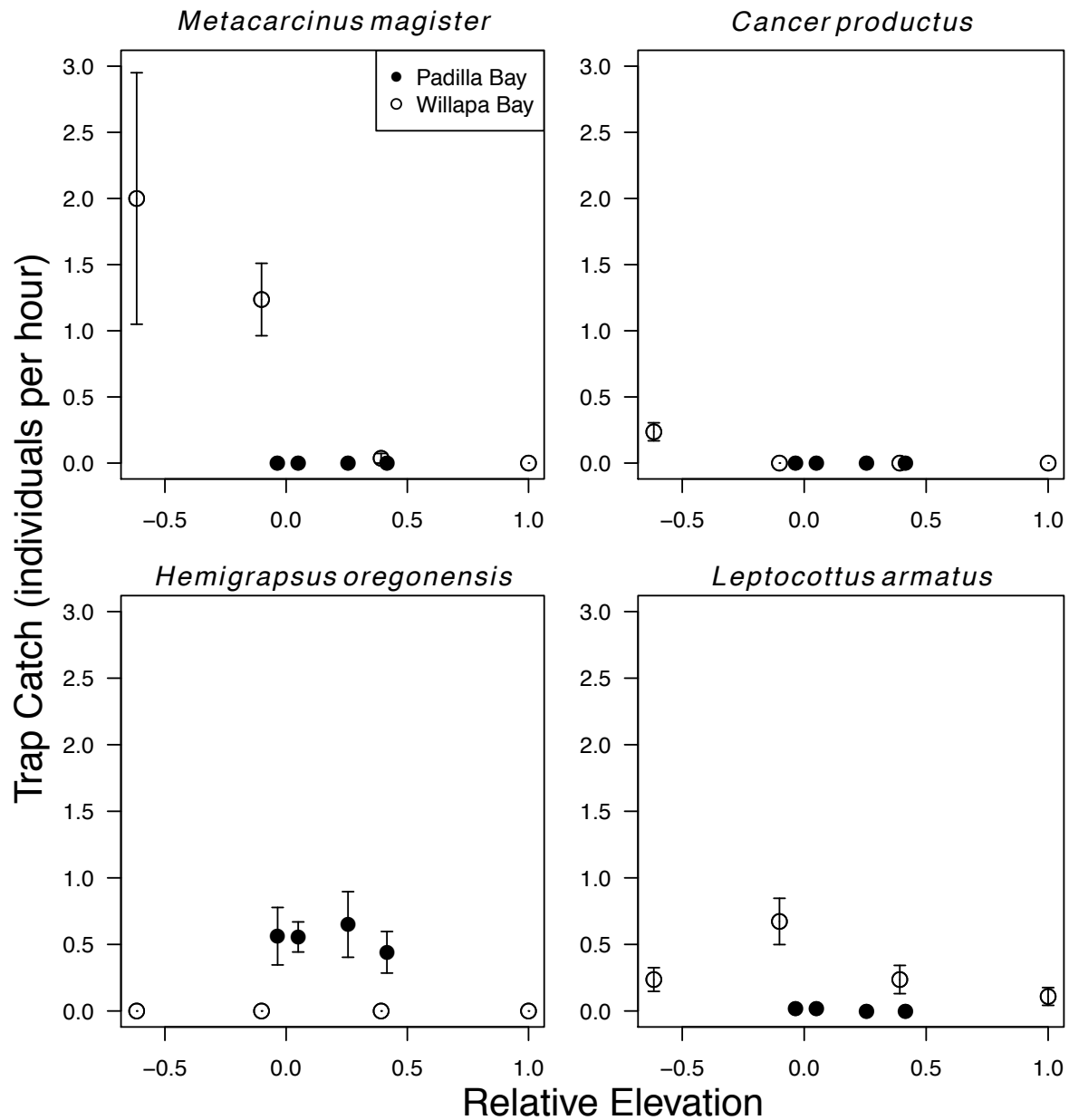
Figure 2.7. Average Fukui trap catches (1 SEM), standardized by effort, of four species of potential predators of *Batillaria attramentaria* as a function of tidal elevation relative to local observed distribution of snails (0 = lower limit, 1 = upper limit). Traps were set at lowest, fourth, seventh, and highest tethering positions at each site. Filled symbols represent catches at Padilla Bay, and open symbols indicate catches in Willapa Bay, WA.

the deepest and 4^(th) deepest tethering positions). Cancrids were entirely absent from traps deployed in Padilla Bay, despite the longer trap deployment, and greater inundation time. The only crabs captured in traps at that site were grapsid crabs *(Hemigrapsus oregonensis)*; their abundance was low and did not appear to vary with tidal elevation. Staghorn sculpin (*Leptocottus armatus*) were captured at both embayments. At Willapa Bay, they appeared in traps at all elevations, and were most abundant in the middle of the range. The abundance of sculpins was lower overall at the site of the older snail population, and they were captured only at the deepest two trapping positions.

2.4.2.2 Tethering

The effects of predators on tethered snails differed substantially between the two sites (Figure 2.8). On average, snails from Willapa Bay were much more likely to be attacked or consumed by predators, even in our model of predator effects in which the deepest three positions, where predation was greatest, were removed from the analysis (see above, Table 2.3). Moreover, where the ranges overlapped, the smallest size class of snails showed more evidence of predator damage or predator-related mortality than the largest size class (Table 2.3). The effect of relative

2.4.2.3 Laboratory experiment

In the laboratory predation experiment, mortality and shell damage in the remaining enclosures was greatest for small and medium snails (Figure 2.9), with an average proportion of 0.23 and 0.20 snails affected by predators, respectively. Mortality and shell damage for large snails was significantly lower than for small snails, only an average proportion of 0.05 snails were either damaged or consumed by crabs (Table 2.4). All dead *Batillaria* showed evidence of crab predation (e.g., broken/crushed shells), and no mortality occurred in the predator-free control enclosure.
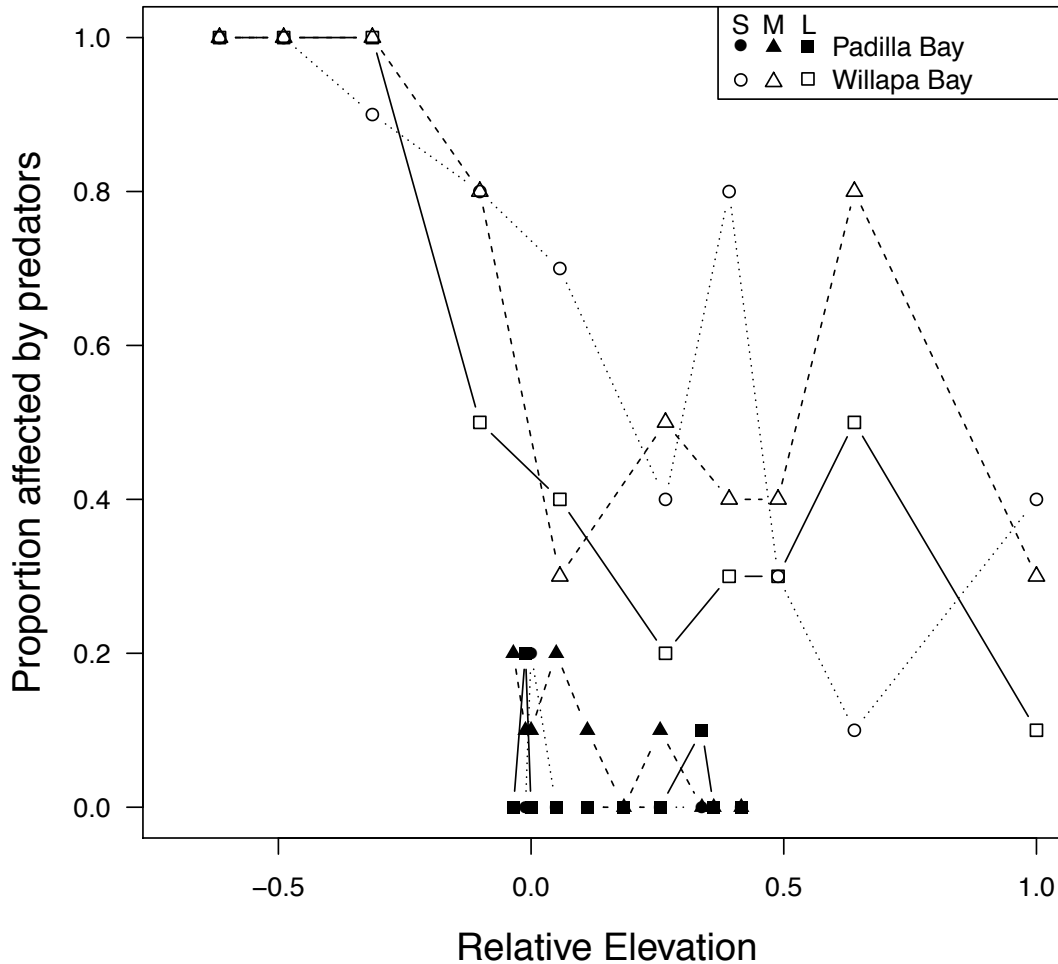
Figure 2.8 Proportion of snails of three size classes of *Batillaria attramentaria* damaged or killed by predators in tethering study as a function of tidal elevation relative to local observed distribution of snails (0 = lower limit, 1 = upper limit). Open symbols are observations from the Willapa Bay, filled symbols represent Padilla Bay, WA. Size class: small = circle/dotted line, medium = triangle/dashed line, large = square/solid line).

Table 2.3 Generalized linear model of number of snails affected by predators based on site, snail size class, and relative elevation. The reference group for the GLM comparison of size was the small size class.

Factor	Estimate	SEM	*Z*	*p*
Relative elevation	-2.401	1.442	-1.665	0.096
Site	-4.757	1.555	-3.059	**0.002**
Size class (medium)	-0.692	0.628	-1.102	0.271
Size class (large)	-1.388	0.626	-2.218	**0.027**
Relative elevation x Site	-37.999	50.179	-0.757	0.449
Relative elevation x Size (medium)	0.560	1.974	0.284	0.777
Relative elevation x Size (large)	0.667	2.007	0.333	0.740
Site x Size (medium)	2.596	1.661	1.563	0.119
Site x Size (large)	1.942	1.763	1.101	0.271
Site x Size (medium) x Relative elevation	33.379	50.304	0.664	0.507
Site x Size (large) x Relative elevation	37.255	50.353	0.740	0.460
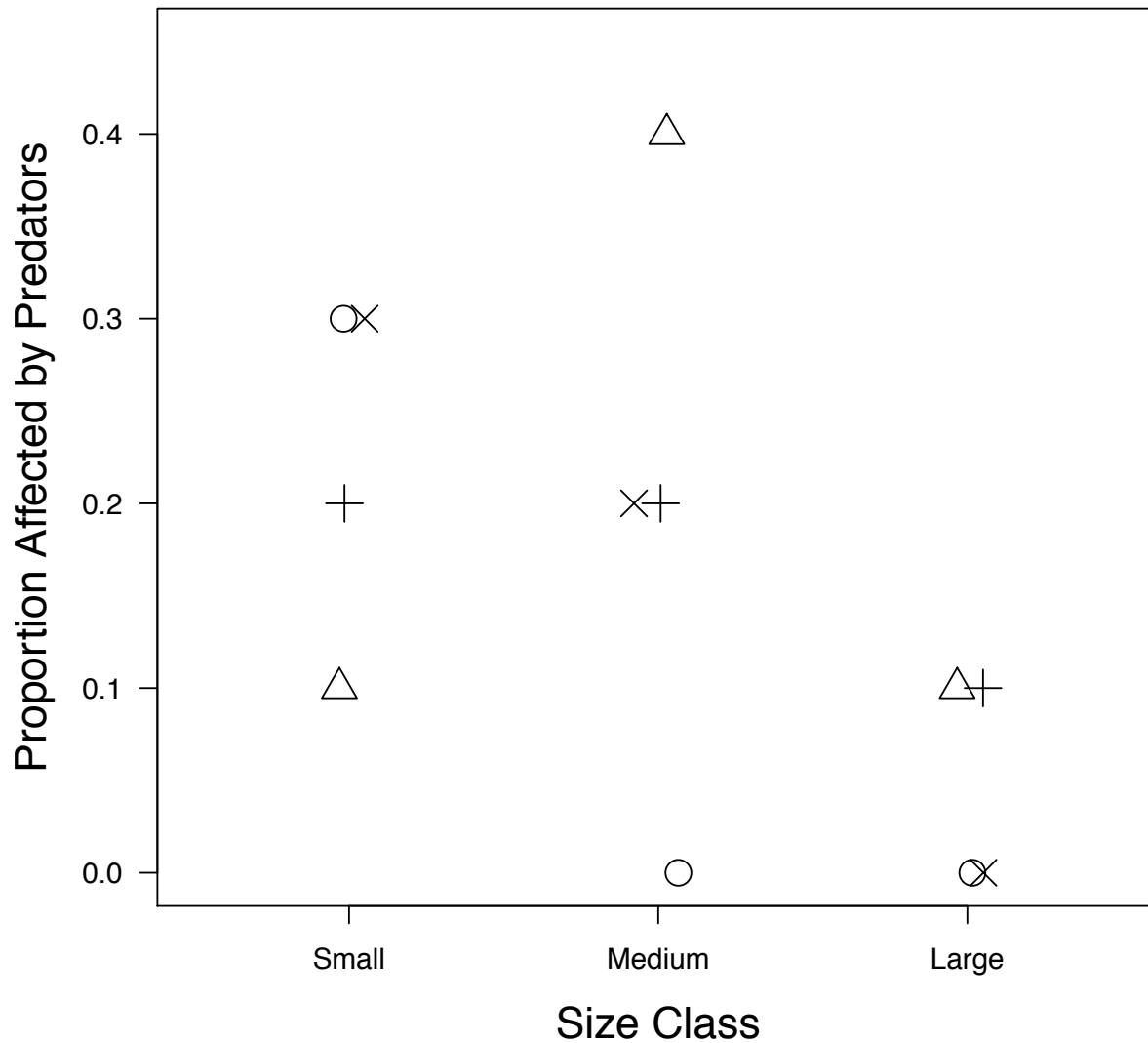
Figure 2.9 Proportion of snails of three size classes of *Batillaria attramentaria* damaged or killed by *Cancer* (*Metacarcinus*) *magister* in size-selectivity laboratory experiment. A different symbol has been assigned to each replicate and x axis is jittered for visibility.

Table 2.4. Generalized linear mixed effects model of the number of snails affected by crabs, *Cancer (Metacarcinus) magister* (predation-related damage and mortality) in laboratory experiment based on snail size class. The reference group for factor of size was the small size class, and the p value compares intercept to 0.

Size Class	Estimate	SEM	Z	p
Small (reference group)	-1.237	0.379	-3.266	**0.001**
Medium	-0.150	0.547	-0.273	0.785
Large	-1.708	0.818	-2.987	**0.037**

2.5 Discussion

The apparent cause of differences in the distribution and density between the two populations of *Batillaria* is variation in top down control by native predators at the two sites, rather than an effect of historical context or infection by co-evolved parasites. We observed the strongest support for the hypothesis that predators, namely cancrid crabs, influence the abundance, vertical range, and size distribution of *Batillaria* in Willapa Bay, but do not play a substantial role in controlling the population in Padilla Bay. The effects of predators on tethered snails were, overall, greater in Willapa Bay, and they increased sharply near the lower limit of observed snail distribution at that site as well. By contrast, tethered snails in Padilla bay experienced very low mortality or damage across the entire vertical gradient.

Trapping surveys suggested that predation effects were strongly driven by cancrid crabs, and the magnitude of predation effects corresponded most closely with the abundance of *Cancer (Metacarcinus) magister*, the Dungeness crab. The only crabs captured in Padilla bay, Grapsid crabs, are likely too small to effectively prey on the larger snails typically found at low elevations, while both *M. (C.) magister* and *C. productus* are highly efficient predators of *Batillaria* (E.W. Grason and P.S. McDonald, unpublished data). Moreover, previous work has demonstrated that the morphology and biomechanics of grapsid claws, unlike those of cancrids, are not well-suited for crushing snail prey (Yamada and Boulding 1998). In addition, results of the laboratory predation trials corroborated patterns observed in the tethering experiment; *M. magister* consumed all size classes of *Batillaria* but consumed significantly more small and medium snails compared to large snails, in spite of the fact that crabs captured from the field tethering sites at Willapa Bay were smaller than the crabs used in the laboratory study (66 mm

compared to >100 mm). Predation by *M. (C.) magister* could therefore also explain why the smallest size class was extremely rare at the Willapa Bay site when we collected snails for the tethering study. Staghorn sculpin, *Leptocottus armatus*, are unlikely to significantly impact *Batillaria* populations, as their variation in abundance did not correlate with predator effects on tethered snails. Moreover, previous gut content analyses have failed to detect evidence that *L. armatus* commonly include snails in their diet (McPeek et al. 2014), despite co-occurring with high *Batillaria* densities in some areas (P.S. McDonald, pers. obs.).

Notably, we did not observe a dramatic increase in predator effects on *Batillaria* in Padilla Bay below the lower limit of observed snail distribution, as we did in Willapa Bay, possibly because it was logistically infeasible to tether snails much deeper than the lower limit in Padilla Bay. Located within Puget Sound, Padilla Bay has much longer inundation times for the same elevations as Willapa Bay, a coastal estuary (Figure 2.2), making deeper sites rarely accessible when accessing from the shore. This prevents us from drawing strong conclusions about the effects of predators below this limit. Previous surveys have corroborated our observation that cancrids are very rare at intertidal elevations in Padilla Bay (Dinnel et al. 1986). Cancrids that forage in intertidal habitats during high tides typically migrate to small side channels during low tides (Holsman et al. 2006). Densities of *M. magister* in such side channels are 2 to 7 times greater in Willapa Bay (500 – 1,700 ha^{-1}, Rooper et al. 2002) than in Padilla Bay (250 ha^{-1}, Dinnel et al. 1986) suggesting that, below the current lower limit of *Batillaria* distribution, crab predation pressure is likely much greater on the younger population in Willapa Bay.

Surveys over the five years of this study do not support the hypothesis that the younger population (Willapa Bay) of *Batillaria* is restricted to higher elevations because it is dispersal-limited or in an exponential growth phase. We were unable to detect a change in the lower limit of distribution for snails in Willapa Bay, partially due to data limitation. Mark-release-recapture studies have shown that *Batillaria* can travel at least 15 cm per day (Miura et al. 2006). At this rate, which is likely conservative, the population could have moved at least 219 horizontal meters deeper, to a depth of about 0.9 m above MLLW, which is the elevation where 100% of snails in the tethering experiment were damaged or killed by predators. In 2011, *Batillaria* were never observed deeper than 1.0 m above MLLW, despite extensive searching. Moreover, snails from the younger population did not demonstrate increasing density over time as would be predicted under the exponential growth hypothesis. Density, when averaged across the entire range, was relatively constant at that site across the 5-year study period.

In addition, infection by co-evolved parasitic trematodes does not explain differences in elevation or density of snails, as infection rates were similar in the two populations. Infection rates in both populations were similar to those reported for the native range (Miura et al. 2006). Notably, however, we observed a much lower frequency of infection than was reported for Padilla Bay in 2000 (Torchin et al. 2005). Given that we observed stable infection rates since 2007 (P.S. McDonald, unpublished data), previous estimates may have been derived from subsamples of the total population, particularly lower and/or larger individuals that exhibited higher infection rates in the present study.

We did not investigate the role of other possible influences on vertical distribution, such as food availability or abiotic stresses. We believe that it is unlikely that abiotic factors were imposing strong population regulation on snails over the vertical ranges at which the tethering experiment was conducted, which encompassed nearly all of the snails' realized ranges, as well as a portion of deeper habitat. Only two of the 600 snails that were tethered appear to have died without evidence of attempted predation over the 6-8 weeks of the study. While it seems clear that crabs prevent *Batillaria* from extending their range deeper in Willapa Bay, the possibility that food availability also limits the depth at which snails can live in Padilla Bay remains untested. We would expect light limitation on growth of benthic diatoms to increase with depth and immersion time, as well as from shading by native eelgrass (*Zostera marina*). Indeed, observational evidence suggests that the lower limit of snails was often very close to the upper distributional limit of *Z. marina* in Padilla Bay (E.W. Grason, unpublished data).

Together our findings argue that abiotic factors and historic context alone are not sufficient predictors of invasion success in all cases. Concordant with previous studies, we observe that novel predators are not likely to preclude establishment at an invasion site, but they can influence success at one or more later stages of invasion, both spread and impact. In many cases, top-down control by native predators is observed to be context dependent with implications for variability of invasion success. For instance, an estuarine gradient influences the abundance of native crabs (including *Cancer productus*, also in our study) that prey on invasive European green crab (*Carcinus maenas*), such that the invasive crab is only found in more physiologically stressful, low-salinity habitats (Hunt & Yamada 2003; Jensen et al. 2007). In the San Juan Islands, the invasive clam, *Nuttalia obscurata*, is limited to soft-substrate habitats in which they can

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effectively bury to avoid predation by native *C. productus* (Byers 2002). Indeed, Colautti et al. (2006) noted, in a meta-analysis of invasion characteristics, that the only feature of the invasion process that significantly reduced the success of an introduced species at the "abundance/impact stage" was predation. Thus, spatial and temporal variability in native species that are likely to prey on a potential invasive species should be incorporated into invasion risk assessments.

Lastly, we have observed that residence time does not explain range or niche filling in terms of intertidal distribution. Distinct processes most likely influence the abundance and spread of an invasive species at different spatial scales. Namely, while residence time might influence range expansion on a large spatial scale, other processes, such as species interaction could be more influential in determining distribution at the local scale, even among dispersal limited-species.

CHAPTER 3

DOES CO-HISTORY CONSTRAIN INFORMATION USE? EVIDENCE FOR GENERALIZED RISK ASSESSMENT IN NON-NATIVE PREY

3.1 Abstract

Though prey use a variety of information sources to assess predation risk, evolutionary co-history with a predator could constrain information use, and non-native prey might fail to recognize risk from a novel predator. Non-native prey could compensate via generalized risk assessment, relying on general alarm signals from injured conspecifics rather than cues from predators. I tested the influence of shared predator-prey history on information use, comparing responses among three native and four non-native prey species to chemical cues from a native predator and cues from injured conspecific prey. Non-native prey demonstrated information generalism: (1) responding stronger to alarm cues released by injured conspecific prey than to the predators, and (2) responding similarly to alarm cues as to cues from predators consuming injured conspecific prey. By contrast, native prey required multiple information sources to elicit the greatest defense. The influence of other sources of chemical information was not predicted by co-history with the predator: only one non-native snail responded to the predator; digestion was only important for two native species; the identity of injured prey was important for all prey; and predator and prey cues contributed additively to prey response. Information generalism, hypothesized to be costly in co-evolved interactions, could facilitate invasions as a driver of, or response to, introduction to novel habitats.

3.2 Introduction

Predation-threat recognition is ubiquitous in both plants and animals, and resulting defenses can exert a strong influence on ecological dynamics, community structure, and ecosystem function (Werner and Peacor 2003; Peckarsky et al. 2008; Schmitz et al. 2008). Prey (including plants) commonly respond to a wide range of information modalities in assessing threats, including visual (Blumstein et al. 2000; Cooper 2009), auditory (Moiseff et al. 1978; Lohrey et al. 2009), olfactory/chemical (Hay 2009; Ferrari et al. 2010), and mechanical/tactile cues (e.g., Hazlett and McLay 2000; León et al 2001; Warkentin 2005). Informative cues can originate either from the predator itself (Kats and Dill 1998), from other conspecific or heterospecific prey (Chivers and Smith 1998; Schoeppner and Relyea 2009*a*), or from the interaction between predator and prey (e.g., fecal material, "altered" prey cues (Jacobsen and Stabell 1999; Agarwala et al. 2003; Schoeppner and Relyea 2009*b*)).

The relative value of these signals in risk assessments varies based on two qualities: 1) the quantity of information in the signal, and 2) the level of predation risk associated with the information. This is an extension of the "threat-sensitivity hypothesis", made popular by Helfman (1989), which posited that prey should demonstrate graded responses to risk cues based on the magnitude of threat indicated by any particular cue, optimizing the tradeoff between increased probability of survival and increased fitness cost incurred by engaging in defenses. This hypothesis predicts that cues, or cue combinations, indicating a greater probability of predation should elicit a greater magnitude of defense, and has been well supported empirically (e.g., Schoeppner and Relyea 2008; Hill and Weissburg 2014; Turney and Godin 2014). The amount of information contained in a cue should influence the prey's certainty of predation risk,

and suggest an appropriate response, and thus alters the value of the risk information for a given cue. However, the relative value of different cues remains unresolved, and most certainly varies across ecological and evolutionary contexts. The majority of experiments on risk assessment are conducted with only one or two species, and test a small number of cues, limiting generalization about information use in risk assessments. Moreover, interpreting a lack of response to a given cue is difficult because prey could fail to respond to a cue for multiple reasons: an inability to recognize the cue, an inability to mount the defense, constraints of a fitness trade off, or because that cue alone, in the individual's or population's history, has not been an accurate predictor of predation risk (Carthey and Banks 2014). Disentangling these possibilities presents an ongoing experimental challenge.

One leading assumption is that, in contrast to alarm cues originating from prey, cues originating from predators are more "informative" because they could indicate the predator's attack strategy, location, and even motivation state (Kats and Dill 1998; Bourdeau 2010a). For this reason, researchers have proposed that cues from injured conspecifics are less useful indicators of risk than cues from predators, and, therefore, it should be costly for prey to respond to general injury cues without additional information indicating which type of defense would maximize the probability of surviving (Sih et al. 2010). Supporting this hypothesis, many researchers have noted that prey only engage in defenses when multiple cues are combined (Alexander and Covich 1991a; Bourdeau 2010a), or that cues emanating from injured conspecific prey fail to elicit any defense at all (e.g., Slusarczyk 1999; Griffiths and Richardson 2006; Dalesman et al 2007). A key factor in the information content of an alarm cue is likely to be whether the cue is released actively or passively (Fraker et al. 2009). For instance vocal alarm signals from birds or

prairie dogs contain information about the type and location of predation threats present (Kiriazis and Slobodchikoff 2006; Templeton and Greene 2007).

Evidence of digestion and predator diet could also provide valuable information about risk. Many prey respond stronger to cues from predators fed a conspecific prey than to cues from predators fed a heterospecific prey (Alexander and Covich 1991*a*; Jacobsen and Stabell 2004; Laforsch et al. 2006; Schoeppner and Relyea 2009*a*). Presumably, information about prey identity tells the responding prey about the diet preferences of the predator, and a predator that has the capability and motivation to consume a conspecific should indicate a greater risk. On the other hand, it is less intuitive to predict how prey should respond to digestion *per se*. Detecting that the predator has digested conspecifics could be more informative than simultaneously detecting a predator along with cues from injured conspecifics, because digestion provides evidence of a causal link between the predator and injured prey cues. But digestion could also indicate lower risk because the predator might be satiated. In the limited number of experiments that have addressed this question explicitly, digestion increased the magnitude of prey response relative to combined cues from predators and injured prey (Jacobsen and Stabell 2004; Schoeppner and Relyea 2009*b*).

One constraint that can undoubtedly influence the information that prey use to assess risk is the length of evolutionary history the prey shares with the predator (Payne et al. 2004). In species introductions, for example, prey have been exposed to a predator for only a relatively short time, and might not have the ability to recognize cues produced by that predator (Carthey and Banks 2014). Failure to demonstrate any appropriate defense to the novel predation threat could have

substantial consequences for the success and impacts of an invasion, regardless of whether the prey is the native or the non-native species in the interaction (Sih et al. 2010). Perhaps the best-known example is the brown tree snake invasion on the island of Guam, which locally extirpated local and endemic prey unfamiliar with a threat from an arboreal snake (Fritts and Rodda 1998).

Non-native prey could theoretically compensate for an inability to recognize cues from a predator by relying on cues generated by injured conspecifics in assessing predation risk (the generalized risk assessment hypothesis discussed by Sih et al. (2010)), because the latter response is not similarly constrained by evolutionary co-history. Generalized risk assessment would mean that cues providing little information about the nature of the threat were relatively more important in generating the total response to the predation threat, and does not require that the introduced population recognize the novel predator *per se*. A growing number of studies has addressed whether prey recognize novel predators and/or novel predation threats (for a recent review, see Carthey and Banks 2014), but few have investigated the role of general cues in novel predator-prey interactions (Grason and Miner 2012; Bourdeau et al. 2013).

Information generalism in risk assessment, like dietary and habitat generalism, could predispose species to be successful at invading novel habitats. Species that utilize generalized risk assessment might be able to reduce biotic resistance enough to persist upon arrival in a novel predation regime, and could be characterized as high-risk invaders. Alternatively, the introduction event itself might impose selection for generalized risk assessment if novel native predators consume all individuals that require information about familiar predators, and those that are wary of cues from injured conspecifics survive. These scenarios are non-mutually

exclusive, and their relative importance could have substantial implications for identifying and managing invaders and biocontrol agents.

I tested how information used in risk assessments varies with evolutionary co-history by assaying behavioral defenses of three species of native, and four species of non-native prey, in response to a single native predator. I hypothesized (Table 3.1) that: 1) the shorter evolutionary co-history with the predator would mean that non-native prey were less likely than native prey to respond defensively to that predator, 2) a response to general risk cues (passively-released injury cues) would be uncommon or of low magnitude in native prey compared to non-native prey, because it only potentially carries a fitness advantage when prey cannot recognize the predator, 3) combining multiple cue types increases the information available to prey non-linearly only if prey can recognize both cues, and therefore, native prey should be more likely than non-native prey to show a synergistic response to a combination of predator-released and injured prey cues, 4) information generalism, defined as the greater relative importance of general cues in driving the full risk assessment, would be more common in non-native than native prey. That is, even where prey respond to both general injury cues, and cues from the predator itself the general cues elicit a stronger response in native prey than the predator cues. In addition, this multi-species, multi-cue experiment enabled me to test further hypotheses that are related to information use in risk assessment: 5) digestion by the predator increases the perception of risk, and 6) digestion of conspecific prey elicits a stronger response than digestion of heterospecific prey. The expectations about the role of evolutionary co-history in informing these last 2 predictions is less clear, but very few multi-species studies have been conducted to address these questions, let alone studies comparing native and non-native species. Thus, this study represents an

opportunity to investigate interspecific variation in use of information in a more robust way than has previously been attempted.

Table 3.1 Hypotheses, predictions, and analyses used to test information use in native and non-native prey.

Hypothesis	Analysis Type	Predictions (Treatment comparisons)	
		Native Prey	Non-Native Prey
1. Non-native prey are less likely to respond to a predator than native prey	2-way GLMM	Significant effect of predator cues	No effect of predator cues
2. Non-native prey are more likely to respond to general cues from injured conspecifics than native prey.	2-way GLMM	No effect of injured conspecifics	Significant effect of injured conspecifics
3. Native prey are more likely than non-native prey to respond synergistically to combined predator and general injured conspecific cues	2-way GLMM	Positive interaction term	No, or negative interaction term
4. Information generalism is more common in non-native than native prey	a. Linear contrast	$P > IC$ or $P \approx IC$	$P < IC$
	b. Linear contrast	$P \times IC > IC$	$P \times IC \approx IC$
5. Digestion of prey increases assessment of risk regardless of origin of prey.	Linear contrast	$P + IC < P \times IC$	$P + IC < P \times IC$
6. Digestion of conspecific prey increases assessment of risk relative to digestion of heterospecific prey.	Linear contrast	$P \times H < P \times IC$	$P \times H < P \times IC$

3.3 Methods

To test whether predator-prey co-history influences risk assessment, I compared information use of chemical cues among 7 species of marine snail (3 native and 4 non-native) in response to a single crab predator (red rock crab, *Cancer productus* Randall) native to coastlines of the northeastern Pacific Ocean. In separate mesocosm experiments, each snail species was exposed to six predation cue treatments (Table 3.2): a control treatment with no added cues, cues from an unfed predator only, cues from injured conspecific prey only, an additive combination of unfed predator and injured conspecific prey cues, a consumptive combination of predator and injured conspecific prey, or a general digestive cue treatment in which a "bland" food was fed to predators. I quantified responses to cues by observing snail avoidance behavior three times per week for multiple weeks (Table 3.3).

The four non-native snail species (*Ilyanassa obsoleta* Say, and *Urosalpinx cinerea* Say, from the western Atlantic Ocean, and *Ocenebra inornata* Récluz, and *Batillaria attramentaria* Sowerby from the western Pacific, hereafter referred to by genus names) share a similar history in Washington State, having been introduced unintentionally as hitchhikers along with non-native oysters imported in the 1920's (Wonham and Carlton 2005). The ca. 100 years since introduction, all of the non-native species has had at most 50 generations in the new habitat. None of the three

Table 3.2 Six cue treatments were applied to each snail species consisting of varying combinations of component cues.

Treatment	Abbreviation	Predator	Injured Conspecifics	Fed
Control	Control	-	-	-
Predator	P	+	-	No
Injured Conspecifics	IC	-	+	-
Additive Combination	P + IC	+	+	No
Consumptive Combination	P × IC	+	+	IC
General Digestion	P × H	+	-	Fish

Table 3.3 Ecological and experimental metadata for each of seven species of marine snail in behavioral experiments. In all experiments, each treatment was replicated in 8 bin systems.

Species	Native range	Collection Locality	Collection Habitat	Dates	Focal Snails (#)	Experiment Length (#D)	Total observations (#)	IC used (#)	Snail Length (mm)	Crab Carapace Width (mm)	Food Provided	Type of Avoidance
Nucella lamellosa	Northeast Pacific	Anacortes, WA 48.509228°, -122.683886°	Cobble	8/7 – 9/18/2013	1	42	18	2	28.4 SD: 4.7 N: 54	109.2 SD:12.3 N: 27 109.2	Mussels (30.9 mm, SD: 3.6, N: 46)	Hiding
Littorina sitkana	Northeast Pacific	Anacortes, WA 48.509228°, -122.683886° Case Inlet, WA	Cobble	10/2 – 10/28/2013	10	26	12	5	10.4 SD: 1.4 N: 103	109.2 SD:12.3 N:27 102.0	Diatoms on slides	Both
Alia carinata	Northeast Pacific	47.372406° -122.816353°	Eelgrass/mud	6/2- 6/18/2014	10	16	7	10	8.8 SD: 0.7 N:100	102.0 SD: 19.3 N:32	None/Ambient diatoms	Fleeing
Batillaria attramentaria	Western Pacific	Samish Bay, WA 48.577523° -122.485835°	Oyster bottom culture beds	5/31- 6/12/2013	10	13	6	5	30.9 SD: 3.4 N: 99	109.2 SD:12.3 N: 27 102.0	None/Ambient diatoms	Both
Ilyanassa obsoleta	Western Atlantic	Nemah, WA 46.547293° -123.899923°	Mudflat	5/4 – 5/23/14	3	19	9	3	22.1 SD 2.1 N: 50	109.2 SD: 19.3 N:32	Diatoms on slides	Hiding
Ocenebra inornata	Western Pacific	Samish Bay, WA 48.577523° -122.485835°	Oyster bottom culture beds	6/21-7/26/13	1	36	16	2	29.4 SD: 3.6 N:109	109.2 SD:12.3 N: 27	Mussels (33.4 mm, SD: 4.1, N: 27)	Hiding
Urosalpinx cinerea*	Western Atlantic	Willapa Bay, WA 46.420718° -123.933877°	Oyster hummocks	8/26 – 9/11/09	1	16	8	2	27.3 SD: 3.7 N: 48	108.0 SD: 13.6 N: 36	Oysters (10 – 25 mm)	Both

* Data reproduced from Grason and Miner (2012)

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native snails (*Littorina sitkana* Philippi, *Nucella lamellosa* Gmelin, and *Alia carinata* Hinds) is known to be invasive elsewhere. However, whether or not they have been introduced along other coastlines is also not known. The native crab, *Cancer productus*, is a locally-abundant predator in intertidal and subtidal habitats. With strong, crushing claws, *C. productus* is a significant predation threat to snails (Yamada and Boulding 1998), and structures intertidal communities via consumption (Yamada and Boulding 1996).

Previous research has explored defensive responses of *Urosalpinx*, *Nucella*, and *Littorina*, to Cancrid crab predation cues. All three species respond defensively when they detect *C. productus* preying on conspecific snails (Appleton and Palmer 1988; Yamada et al. 1998; Grason and Miner 2012), but the organismal source of the inducing cue and the response measured has varied. For instance, *Nucella lamellosa* native to Washington State developed the greatest morphological shell defenses when exposed to cues from *C. productus* consuming conspecific snails, and, to a lesser extent, crabs alone, but did not change shell morphology in response to cues from injured conspecifics (Appleton and Palmer 1988; Bourdeau 2010*a*). Notably, the proximal cause of the change in shell characteristics might have been starvation due to reduced foraging rates of snails exposed to predation cues; starved *Nucella* produced shells similar to those exposed to cues of crabs fed conspecific snails (Bourdeau 2010*b*). On the other hand, *Littorina sitkana*, also native, responded behaviorally to *C. productus* (hiding or climbing out of the water, depending on the population) only when crabs were fed conspecific snails (Yamada et al. 1998). The authors inferred that the snails were therefore responding to alarm cues from injured conspecific snails, though this was not directly tested. Risk responses of *Urosalpinx cinerea* to co-evolved predators have not been examined, but several experiments document the

response of non-native populations to novel predator cues. *Urosalpinx* reduces feeding and increases predator avoidance behavior in response to crabs (*Carcinus maenas, Cancer productus, and Cancer antennarius*) consuming conspecifics, but conflicting evidence exists as to whether snails recognized the crabs themselves, or were responding to other chemical information. In one study, *Urosalpinx* did not respond to *C. antennarius* alone (Kimbro et al. 2009), but a separate study was able to detect avoidance behavior in response to both *C. antennarius* and *C. maenas* (Blum 2012). Additionally, *Urosalpinx* from Washington State spend the more time hiding when presented to cues from *C. productus* alone (Grason and Miner 2012).

3.3.1 Collection and Husbandry

Snails were collected by hand from multiple localities in Washington State as they were required for experiments (collection localities and snail sizes can be found in Table 3.3). Non-native snails were housed in closed-circulating aquaria, while native snails were kept in flow-through seawater tables at Shannon Point Marine Center (SPMC), in Anacortes, WA. All snails in holding were exposed to the same source water from the flow-through system at SPMC which draws from the local beach. While in holding, snails were fed, *ad libidum*, on barnacles and bivalves, for predatory snails, or macroalgae and naturally-recruiting diatoms, for herbivorous snails. No snails were directly exposed to cues from *C. productus* while in holding, and snails were not kept in holding for more than 3 weeks before being used in experiments.

Crabs were collected intertidally, by hand, at SMPC and Shilshole Marina (Seattle, WA) and housed in flow-through sea tables at SMPC. Crabs were fed frozen fish (*Tilapia sp.* or *Pangasius sp.*) or mussels (*Mytilus spp.*) several times weekly. The experiments included both male and female crabs of a broad range of sizes (70 – 150 mm carapace width), because even young

individuals of this species are capable of crushing the shells of all sizes of snails (Author, pers. obs.). Crabs were starved for at least 48h before subsequent experiments to clear the gut.

3.3.2 Mesocosm Experiments

Experiments were conducted in laboratory mescosoms separately for each snail species over multiple years, but all experiments used the same design and equipment. Cue treatments were applied using a coupled, flow-through, bin system (Figure 3.1). Each coupled bin-system was randomly assigned to one of the six cue treatments; eight replicate bin-systems were used per cue treatment for a total of 48 bin-systems per experiment. Focal snails, those on which behavioral observations were made, were isolated from cue sources in the downstream bin, and were provided with food and a refuge (several pieces of oyster shell) placed at opposing ends of the bin to force a trade-off decision.

I applied cue treatments upstream of the focal snails. In treatments that included crabs, a single, native crab predator (*Cancer productus*) was enclosed in the upstream bin, and fed or starved as appropriate for the treatment (Table 3.2). To generate cues of injured conspecifics that were not consumed by predators (IC and P + IC treatments), conspecific snails were lightly crushed, sufficiently to inflict shell and tissue injury, but not to liquefy snails. Injured snails were then wrapped in a mesh pouch, which was attached to the inflow of the downstream bin, so that cues from injured snails were dispersed thoroughly throughout the bins with focal snails, but upstream crabs were prevented from detecting those same cues. In treatments where crabs were fed conspecific snails, the snails were first injured in the same manner as above before being added to the upstream bin with the crab. The "bland" diet for crabs consisted of frozen fish fillet
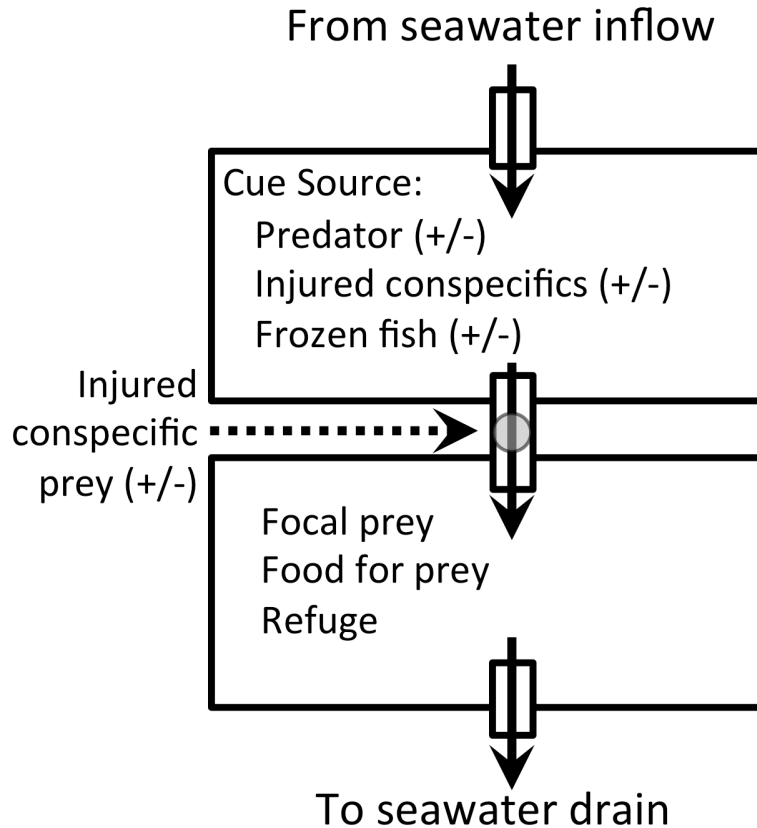
Figure 3.1 Schematic diagram of coupled, flow-through mesocosm system used in cue experiments. Each replicate bin system (n = 8) had constantly flowing seawater, and manipulated cue treatments upstream of the focal prey on which behavior observations were taken.

(*Tilapia sp.* or *Pangasius sp.*) similar in mass to the snail body tissue used for injury cues (IC, P + IC, and P × IC treatments).

Behavioral observations and reapplication of cue treatments occurred three times per week, the observations taking place prior to reapplication of cue treatments to minimize the effect of the disturbance on the behavior of the organisms. On those days, crabs were fed diets appropriate for the treatment and injured conspecific pouches were replaced. In order to avoid the over-accumulation of cues, I removed crab waste and shell debris from all bins with crabs at least twice weekly. Prior to experiments, crabs were starved for at least 48h, but snails were allowed

to feed on bivalves or algae as appropriate for each species. Flow rates were maintained in the system at approximately 2 Lm^{-1}.

I assessed responses to risk cue treatments by observing the proportion of time focal snails in the downstream bin were engaged in predator avoidance behavior. Snails typically avoid predator encounters in two ways, either by hiding, or by attempting to crawl out of the water (e.g., Hadlock 1980; Alexander and Covich 1991; Turner et al 1999). During observations, the location of each focal snail was categorized as either flight (emersion), hiding (under or behind the refuge, or behind other structure in the bin), or neither (feeding or crawling in any open area of the bin). *Batillaria* commonly buries in response to predators (Wells 2013); in that experiment I added a layer of clean play sand to the bin, at a depth of approximately 1.5 cm. *Batillaria* that were partially or fully buried in the sand were considered to be hiding. Another snail species, *Littorina*, would often climb onto the underside of the lid of the bin and fall off when I removed the lid to record observations. Such snails could be found oriented on the bottom of the bin with their operculum facing upwards, and were also considered to be attempting to flee via emersion.

The number of focal snails in each replicate downstream bin was consistent for each species, i.e., within each experiment, but varied among species to partially account for variations in natural density (Table 3.3). Thus, the whelk species, which occur in relatively lower densities, were isolated as individual focal snails in each replicate downstream bin. By contrast, groups of 10 *Alia* were placed in each replicate downstream bin for that experiment, because that species occurs in densities 100x greater in situ.

3.3.3 Analysis

It is unlikely that all species of marine snail have evolved the same types of avoidance behaviors because ecological contexts and shell morphology likely make different types of behavioral defenses, such as emersion and refuge use, more or less valuable to each species or population. I am aware of no published information documenting effect of behavioral defenses on survivorship in the presence of predators for these species. To avoid biasing the interpretation of behavior based on a prior expectation of what is believed to be adaptive, I calculated predator avoidance behavior separately for each snail species.

Avoidance behavior was defined as the location metric (either hiding, fleeing, or the sum of both as indicated in Table 3.3) that yielded the largest effect size for the full information treatment (predators eating conspecifics, abbreviated as $P \times IC$). Effect size was calculated as the odds ratio of the response to the $P \times IC$ treatment (predators consuming injured conspecific prey) relative to the control (no cues). For example, avoidance behavior for *Alia* was defined as the number of snails fleeing in each bin on a given day, because the location metric that maximized the difference between the $P \times IC$ and Control treatments was flight only. By contrast, the location metric for which the greatest effect size of the $P \times IC$ treatment was observable for *Littorina* was the sum of snails fleeing and hiding. The metric that yielded the greatest effect size was used for avoidance for all comparisons for that species.

To test hypotheses about influence of evolutionary co-history of predator and prey on information use, I compared the proportion of time prey spent avoiding between native and non-native species (Table 3.3). Separate binomial generalized linear mixed effects models (GLMMs)

of avoidance were constructed for each species, where the response variable number was the number of snails avoiding in each bin on a given observation day, with the number total number of snails in the observation bin (Table 3.3) as the number of trials. Thus, the number of snails avoiding each day was the number of successes, and the number of snails not avoiding was the number of failures. The GLMMs also included replicate bin as a randomly varying intercept to account for the repeated measures structure of multiple observation days within each experiment (Table 3.3). A two-factor implementation of this model, using the control, the crab only, injured conspecifics only, and the additive combination (P + IC, as the true factorial combination of the two cues) treatments, tested the separate and combined effects of the main constituent cues (Hypotheses 1 – 3). I further used linear contrasts to address additional *a priori* questions about the importance of digestion, and the relative influence of constituent cues (Table 3.3, Hypotheses 4 - 6).

Because the experiments were conducted separately for each species, I used meta-analysis to test for differences between native and non-native species as a group. Effect size was calculated for each species, for each hypothesis test, as the parameter estimate from the GLMM described above divided by the estimated standard error from the model. Thus, replication for the meta-analysis comparison was three natives, and four non-native species. The difference between native and non-native species was then tested for each hypothesis with t-tests (n = 3 for natives, n= 4 non-natives). This approach has the benefit of incorporating the variation in estimates (standard error) and random effects into effect sizes. All analyses were conducted in R (R Development Core Team 2013) using the lme4 package (Bates et al. 2015).

3.4 Results

All seven species of prey, regardless of status as a native, increased the proportion of time spent avoiding in response to a predation threat from native *Cancer productus* when predatory crabs were allowed to attack, consume, and digest conspecific prey (Figure 3.2). The magnitude of the response to the full predation cue (P × IC) relative to the no-cue control treatment varied greatly among species, ranging from a factor of 1.3 (*Littorina*, Figure 3.2f) to 17.2 (*Alia*, Figure 3.2e).

Observations supported only a subset of my predictions about the role of evolutionary co-history in risk assessment. Native and non-native prey did not differ significantly in their response to cues from an unfed predator (Figure 3.3, Hypothesis 1, t-test: $t = -0.304$, $P > 0.10$). Though all prey species showed a trend toward increasing time avoiding in the predator only treatment relative to the control, the magnitude of this increase was only significant for one non-native species, *Urosalpinx* (Figure 3.2).

Non-native prey responded defensively to general cues from injured conspecifics more frequently, and at a greater magnitude, than native prey (Figure 3.3, Hypothesis 2, t-test: $t = 3.232$, $P = 0.023$). Two of the three native species did not increase hiding when they were exposed to cues emanating from injured conspecifics, and the third, *Littorina*, responded in the opposite direction as would be expected for a defensive response (Figure 3.2f,), i.e., they spent less time hiding or fleeing when they detected cues of injured conspecifics than when those cues were absent.
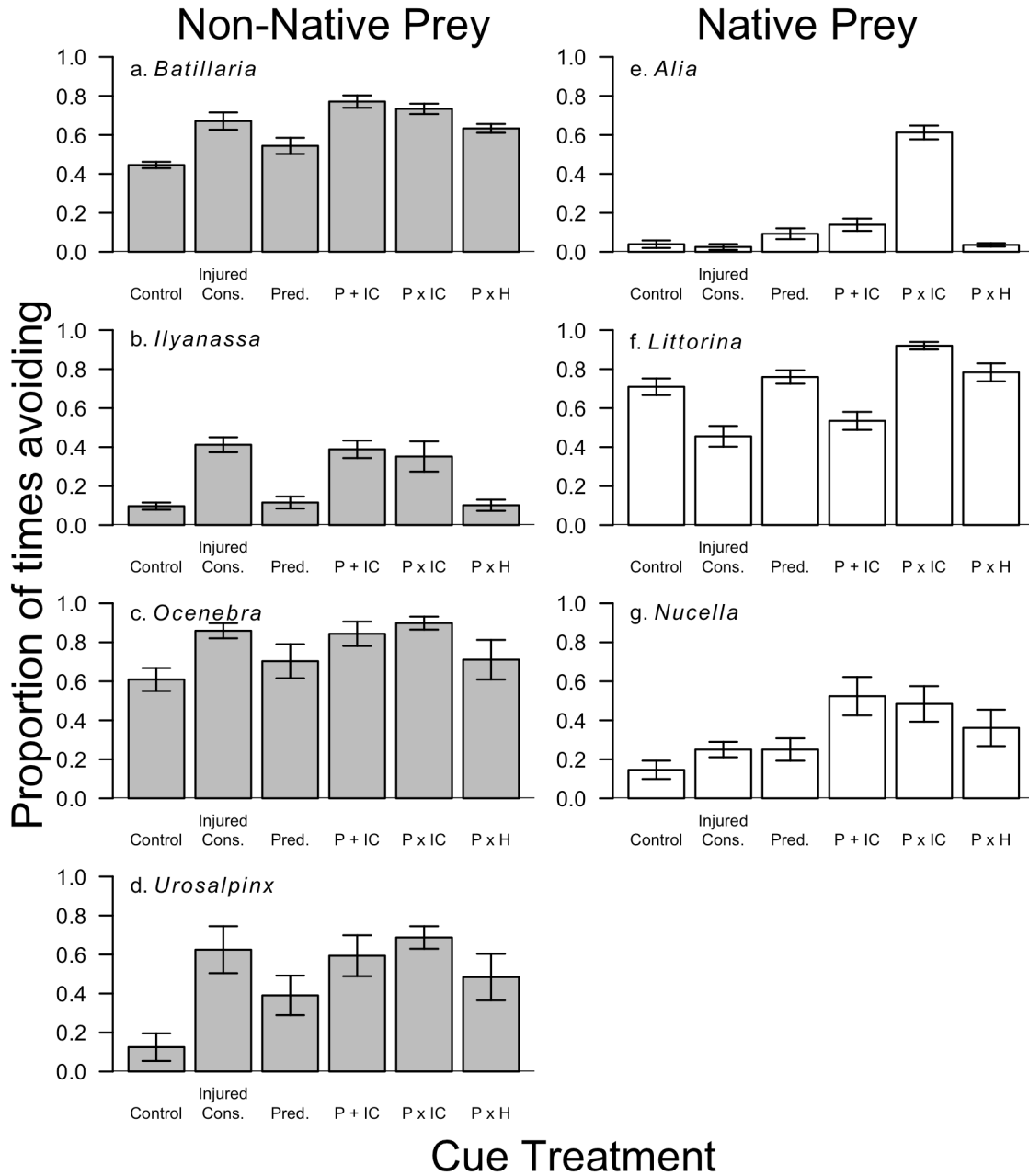
Figure 3.2 Avoidance behavior in four non-native (panels a-d, gray bars) and three native (panels e-g, white bars) snail species, a. *Urosalpinx cinerea*; b. *Ocenebra inornata*; c. *Batillaria attramentaria*; d. *Ilyanassa obsoleta*; e. *Nucella lamellosa*; f. *Littorina sitakana*; g. *Alia carinata* in response to cues from a novel native predatory crab, *Cancer productus.*
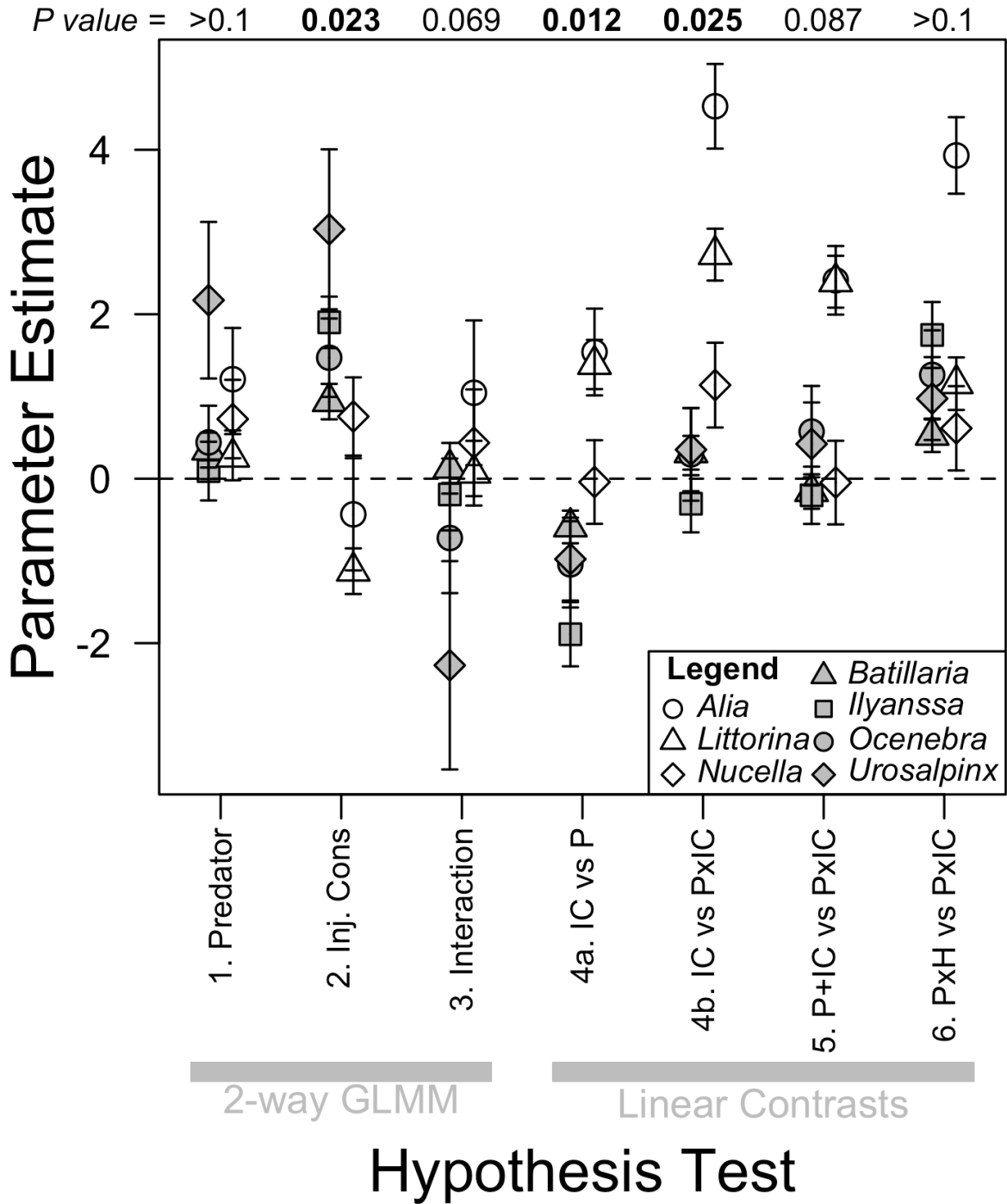
Figure 3.3 Parameter estimates (+/- estimate standard error) for GLMM models of hypothesis tests. Filled symbols are non-native species, and open symbols are native species; native and non-native points are jittered slightly for visibility. Bold *P*-values indicate a significant difference between native and non-native species for that test. For linear contrasts, a positive parameter estimate indicates the second treatment listed generates greater avoidance behavior, i.e. *Alia* avoids more in response to predators consuming conspecifics (P × IC) than to cues from injured conspecifics alone (IC), while *Ilyanassa* avoids more in response to cues from injured conspecifics (IC) than cues from an unfed predator (P).

Contrary to my prediction, native and non-native prey did not differ based on how they responded to combined constituent risk cues (Figure 3.3, Hypothesis 3, t-test: $t = -2.310$, $P = 0.069$). For all seven prey species tested, adding the component risk cues together (P + IC) increased avoidance linearly relative to the cues when applied separately. One non-native species, *Urosalpinx*, showed a trend toward an antagonistic response to the combination treatment (Figure 3.3), but the interaction term of the two-way GLMM was not distinguishable from zero.

Non-native species diverged from native species in that the former demonstrated information generalism; avoidance behavior by all non-native prey assayed was driven primarily by general cues from injured conspecifics, which were the most important source of information in their risk assessment. Information generalism was not shown by any of the native species. Two pieces of evidence support this inference. First, for non-native prey species, cues from injured conspecifics provoked a greater defensive response than cues from the predator itself (Figure 3.3, Hypothesis 4a, t-test: $t = -3.858$, $P = 0.012$). By contrast, prey that shared an evolutionary history with the crab (natives) either showed greater avoidance to cues from the predator (P) than to cues from injured conspecific cues (*Littorina* and *Alia*) or responded similarly to the two component cues (*Nucella*). As a group, non-natives spent more time than natives avoiding predators when they detected cues from injured conspecifics alone compared to when they detected cues from the predator alone. Secondly, for all non-native prey, and only for non-native prey, avoidance in response to chemical cues from injured conspecific prey (IC) was statistically indistinguishable from avoidance in response to the full predation cue (P × IC) (Figure 3.3, Hypothesis 4b, t-test: $t = -3.181$, $P = 0.025$). Cues from injured conspecifics alone were sufficient to elicit the maximum increase in avoidance observed. Conversely, all native prey in the consumptive predation

treatment (P × IC) spent more time avoiding than they did in the injured conspecific cue treatment (IC).

Digestion of conspecific prey only increased avoidance behavior for two species (*Littorina* and *Alia*), both native, but the difference between native and non-native prey in terms of the importance of digestion was not significant (Figure 3.3, Hypothesis 5, t-test: $t = -2.127$, P 0.087). For *Littorina*, the avoidance response reversed direction in response to digestion; this species spent *less* time avoiding when predator and injured conspecific cues were additively combined (P + IC) compared to the control treatment, but spent *more* time avoiding than the control when crabs digested conspecifics (P × IC, Figure 3.2f). While digestion itself was not always important to risk assessment, the identity of prey being digested was generally important to the prey tested (Figure 3.3, Hypothesis 6, t-test: $t = 0.853$, $P > 0.10$), and prey spent more time engaging in avoidance behavior when they detected predators consuming conspecifics than when predators were fed fish. The magnitude of increase in avoidance in response to predator diet was similar between native and non-native prey, and there was no statistical difference in effect size of the contrast between the P × H and P × IC treatments between native and non-native prey.

3.5 Discussion

I observed that native and non-native prey diverge in their use of general cues in assessing risk from a native predator, and that information generalism was a shared trait among all of the non-native snails assayed here. While all species of prey demonstrated avoidance behavior in response to chemical cues from a native predator attacking, consuming, and digesting prey (P × IC, Figure 3.2), non-native prey employed a generalized risk assessment strategy, based primarily on strong responses to general alarm cues from injured conspecifics. Conversely, alarm

cues from injured conspecific prey did not cause native prey to increase their avoidance behavior, and those species required multiple sources of information to engage in the greatest magnitude of observed defensive behavior. Patterns of response to the predator by itself, and the importance of digestion, were variable among prey species independent of evolutionary co-history with the predator, indicating that some aspects of risk assessment might be more important in novel predator-prey interactions than others. The only source of information that increased avoidance for every single species was prey identity. Multiple species comparisons in similar predator-prey interactions are a critical first step in identifying how information use might constrained by ecology or evolution, and where varying contexts can select for divergent strategies.

3.5.1 Generalized risk assessment in non-native snails

These results stand as the strongest support to date for general information use by non-native species (Sih et al. 2010). Evidence for generalized risk assessment by non-native snails is found in the relative importance of chemical cues originating from injured conspecifics. Alarm cues, though hypothesized to be uninformative about the nature of the threat, were both necessary and alone sufficient to explain the greatest magnitude of avoidance behavior observed for any of the non-native species.

Generalized risk assessment could result in what has been referred to as level 4 naïveté (Carthey and Banks 2014), where prey respond appropriately and effectively to a novel predation threat, but incur excess non-lethal effects because they over-invest in defense. Indeed, I have observed that for nearly all species, increased avoidance was correlated with significantly reduced feeding rates (E. Grason, unpublished data). If this carries sufficient cost and does not improve survival compared to a more specific risk recognition (requiring predator cues), relative influence of

general cues in risk assessments should decrease over time, and generalized risk assessment could disappear entirely in introduced populations. Each of the four non-native species assayed here has been present in Washington State for as many as 50 generations. However, because very little is known about the selective pressures of predators on non-native populations and the costs of defensive behavior, it is unclear whether this constitutes sufficient opportunity for evolution to refine risk assessment, or whether other factors are contributing to the maintenance of generalized risk assessment.

There are several important caveats in evaluating support for the role of information generalism in biological invasions. First, although the native species of prey assayed here are not known to be invasive elsewhere, an additional test of this hypothesis would include known failed invaders, which would be expected to require combined cues or respond stronger to cues from predators than cues from injured conspecific prey. Second, information generalism could extend beyond responses to injured conspecifics. Increased support for the importance of this trait in invasions would be found if non-natives defend in response to cues from a wider range of injured heterospecific prey than natives. Finally, it will be critical to determine whether native populations of the non-native species assayed here also demonstrate generalized risk assessment when faced with predators from their native range, with which they share a longer evolutionary history. I am aware of no published studies testing defensive responses to cues from predators in the native range, but studies on three species' responses to non-native predators do exist. *Ilyanassa* has been observed to spend more time hiding when exposed to chemicals from crushed conspecifics, but not cues from *Carcinus maenas* L., itself introduced to the native range of *Ilyanassa* (Atema and Stenzler 1977), and no treatment of the full predation cue was tested for

comparison. Thus, while this study makes clear that *Ilyanassa* does respond to general risk cues, it does not fully test for generalize risk assessment. *Batillaria,* in a separate invasive population in California, apparently do demonstrate generalized risk assessment. Snails in experiments hid similarly in response to cues from injured conspecifics and cues of non-native *C. maenas* consuming conspecifics, but not to cues from the crab itself (Wells 2013). Lastly, as described above, evidence for recognition of novel crabs by *Urosalpinx* is equivocal (Kimbro et al. 2009; Blum 2012). An observed response to injured conspecifics alone is not sufficient evidence for generalized risk assessment, rather that response must be compared to the magnitude of responses to other predation cues.

3.5.2 Responses to the predator

Contrary to my prediction, response to native predator cues was not explained by whether or not the prey was also native. With the exception of one non-native prey, *Urosalpinx*, none of the prey increased the proportion of time avoiding in response to cues from the crab alone. I expected that native snails would be more likely than non-native snails to respond to cues from the unfed predator, because (1) predator cues are believed to be accurate and informative indicators of predation risk, and (2) non-native snails might not have evolved the ability to detect the novel predator.

Perhaps more surprising than the fact the one non-native prey species did hide in response to a novel native crab predator is the fact that the majority of native snails did not change their defensive behavior in the presence of a predator. The failure to respond to a given cue or combination of cues admits multiple possibilities: 1) an inability to recognize the cue, 2) a low probability of risk associated with that cue, 3) a constrained fitness trade-off, or 4) an inability to

mount a defense. Because all snails increased avoidance in response to at least one of the cue treatments, the last possibility can be ruled out. Support for the third possibility would be observed if prey had reduced their avoidance behavior during longer experiments, because they needed to emerge in order feed (i.e. the life versus lunch hypothesis). However, longer experiments were not associated with a reduction in hiding behavior (E. Grason, unpublished data). It is notable that these experiments did not enable me to distinguish between the first two explanations. While it seems unlikely that native snails would not have evolved the ability to detect a co-evolved predator if that cue indicated risk, it is also possible that selection has acted on the chemical cues released by the crab to reduce their detectability by prey (Havel 1987). These are promising areas of future research.

It is worth noting that I measured only one type of anti-predator response - avoidance behavior – and it is possible that prey were indeed responding to the predator cues, but via an unmeasured behavior. Different types of anti-predator defenses, behavior, morphological, and life-history, are likely differentially valuable in different contexts, and therefore might be differentially responsive to different information sources and cue types. Moreover, organisms can make trade-offs between investing in different types of defense. Prey that have invested in thicker shells might not need to reduce their time foraging in the open if their shell is and effective defense (Rundle and Brönmark 2001). All prey assayed in this study were collected from areas where crabs are known to be common, which ensures that inference about whether natives and non-natives differ in this regard is not confounded by previous experience with the predator, because all prey had similar environmental exposure. An additional test for response to predator cues could use laboratory-reared naïve prey.

Apparent recognition of native *C. productus* by the non-native *Urosalpinx* echoes other

observations of non-naïve non-native species (Pearl et al. 2003; Freeman and Byers 2006).

However the basis for the recognition ability observed here remains uncertain. Several potential,

non-mutually exclusive, explanations exist: 1) rapid-adaptation in the <50 generations since

introduction (Freeman and Byers 2006); 2) associative learning (Hazlett et al. 2002; Ferrari et al.

2008); and 3) recognition via similarities to co-evolved predators of the same "archetype" in the

native habitat (Carthey and Banks 2014). Crabs of the genus *Cancer* overlap in geographic range

with native populations of all the non-native species (though it is unknown whether the source

populations for the invasions occurred within those ranges). There is evidence that *Urosalpinx*

from another part of the non-native range are able to recognize several species of crab with

which it shares no, or a very short, evolutionary history (*Romaleon antennarium* Stimpson and

Carcinus maenas), lending support for the importance of archetypes for that species (Blum 2012).

However, additional exploration of behavior of *Urosalpinx* from the native range, as well as

naïve, laboratory-reared individuals, are necessary to determine support for any of these

mechanisms. Neophobia, an aversion to any novel sensory stimulus, is an unlikely explanation

because crabs are present at sites where *Urosalpinx* was collected, and thus snails used in the

experiment have most certainly been exposed to those cues.

3.5.3 Responses to multiple cues and digestion of prey

Many previous studies have separately argued for the importance and universality of either

responses to predator cues (Kats and Dill 1998) *or* responses to alarm signals (Chivers and Smith

1998), but studies rarely test both *and* their combination. Responses to combined or multiple

cues are not always linear (Bourdeau 2010*a*; Grason and Miner 2012), meaning that inference

from partial treatment combinations can lead to erroneous conclusions. Previous research

concluded that because *Littorina sitkana* did not respond defensively to cues from *C. productus*, but did defend when conspecifics were fed to crabs, that the defense was driven by alarm cues from conspecifics (Behrens Yamada 1989). I have shown that this is not the case, as avoidance behavior decreased when *Littorina* was exposed to injured conspecific cues, regardless of whether or not a crab was present, indicating that digestion of conspecifics is also required to reverse the response to injured conspecifics. This reduction of avoidance in response to injured conspecifics is somewhat perplexing, but robust across multiple experiments (E. Grason, unpublished data), and underscores the fact that multiple selective pressures are likely operating on intra- and inter-specific signals.

My prediction that increased information content of combined cues should increase risk perception non-linearly (synergistically) for native prey, but additively for non-native prey, was not supported. To the contrary, all species responded additively to the combined cue treatment (non-significant interaction), including all of the non-native snails that failed to recognize the predator. This is perhaps not surprising if those prey species are truly unable to recognize the predator, in which case only an additive response would be expected. The predominance of additive interactions observed here suggests that additivity might be the null expectation in response to combined information sources, even where both constituent cues also elicit a response.

3.5.4 Conclusions

This study underscores the value of multi-cue, multi-species experiments to informing the theoretical framework on how the influence of risk information can change within and among species, over time, and across environmental contexts (Hoverman et al. 2005). A subset of the

response patterns that I observed related to general information originating from injured conspecifics were distinct between native and non-native prey, suggesting that shared evolutionary history of predator and prey could place a constraint on the use of general information. By contrast, the cues for which responses varied irrespective of status as native or non-native might be those for which selection depends on ecological or evolutionary contexts not explored here. These offer a promising avenue for future research into which factors influence whether responses to the predator, responses to alarm cues, and the importance of digestion are relatively more or less valuable in risk assessments.

I found strong support for the hypothesis that prey can circumvent evolutionary constraints of predator naïveté by using cues thought to be maladaptive in co-evolved interactions (Sih et al. 2010). These results also suggest that this trait might play a role in facilitating biological invasions. The impact of generalized risk assessment, relative to other patterns of information use, on community dynamics remains an open and inviting question. Nevertheless, understanding how prey use information to assess predation risk is critical to precisely characterizing the selective forces operating on predator-prey arms races. Biological invasions offer an excellent opportunity to investigate these questions because selection can be strong in novel interactions and community perturbations are often readily apparent.

WORKS CITED

Abrams, P. A. 2007. Defining and measuring the impact of dynamic traits on interspecific interactions. Ecology 88:2555–2562.

Agarwala, B. K., H. Yasuda, and Y. Kajita. 2003. Effect of Conspecific and Heterospecific Feces on Foraging and Oviposition of Two Predatory Ladybirds: Role of Fecal Cues in Predator Avoidance. Journal of Chemical Ecology 29:357–376.

Alexander, J. E., and A. P. Covich. 1991a. Predator avoidance by the fresh-water snail Physella virgata in response to the crayfish *Procambarus simulans*. Oecologia 87:435–442.

Alexander, J. E., and A. P. Covich. 1991b. Predation risk and avoidance-behavior in 2 fresh-water snails. Biological Bulletin 180:387–393.

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

Atema, J., and D. Stenzler. 1977. Alarm substance of the marine mud snail, *Nassarius obsoletus*: biological characterization and possible evolution. Journal of Chemical Ecology 3:173–187.

Bates, D., M. Maechler, B. Bolker, S. Walker, R. H. B. Christensen, H. Singmann, B. Dai, and G. Grothendieck. 2015. lme4: Linear Mixed-Effects Models using "Eigen" and S4.

Behrens Yamada, S. 1989. Are direct developers more locally adapted than planktonic developers? Marine Biology 103:403–411.

Bender, E. A., T. J. Case, and M. E. Gilpin. 1984. Perturbation Experiments in Community Ecology - Theory and Practice. Ecology 65:1–13.

Bergstrom, D. M., A. Lucieer, K. Kiefer, J. Wasley, L. Belbin, T. K. Pedersen, and S. L. Chown. 2009. Indirect effects of invasive species removal devastate World Heritage Island. Journal of Applied Ecology 46:73–81.

Blum, J. C. 2012. Temperature sensitivity and predation risk cue detection in native and introduced populations of the Atlantic oyster drill, *Urosalpinx cinerea*. Master's Thesis, University of California Davis, Davis, CA.

Blumstein, D. T., J. C. Daniel, A. S. Griffin, and C. S. Evans. 2000. Insular tammar wallabies (*Macropus eugenii*) respond to visual but not acoustic cues from predators. Behavioral Ecology 11:528–535.

Bolker, B. M., M. E. Brooks, C. J. Clark, S. W. Geange, J. R. Poulsen, M. H. H. Stevens, and J.-S. S. White. 2009. Generalized linear mixed models: a practical guide for ecology and evolution. Trends in Ecology & Evolution 24:127–135.

Bourdeau, P. E. 2010a. An inducible morphological defence is a passive by-product of behaviour in a marine snail. Proceedings of the Royal Society B-Biologial Sciences 277:455–462.

Bourdeau, P. E. 2010b. Cue reliability, risk sensitivity and inducible morphological defense in a marine snail. Oecologia 162:987–994.

Bourdeau, P. E., K. L. Pangle, E. M. Reed, and S. D. Peacor. 2013. Finely tuned response of native prey to an invasive predator in a freshwater system. Ecology 94:1449–1455.

Buhle, E. R. 2007. The role of nonlinear feedbacks in the establishment, impact, and control of marine invasive species. Doctoral Dissertation, University of Washington, Seattle, WA.

Buhle, E. R., M. Margolis, and J. L. Ruesink. 2005. Bang for buck: cost-effective control of invasive species with different life histories. Ecological Economics 52:355–366.

Buhle, E. R., and J. L. Ruesink. 2009. Impacts of invasive oyster drills on Olympia oyster (*Ostrea lurida* Carpenter 1864) recovery in Willapa Bay, Washington, United States. Journal of Shellfish Research 28:87–96.

Burnham, K. P., and D. R. Anderson. 2002. Model Selection and Multimodel Inference: A Practical Information-Theoretic Approach. Second. Springer, New York.

Byers, J. E. 1999. The distribution of an introduced mollusc and its role in the long-term demise of a native confamilial species. Biological Invasions 1:339–352.

Byers, J. E. 2002. Physical habitat attribute mediates biotic resistance to non-indigenous species invasion. Oecologia 130:146–156.

Byers, J. E., and L. Goldwasser. 2001. Exposing the mechanism and timing of impact of nonindigenous species on native species. Ecology 82:1330–1343.

Byers, J. E., R. S. Smith, J. M. Pringle, G. F. Clark, P. E. Gribben, C. L. Hewitt, G. J. Inglis, E. L. Johnston, G. M. Ruiz, J. J. Stachowicz, and Bishop, Melanie J. (2015). Invasion Expansion: Time since introduction best predicts global ranges of marine invaders. Scientific Reports 5: Article 12436.

Carthey, A. J. R., and P. B. Banks. 2014. Naivete in novel ecological interactions: lessons from theory and experimental evidence. Biological Reviews 89:932–949.

Chalcraft, D. R., C. A. Binckley, and W. J. Resetarits. 2005. Experimental venue and estimation of interaction strength: Comment. Ecology 86:1061 – 1067.

Chapman, W. M., and A. H. Banner. 1949. Contributions to the life history of the Japanese oyster drill, *Tritonalia japonica* (= *Ceratostoma inornatum*), with notes on other enemies

of the Olympia oyster, *Ostrea lurida*. Pages 169–200. Biology Bulletin, Washington Department of Fisheries.

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

Colautti, R. I., I. A. Grigorovich, and H. J. MacIsaac. 2006. Propagule Pressure: A Null Model for Biological Invasions. Biological Invasions 8:1023–1037.

Connell, J. H. 1961. The Influence of Interspecific Competition and Other Factors on the Distribution of the Barnacle Chthamalus Stellatus. Ecology 42:710–723.

Cooper, W. E. 2009. Rapid covering by shadow as a cue to predation risk in three lizard species. Behaviour 146:1217–1234.

Courchamp, F., M. Langlais, and G. Sugihara. 1999. Cats protecting birds: modelling the mesopredator release effect. Journal of Animal Ecology 68:282–292.

Dalesman, S., S. D. Rundle, and P. A. Cotton. 2007. Predator regime influences innate anti-predator behaviour in the freshwater gastropod *Lymnaea stagnalis*. Freshwater Biology 52:2134–2140.

Dinnel, P. A., R. O. McMillan, D. A. Armstrong, T. C. Wainwright, A. J. Whiley, R. Burge, and R. Bumgarner. 1986. Padilla Bay Dungeness Crab, *Cancer magister*, habitat study. Technical Report, Padilla Bay National Estuarine Research Reserve.

Ferrari, M. C., F. Messier, and D. P. Chivers. 2008. Can prey exhibit threat-sensitive generalization of predator recognition? Extending the Predator Recognition Continuum Hypothesis. Proceedings of the Royal Society B: Biological Sciences 275:1811–1816.

Ferrari, M. C. O., B. D. Wisenden, and D. P. Chivers. 2010. Chemical ecology of predator-prey interactions in aquatic ecosystems: a review and prospectus. Canadian Journal of Zoology 88:698–724.

Fraker, M. E., F. Hu, V. Cuddapah, S. A. McCollum, R. A. Relyea, J. Hempel, and R. J. Denver. 2009. Characterization of an alarm pheromone secreted by amphibian tadpoles that induces behavioral inhibition and suppression of the neuroendocrine stress axis. Hormones and Behavior 55:520–529.

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

Fritts, T. H., and G. H. Rodda. 1998. The role of introduced species in the degradation of island ecosystems: A case history of Guam. Annual Review of Ecology and Systematics 29:113–140.

Grason, E. W., and B. G. Miner. 2012a. Behavioral plasticity in an invaded system: non-native whelks recognize risk from native crabs. Oecologia 169:105–115.

Grason, E. W., and B. G. Miner. 2012b. Preference alters consumptive effects of predators: top-down effects of a native crab on a system of native and introduced prey. PLoSONE 7:e51322.

Griffiths, C. L., and C. A. Richardson. 2006. Chemically induced predator avoidance behaviour in the burrowing bivalve *Macoma balthica*. Journal of Experimental Marine Biology and Ecology 331:91–98.

Hadlock, R. P. 1980. Alarm Response of the Intertidal Snail *Littorina littorea* (l.) to Predation by the Crab *Carcinus maenas* (l.). The Biological Bulletin 159:269–279.

Hall, R. J. 2011. Eating the competition speeds up invasions. Biology Letters 7:307–311.

Havel, J. E. 1987. Predator-induced defenses: a review. Pages 263–278 Predation: Direct and Indirect Impacts on Aquatic Communities. University Press of New England, Hanover, New Hampshire.

Hay, M. E. 2009. Marine chemical ecology: Chemical signals and cues structure marine populations, communities, and ecosystems. Annual Review of Marine Science 1:193–212.

Hazlett, B. A., P. Acquistapace, and F. Gherardi. 2002. Differences in memory capabilities in invasive and native crayfish. Journal of Crustacean Biology 22:439–448.

Hazlett, B.A., and C. McLay. 2000. Contingencies in the behaviour of the crab *Heterozius rotundifrons*. Animal Behaviour 59:965–974.

Helfman, G. S. 1989. Threat-Sensitive Predator Avoidance in Damselfish-Trumpetfish Interactions. Behavioral Ecology and Sociobiology 24:47–58.

Hill, J. M., and M. J. Weissburg. 2014. Crabs interpret the threat of predator body size and biomass via cue concentration and diet. Animal Behaviour 92:117–123.

Holsman, K. K., P. S. McDonald, and D. A. Armstrong. 2006. Intertidal migration and habitat use by subadult Dungeness crab Cancer magister in a NE Pacific estuary. Marine Ecology Progress Series 308:183–195.

Hoverman, J. T., J. R. Auld, and R. A. Relyea. 2005. Putting prey back together again: integrating predator-induced behavior, morphology, and life history. Oecologia 144:481–491.

Hunt, C. E., and S. B. Yamada. 2003. Biotic resistance experienced by an invasive crustacean in a temperate estuary. Biological Invasions 5:33–43.

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

Jacobsen, H. P., and O. B. Stabell. 2004. Antipredator behaviour mediated by chemical cues: the role of conspecific alarm signalling and predator labelling in the avoidance response of a marine gastropod. Oikos 104:43–50.

Jensen, G. C., P. S. McDonald, and D. A. Armstrong. 2007. Biotic resistance to green crab, *Carcinus maenas*, in California bays. Marine Biology 151:2231–2243.

Kats, L. B., and L. M. Dill. 1998. The scent of death: Chemosensory assessment of predation risk by prey animals. Ecoscience 5:361–394.

Kimbro, D. L., E. D. Grosholz, A. J. Baukus, N. J. Nesbitt, N. M. Travis, S. Attoe, and C. Coleman-Hulbert. 2009. Invasive species cause large-scale loss of native California oyster habitat by disrupting trophic cascades. Oecologia 160:563–575.

Kiriazis, J., and C. N. Slobodchikoff. 2006. Perceptual specificity in the alarm calls of Gunnison's prairie dogs. Behavioural Processes 73:29–35.

Kolar, C. S., and D. M. Lodge. 2001. Progress in invasion biology: predicting invaders. Trends in Ecology & Evolution 16:199–204.

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

León, J., E. Rojo, and J. J. Sánchez-Serrano. 2001. Wound signalling in plants. Journal of Experimental Botany 52:1–9.

Levine, J. M., P. B. Adler, and S. G. Yelenik. 2004. A meta-analysis of biotic resistance to exotic plant invasions. Ecology Letters 7:975–989.

Lima, S. L. 1998. Nonlethal effects in the ecology of predator-prey interactions - What are the ecological effects of anti-predator decision-making? Bioscience 48:25–34.

Lockwood, J. L., P. Cassey, and T. Blackburn. 2005. The role of propagule pressure in explaining species invasions. Trends in Ecology & Evolution 20:223–228.

Lohrey, A. K., D. L. Clark, S. D. Gordon, and G. W. Uetz. 2009. Antipredator responses of wolf spiders (Araneae: Lycosidae) to sensory cues representing an avian predator. Animal Behaviour 77:813–821.

Mack, R. N., D. Simberloff, W. M. Lonsdale, H. Evans, M. Clout, and F. A. Bazzaz. 2000. Biotic invasions: Causes, epidemiology, global consequences, and control. Ecological Applications 10:689–710.

Menge, B. A. 1995. Indirect Effects in Marine Rocky Intertidal Interaction Webs: Patterns and Importance. Ecological Monographs 65:21–74.

Miura, O., A. M. Kuris, M. E. Torchin, R. F. Hechinger, and S. Chiba. 2006. Parasites alter host phenotype and may create a new ecological niche for snail hosts. Proceedings of the Royal Society B: Biological Sciences 273:1323–1328.

Moiseff, A., G. S. Pollack, and R. R. Hoy. 1978. Steering responses of flying crickets to sound and ultrasound: Mate attraction and predator avoidance. Proceedings of the National Academy of Sciences 75:4052–4056.

Moyle, P. B., and T. Light. 1996. Fish invasions in California: do abiotic factors determine success? Ecology 77:1666–1670.

ONRC. 2008. Willapa Bay 30 foot (9.14 meter) Intertidal Bathymetry - Localized MLLW. University of Washington Olympic Natural Resources Center GIS. Available at: http://www.onrc.washington.edu/clearinghouse/themes/elev/elev_theme.html.

Padilla Bay National Estuarine Research Reserve. 2014. Invasive Species. Available at: www.padillabay.gov/stewardshipinvasive.asp. Accessed 29 October, 2014.

Payne, C. M., C. V. Tillberg, and A. V. Suarez. 2004. Recognition systems and biological invasions. Annales Zoologici Fennici 41:843–858.

Pearl, C. A., M. J. Adams, G. S. Schuytema, and A. V. Nebeker. 2003. Behavioral responses of anuran larvae to chemical cues of native and introduced predators in the Pacific Northwestern United States. Journal of Herpetology 37:572–576.

Peckarsky, B. L., P. A. Abrams, D. I. Bolnick, L. M. Dill, J. H. Grabowski, B. Luttbeg, J. L. Orrock, S. D. Peacor, E. L. Preisser, O. J. Schmitz, and G. C. Trussell. 2008. Revisiting the classics: Considering nonconsumptive effects in textbook examples of predator-prey interactions. Ecology 89:2416–2425.

Peterson, A. T. 2003. Predicting the Geography of Species' Invasions via Ecological Niche Modeling. The Quarterly Review of Biology 78:419–433.

Polis, G., C. Myers, and R. Holt. 1989. The Ecology and Evolution of Intraguild Predation - Potential Competitors That Eat Each Other. Annual Review of Ecology and Systematics 20:297–330.

Pyšek, P., and V. Jarošik. 2005. Residence time determines the distribution of alien plants. Pages 77–96 Invasive Plants: Ecological and Agricultural Aspects. Birkhauser Verlag, Basel.

R Development Core Team. 2013. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.

Rooper, C. N., D. A. Armstrong, and D. R. Gunderson. 2002. Habitat use by juvenile Dungeness crabs in coastal nursery estuaries. Pages 609–629 Crabs in Cold Water Regions: Biology, Management, and Economics. Alaska Sea Grant College Program, University of Alaska Fairbanks.

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

Schmitz, O. J., J. H. Grabowski, B. L. Peckarsky, E. L. Preisser, G. C. Trussell, and J. R. Vonesh. 2008. From individuals to ecosystem function: Toward an integration of evolutionary and ecosystem ecology. Ecology 89:2436–2445.

Schoeppner, N. M., and R. A. Relyea. 2008. Detecting small environmental differences: risk-response curves for predator-induced behavior and morphology. Oecologia 154:743–754.

Schoeppner, N. M., and R. A. Relyea. 2009a. When should prey respond to consumed heterospecifics? Testing hypotheses of perceived risk. Copeia:190–194.

Schoeppner, N. M., and R. A. Relyea. 2009b. Interpreting the smells of predation: how alarm cues and kairomones induce different prey defenses. Functional Ecology 23:1114–1121.

Sih, A., D. I. Bolnick, B. Luttbeg, J. L. Orrock, S. D. Peacor, L. M. Pintor, E. Preisser, J. S. Rehage, and J. R. Vonesh. 2010. Predator-prey naivete, antipredator behavior, and the ecology of predator invasions. Oikos 119:610–621.

Simberloff, D. 2009. The Role of Propagule Pressure in Biological Invasions. Annual Review of Ecology, Evolution, and Systematics 40:81–102.

Skelly, D. K. 2002. Experimental venue and estimation of interaction strength. Ecology 83:2097–2101.

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

Strauss, S. Y. 1991. Indirect effects in community ecology: Their definition, study and importance. Trends in Ecology & Evolution 6:206–210.

Suwa, T., and S. M. Louda. 2012. Combined effects of plant competition and insect herbivory hinder invasiveness of an introduced thistle. Oecologia 169:467–476.

Templeton, C. N., and E. Greene. 2007. Nuthatches eavesdrop on variations in heterospecific chickadee mobbing alarm calls. Proceedings of the National Academy of Sciences 104:5479–5482.

Thuiller, W., D. M. Richardson, P. Pyšek, G. F. Midgley, G. O. Hughes, and M. Rouget. 2005. Niche-based modelling as a tool for predicting the risk of alien plant invasions at a global scale. Global Change Biology 11:2234–2250.

Torchin, M. E., J. E. Byers, and T. C. Huspeni. 2005. Differential parasitism of native and introduced snails: Replacement of a parasite fauna. Biological Invasions 7:885–894.

Turner, A. M., S. A. Fetterolf, and R. J. Bernot. 1999. Predator identity and consumer behavior: differential effects of fish and crayfish on the habitat use of a freshwater snail. Oecologia 118:242–247.

Turney, S., and J.-G. J. Godin. 2014. To forage or hide? Threat-sensitive foraging behaviour in wild, non-reproductive passerine birds. Current Zoology 60:719–728.

Warkentin, K. M. 2005. How do embryos assess risk? Vibrational cues in predator-induced hatching of red-eyed treefrogs. Animal Behaviour 70:59–71.

Wasson, K., C. J. Zabin, J. Bible, S. Briley, E. Ceballos, A. Chang, B. S. Cheng, A. Deck, E. D. Grosholz, A. Helms, M. Latta, B. Yednock, D. Zacherl, and M. Ferner. 2015. A Guide to Olympia Oyster Restoration and Conservation. Elkhorn Slough National Estuarine Research Reserve.

Wells, E. H. 2013. Evolutionary novelty and naivete in invertebrate predator-prey interactions in a benthic marine community. Doctoral Dissertation, University of California Davis, Davis, CA.

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

White, E. M., J. C. Wilson, and A. R. Clarke. 2006. Biotic indirect effects: a neglected concept in invasion biology. Diversity & Distributions 12:443–455.

White, J., J. L. Ruesink, and A. C. Trimble. 2009. The nearly forgotten oyster: Ostrea lurida Carpenter 1864 (Olympia Oyster) history and management in Washington State. Journal of Shellfish Research 28:43–49.

Whittaker, R. H. 1967. Gradient analysis of vegetation. Biological Reviews 49:207–264.

Wilson, J. R. U., D. M. Richardson, M. Rouget, S. Proches, M. A. Amis, L. Henderson, and W. Thuiller. 2007. Residence time and potential range: crucial considerations in modelling plant invasions. Diversity and Distributions 13:11–22.

Wonham, M. J., and J. T. Carlton. 2005. Trends in marine biological invasions at local and regional scales: the Northeast Pacific Ocean as a model system. Biological Invasions 7:369–392.

Wonham, M. J., M. O'Connor, and C. D. G. Harley. 2005. Positive effects of a dominant invader on introduced and native mudflat species. Marine Ecology Progress Series 289:109–116.

Wootton, J. T. 1994a. The nature and consequences of indirect effects in ecological communities. Annual Review of Ecology and Systematics 25:443–466.

Wootton, J. T. 1994b. Putting the pieces together - Testing the independence of interactions among organisms. Ecology 75:1544–1551.

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

Yamada, S. B., S. A. Navarrete, and C. Needham. 1998. Predation induced changes in behavior and growth rate in three populations of the intertidal snail, *Littorina sitkana* (Philippi). Journal of Experimental Marine Biology and Ecology 220:213–226.

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

Yamada, S. B., and C. S. Sankurathri. 1977. Direct development in the intertidal gastropod Batillaria zonalis (Bruguiere, 1792). Veliger 20:179.

Zenni, R. D., and M. A. Nuñez. 2013. The elephant in the room: the role of failed invasions in understanding invasion biology. Oikos 122:801–815.