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ORIGINAL PAPER

Cue specificity of predator-induced phenotype in a marine snail: is a crab just a crab?

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Abstract

A wide range of taxa have been shown to display inducible, phenotypically plastic responses to known predators. Most studies of inducible defenses include only known predators but not non-predatory species in experimental designs, precluding tests of specificity for these responses. We tested the specificity of predator-induced defenses in the marine snail *Nucella lamellosa,* when exposed to chemical cues from potential crab predators as well as more distantly related non-predatory crabs that co-occur with this snail. Surprisingly, all crabs tested, even those that are not predators, triggered the common induced response of a reduction of soft-tissue mass relative to control animals, likely reflecting a reduction in snail feeding activity. In contrast, only *N. lamellosa's* major predator, *Cancer productus,* triggered the production of a thicker apertural lip. Increased thickening of the apertural lip may be an adaptive response specific to *C. productus*, which uses shell-breaking at the apertural lip (i.e., shell-peeling) as their main form of attack. Apertural lip thickening appeared to be due to reallocation of shell material (i.e., a change in shell shape) rather than an increase in shell deposition. Our findings demonstrate the importance of determining the specificity of cues triggering inducible responses in prey, and the mechanisms that underlie these plastic responses, as the responses to general versus specific cues may limit the adaptive value of an inducible defense.

Introduction

Predator-induced changes in prey behavior, morphology, and life history can have important ecological consequences at the level of species interactions, population and community dynamics, and ecosystem function (Miner et al. 2005). Therefore, it is important to understand the environmental conditions that favor inducible defenses. Theory predicts that these conditions include: spatial and/or temporal variation in predation risk, costs associated with the expression of the defense, reliable cues that indicate the presence of predators or risk of predation (reviewed in Tollrian and Harvell 1999), and the ability of prey to respond with an effective defense within a short time frame relative to environmental change (Padilla and Adolph 1996; Gabriel et al. 2005). Although each of these conditions has been examined to some extent, we generally know very little about the specificity of cues that indicate predation risk and trigger inducible defenses.

In aquatic systems, water-borne chemical cues released by predators often induce defensive responses in prey (Dodson et al. 1994; Chivers and Smith 1998; Bronmark and Hansson 2000). If highly correlated with predation risk, these cues can provide reliable information about the level of threat in the environment. Previous studies of inducible defenses have examined the role of predator cue concentrations (e.g., Harvell 1998; Hawkins et al. 2007), the role of cues associated with damaged and consumed conspecific prey (e.g., Trussell and Nicklin 2002; Schoeppner and Relyea 2005; Laforsch et al. 2006; Bourdeau 2010a), and cues from multiple predatory species (e.g., Relyea 2003; Teplitsky et al. 2004; Bourdeau 2009). However, few studies have considered whether prey may respond to cues from other, non-predatory species (but see Iyengar and Harvell 2002; Langerhans and DeWitt 2002

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for two notable exceptions). Yet, in nature, prey are exposed to a variety of water-borne cues from both predatory and non-predatory species alike. Thus, understanding the specificity of inducible responses to these different cues will be critical to our understanding of both the evolution of plastic traits and their ecological consequences.

In marine systems, a large number of shelled molluscs (gastropods and bivalves) produce thicker shells in response to water-borne chemical cues from predatory crabs, which has been suggested to be a predator-induced defense specific to crabs that attack these molluscs by crushing and/or peeling their shell (Appleton and Palmer 1988; Palmer 1990; Trussell 1996; Leonard et al. 1999; Caro and Castilla 2004; Dalziel and Boulding 2005; Bourdeau 2009). Previous studies of these inducible shell defenses in molluscs have only examined responses to chemical cues from large predatory species of crabs (Appleton and Palmer 1988; Trussell 1996; Leonard et al. 1999; Caro and Castilla 2004; Dalziel and Boulding 2005; Freeman and Byers 2006; Edgell and Neufeld 2008; Bourdeau 2009), but molluscs often co-exist with, and are likely exposed to, a variety of predatory and non-predatory crab species (Vermeij 1987). Furthermore, many predatory crabs select relatively small molluscan prey (Juanes 1992). Some molluscs survive by growing rapidly until they reach a size refuge at which predator-induced mortality declines significantly (e.g., Paine 1976; Whetstone and Eversole 1981). Thus, other inducible responses, such as rapid growth, might be effective defenses. In addition, smaller decapod crustaceans (e.g., hermit crabs and shore crabs) that attack molluscs via shell entry or 'winkling' (Rochette et al. 2007; Edgell and Rochette 2009) may cause substantial mortality to early juvenile stages (Gosselin and Chia 1995; Gosselin and Rehak 2007), and thus, select for inducible responses like shell elongation and increased retraction depth (Bourdeau 2009; Edgell et al. 2009; Miner et al. 2013).

At present, it is not known whether there are specific cues associated with predatory crab species, or generalized cues associated with all crabs that trigger inducible defenses in molluscs (but see Hooks and Padilla 2014). Here, we tested whether inducible shell defenses and associated changes in soft-tissue growth in *Nucella lamellosa,* one of the most well-studied marine species displaying inducible defenses (Padilla and Sevedo 2013; Bourdeau et al. 2015), are specific to its most common predator, the rock crab *Cancer productus*, or if other co-occurring predatory and non-predatory crabs can induce similar or different responses.

Methods

Study organisms and collection sites

Nucella lamellosa is an intertidal zone snail that produces characteristic inducible defenses, a heavy shell with a thickened apertural lip and apertural teeth, in the presence of water-borne chemical cues from its major crab predator, the red rock crab, *C. productus* (Appleton and Palmer 1988; Edgell and Neufeld 2008; Bourdeau 2009). In addition to co-occurring with *C. productus, N. lamellosa* lives sympatrically with a suite of predatory and non-predatory crabs throughout much its range in the eastern north Pacific, from Alaska to California (Kozloff 1987; Jensen 1995; Collins et al. 1996).

We used five of these sympatric crab species in our study, all of which are common on protected rocky shores in the eastern north Pacific, but which differ in their diets and their ability to eat hard-shelled prey (i.e., durophagy; Kozloff 1987; Jensen 1995). *C. productus* (hereafter *Cancer*), and the pygmy rock crab, *Glebocarcinus oregonensis* (formerly *Cancer oregonensis*), are primarily durophagous and possess relatively large, strong chelae capable of producing powerful crushing forces (Yamada and Boulding 1998; Taylor et al. 2000). Whereas *Cancer* is large, highly mobile, and actively accesses the intertidal zone at high tide and consumes a large number of prey per unit body weight, *Glebocarcinus* is relatively sedentary, is found mostly in shelters in the low intertidal and subtidal zones, and has a small effect on intertidal zone gastropod prey compared to *Cancer* (Robles et al. 1989; Yamada and Boulding 1996, 1998). The purple shore crab, *Hemigrapsus nudus*, is a generalist omnivore; it has smaller, weaker claws than *Glebocarcinus* or *Cancer,* and consumes only the smallest snail species (Yamada and Boulding 1998). We also included two anomuran crabs, neither of which regularly consume hard-shelled prey. The grainyhand hermit crab, *Pagurus granosimanus*, is mainly a scavenger and detritivore; although it can feed opportunistically on *Nucella* hatchlings, it does so only rarely (Gosselin 1997). The porcelain crab, *Petrolisthes eriomerus*, is primarily a suspension feeder and detritivore, and does not use its chelae to feed (Jensen 1995).

We collected 180 juvenile (< 25 mm) *N. lamellosa* from the Westside Preserve, a current-swept shore on the west side of San Juan Island, WA, USA (48°30′26.76″N, 123°8′35.20″W). All crab species used in this study can be found at this site, but are rare (Bourdeau, personal observation). This ensured that experimental snails had little prior field exposure to cues from any of the test crab species. *Cancer* was trap-collected from the pier at the University of Washington's Friday Harbor Laboratories (FHL). All other crabs used in the experiment were collected by hand approximately 16 km from FHL in areas on the south and west sides of San Juan Island, which are exposed to wave action during winter storms (Dayton 1971; Menge 1972).

Experimental design

All experiments were conducted at FHL. Snails were exposed to six treatments: a ‘no cue’ control (no crab), and water-borne cues from each of the five different crab species: *Cancer, Glebocarcinus*, *Hemigrapsus*, *Pagurus,* and *Petrolisthes*. Because of the relatively large number of treatments (six), two replicate aquaria ($30.5W \times 19.1D \times 20.3H$ cm) were used for each treatment. Although more replicate aquaria per treatment would have given us more statistical power, a previous study (Appleton and Palmer 1988) used only two replicates per treatment. They found that *N. lamellosa* reared in the presence of chemical cues from *C. productus* fed fish grew only 78% as much as snails in the absence of these cues (Appleton and Palmer 1988). Furthermore, subsequent studies (Bourdeau 2010a; Bourdeau 2012) have documented that *N. lamellosa* grow roughly 67% (between 45 and 90%) as much as snails in the absence of these cues, indicating an approximate effect size that should be statistically detectable with only two replicates. Fifteen snails, individually numbered with bee tags, were randomly allocated to each replicate aquarium. Snails had the same somatic mass (ANOVA, $F_{5,6} = 1.19$, $P = 0.41$) and shell mass (ANOVA, $F_{5,6} = 1.90$, $P = 0.23$) across treatments at the beginning of the experiment. Because predator biomass has been shown to determine the magnitude of inducible prey responses to predatory crabs (Hill and Weissburg 2013), and because the crab species which we used differed greatly in size, we used different numbers of crabs of each species in an effort to keep the biomass of the crabs used similar among treatments [1 *Cancer* (mean carapace width = 117.33 mm), 3 *Glebocarcinus* (maximum carapace width = 53 mm), 4 *Hemigrapsus* (max. width = 34 mm), 6 *Pagurus* (max. width = 19 mm), and 5 *Petrolisthes* (max. width = 19 mm)]. Crabs were placed in a plastic chamber (1.9 L) fastened to the underside of the lid of each experimental aquarium. Gravity-fed seawater flowed from a header tank into each chamber through a feeding hatch in the lid. Overflow from the chamber provided each replicate aquarium with seawater. This design allowed constant flow-through of crab chemical cues while preventing physical contact between crabs and snails. Snails were fed ad libitum their preferred barnacle prey, *Balanus glandula*, encrusted on small stones. Barnacle-depleted stones were replaced with new barnacle-covered stones as needed, such that the snails were never food limited. Crabs were fed frozen fish (Pacific Dover sole, *Microstomus pacificus*), so that experimental snails were not exposed to the scent of injured snails, which, in conjunction with cues from crabs, can enhance induced shell defenses (Bourdeau 2010a). This was necessary to allow us to isolate the specific effect of each crab species from the effects of a general alarm cue (i.e., crushed or consumed snails) on the inducible responses of the experimental snails. Thawed frozen fish does not induce shell defenses in *N. lamellosa* (Bourdeau 2010a).

Soft-tissue growth and shell morphology

Snails were measured and weighed prior to the beginning and at the end of the experiment, which lasted 60 days. A nondestructive method was used to separate shell mass from soft-tissue mass whereby snails are weighed in air and then weighed submerged in water (Palmer 1982). Shell mass was calculated as $1.572 \times (\text{submerged weight}) + 0.0162$, a species-specific regression equation derived from *N. lamellosa* populations collected near FHL (Palmer 1982). Soft-tissue mass was calculated by subtracting shell mass from the total damp weight in air. Shell length, width, and apertural lip thickness were measured to the nearest 0.01 mm with digital calipers (Fig. 1). Apertural lip thickness was measured at the mid-point of the apertural lip and at the lip suture, and these two values were averaged. A total of three snails died during the course of the experiment.

Statistical analysis

All data were additively coded (+ 1) and then $\log_{10}$-transformed to better meet the assumptions of normality and homoscedasticity for parametric tests (Sokal and Rohlf 1995).

To examine treatment effects on final snail size, we analyzed final shell length with a nested analysis of variance (ANOVA) with cue treatment as a fixed factor and aquarium as a random factor nested within treatment (as snails within a aquarium are not independent). We tested for treatment effects on somatic growth by analyzing final soft-tissue mass with a nested analysis of covariance (ANCOVA) with cue treatment as a fixed factor, aquarium as a random factor nested within treatment, and initial body mass as a covariate to control for initial snail size.

To test for treatment effects on shell thickening, we used a nested ANCOVA on apertural lip thickness with treatment as a fixed factor, aquarium as a random factor nested within treatment, and final shell length as a covariate. To further examine cue treatment effects on shell thickening, we analyzed final shell mass with a nested ANCOVA with cue treatment as a fixed factor and aquarium as a random factor nested within cue treatment, and final shell length as a covariate to account for differences in snail size. For all ANCOVA models, treatment × covariate interactions with $P > 0.10$ were removed from the models (Hendrix et al. 1982).

We made post hoc comparisons of covariate-adjusted means with Fisher’s protected least significant difference test (PLSD). Analyses of variance and post hoc comparison of means were conducted with Statistica (v 6.1) and R 2.14.1

(R Development Core Team 2011), and the Wilcox procedure was performed with the program WILCOX (Quinn and Keough 2002).

Results

Cue treatment had no significant effect on final shell length (ANOVA, $F_{5,6} = 1.18$, $P = 0.42$; Table 1). There was no significant effect of the treatment × initial body mass interaction on final soft-tissue mass (ANCOVA, treatment × initial body mass: $F_{5,160} = 1.71$, $P = 0.14$), so this interaction term was dropped from the model. Treatment did have a significant effect on final soft-tissue mass (Table 2). Post hoc analyses of initial size-adjusted mean soft-tissue mass revealed significant differences between the control and each crab cue treatment, but not among crab cue treatments (Fig. 2).

Apertural lip thickness did not meet the assumption of equal variances (Levene's, $P = 0.027$), even after transformation; but we report the results of the ANCOVA, because it is generally robust to violations of the assumption as long as group sizes are equal and variances are not dramatically different from each other (Sokal and Rohlf 1995). For apertural lip thickness, we found a significant interaction between treatment and the covariate, shell length (Table 3). As a result, we were not able to compare length-adjusted mean shell thickness, because the scaling between it and shell length was not the same across our experimental treatments. We therefore used the Wilcoxon modification of the Johnson–Neyman procedure to determine the range of covariates over which response variables were significantly different among treatments (Huitema 1980). However, only the regression line for *Cancer* differed in slope from the control ($P < 0.05$; Table 3; Fig. 3). Snails exposed to *Cancer* had significantly thicker apertural lips than snails in the no crab treatment when the covariate, final shell length, varied between 27.49 and 30.94 mm (Wilcoxon Johnson–Neyman test; Fig. 3).

Table 1 Final shell lengths (mm) of *N. lamellosa* raised under six treatment conditions: no cue control (no crab), grainyhand hermit crab (*Pagurus*), porcelain crab (*Petrolisthes*), purple shore crab (*Hemigrapsus*), pygmy rock crab (*Glebocarcinus*), and red rock crab (*Cancer*)

Treatment	Mean final shell length (mm)	SD
No crab	32.16	2.94
Petrolisthes	28.92	3.66
Pagurus	27.28	4.62
Hemigrapsus	30.11	4.81
Glebocarcinus	27.55	4.07
Cancer	30.43	3.87

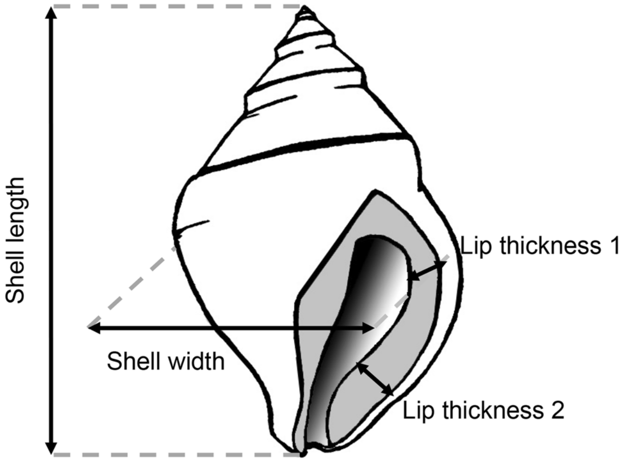

Fig. 1 Linear measurements of the shell of *N. lamellosa*: shell length, maximum shell width perpendicular to shell length, and apertural lip thickness measurements 1 and 2

ANCOVA revealed that the slopes of regressions for final shell mass as a function of shell length were equal for all the treatments (ANCOVA, treatment × final shell length: $F_{5,160} = 1.17$, $P = 0.32$). Hence, the treatment × final shell length interaction term was dropped from the model and we were able to compare size-adjusted mean shell mass, because the scaling between it and shell length was the same across our experimental treatments. There was no significant effect of cue treatment on the final shell mass (Table 4), although responses were highly variable within most treatments (Fig. 4; Table 4).

Discussion

Some aspects of the phenotypic response of *N. lamellosa* were highly specific, restricted to chemical cues from the snail's most dangerous predator, while other aspects of the snail's phenotype changed in response to chemical cues from all of the crabs, independent of the risk which they

Table 2 Results of ANCOVA for log(final soft-tissue mass + 1) of *N. lamellosa* among treatments and aquaria nested within treatments with log(initial soft-tissue mass + 1) as a covariate

Effect	*df*	MS	*F*	*P*
Covariate	1	0.577	141.16	< 0.001
Treatment	5	0.022	4.45	0.048
Aquarium (treatment)	6	0.005	1.23	0.294
Error	165	0.004		

Non-significant covariate-by-treatment interaction terms ($P > 0.10$) were removed from the final model. Initial and final soft-tissue mass originally measured in grams

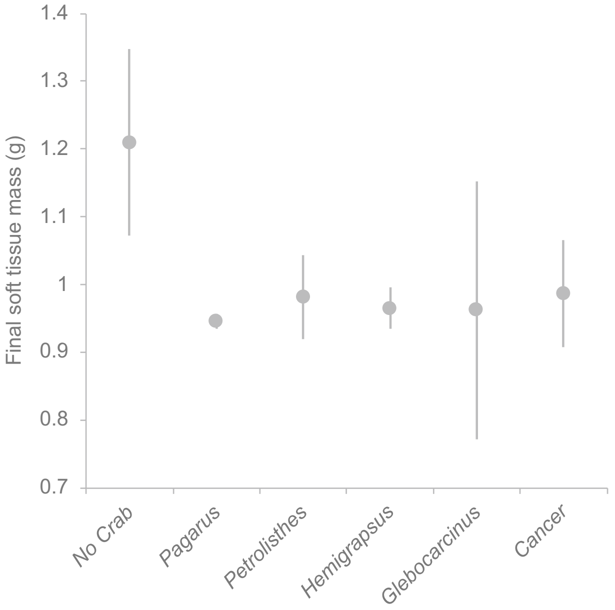

Fig. 2 Final soft-tissue mass (g) of *N. lamellosa* raised under six treatment conditions: no cue control (no crab), grainyhand hermit crab (*Pagurus*), porcelain crab (*Petrolisthes*), purple shore crab (*Hemigrapsus*), pygmy rock crab (*Glebocarcinus*), and red rock crab (*Cancer*). Values are back-transformed least-squares means and 95% confidence intervals of $\log_{10}+1$ transformed data computed for the covariate initial body mass (g) at its mean. All treatment groups were significantly different from the control (PLSD, $P<0.05$), but not from each other (PLSD, $P>0.05$)

posed. A common response of snails exposed to increased risk of predation is a significant reduction in soft-tissue mass, and we observed this across all crab treatments, independent of the predation risk posed by the crab species. Reductions in somatic growth are usually attributed to greater refuge use and reduced feeding under predation risk (Bourdeau 2010b; Bourdeau and Johansson 2012), which would reduce the exposure of snails to potential predators. That all crabs, even those that are omnivores or scavengers, seemed to induce a response in *N. lamellosa* was surprising. This result may indicate that there is some chemical cue associated with all of these sympatric crabs that indicates a habitat with higher risk of predation.

Although size-corrected final shell mass was the same across all experimental treatments, thicker apertural lips in snails exposed to *Cancer* indicate a different geometric allocation of shell mass in those snails. For snails in the control treatment (absence of any crab cues) and in the presence of lower risk or non-predatory crab species, increases in shell mass during the course of the experiment were not concentrated at the apertural lip, whereas snails exposed to the high-risk predator, *Cancer,* allocated shell material preferentially at the apertural lip.

Because theory predicts that inducible defenses are costly (either due to energetic, developmental, or opportunity costs), they should only be deployed in a risk-sensitive manner (i.e., when the risk of predation is high). Therefore, snails were expected to show a strong, specific induced defensive response to predatory crabs, especially in response to *Cancer*, their most important predator, and a weak response or no response to non-predatory species. Although *N. lamellosa* grew less in response to all crab species tested, even those that pose no risk of predation, it only thickened its apertural lip in response to chemical cues from *Cancer,* the species posing the greatest threat of predation. The lip thickening response to *Cancer* is consistent with theoretical expectations, as it would reduce vulnerability to crabs (like *Cancer*) that peel shells from the aperture; however, such shells would still be vulnerable to crushing attacks (Vermeij 1978), a tactic that is also employed by *Cancer* (Zipser and Vermeij 1978). The shell mass of snails exposed to chemical cues from different crabs was not different, and was not different than that of control snails. This result suggests that snails that allocated more shell to the apertural lip in the presence of *Cancer*, sacrificed an overall reinforcement of the shell.

Table 3 Results of regression analyses and ANCOVA for log(apertural lip thickness + 1) for *N. lamellosa* among treatments and aquaria nested within treatments with log(final shell length + 1) as a covariate

Regression		R^2	Effect	ANCOVA			
Treatment	Equation			*df*	MS	*F*	*P*
Log(apertural lip thickness + 1) (y) vs. log(final shell length + 1) (x)							
No crab	$y=0.71x-0.79$	0.42	Covariate	1	0.172	94.85	<0.001
Petrolisthes	$y=0.42x-0.35$	0.22	Treatment	5	0.023	4.99	0.025
Pagurus	$y=0.35x-0.27$	0.36	Aquarium (treatment)	6	0.005	2.49	0.025
Hemigrapsus	$y=0.80x-0.89$	0.64	Treatment × covariate	5	0.006	3.11	0.011
Glebocarcinus	$y=0.43x-0.38$	0.32	Error	160	0.002		
Cancer	$y=0.22x-0.00$	0.11					

Apertural lip thickness and shell length originally measured in millimeters

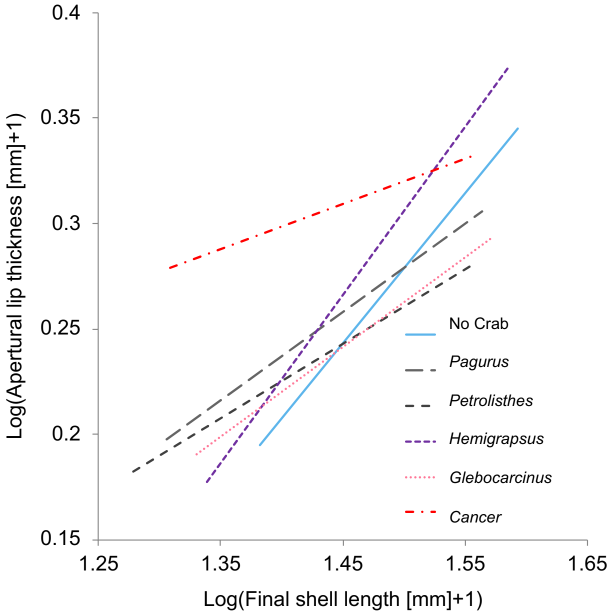

Fig. 3 Relationship between log(apertural lip thickness + 1) and log(final shell length (mm) + 1) of *N. lamellosa* raised under six treatment conditions: no cue control (no crab), grainyhand hermit crab (*Pagurus*), porcelain crab (*Petrolisthes*), purple shore crab (*Hemigrapsus*), pygmy rock crab (*Glebocarcinus*), and red rock crab (*Cancer*). To facilitate visualization, best-fit lines from simple linear regression are shown without associated data points. Best-fit lines are restricted to the range of observed shell lengths

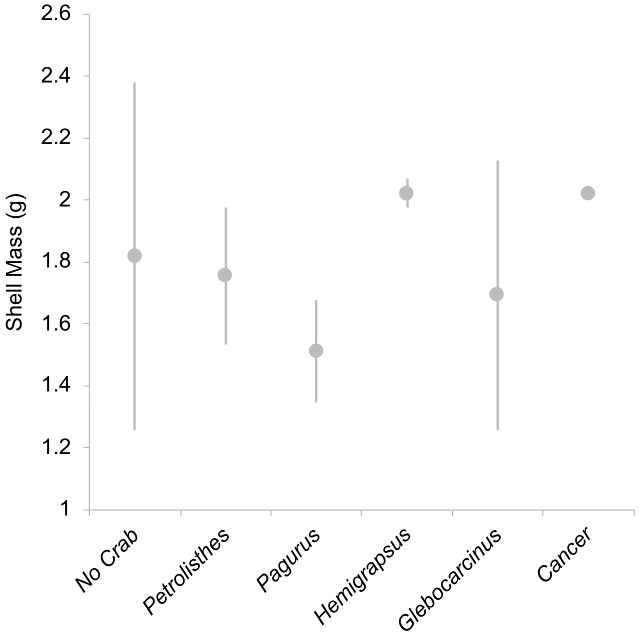

Fig. 4 Final shell mass (g) of *N. lamellosa* raised under six treatment conditions: no cue control (no crab), grainyhand hermit crab (*Pagurus*), porcelain crab (*Petrolisthes*), purple shore crab (*Hemigrapsus*), pygmy rock crab (*Glebocarcinus*), and red rock crab (*Cancer*). Values are back-transformed least-squares means and 95% confidence intervals of $\log_{10} + 1$ transformed data computed for the covariate shell length (mm) at its mean. None of the treatments were significantly different from one another (PLSD $P > 0.05$)

Table 4 Results of ANCOVA for log(final shell mass + 1) of *N. lamellosa* among treatments and aquaria nested within treatments with log(final shell length + 1) as a covariate

Effect	*df*	MS	*F*	*P*
Covariate	1	2.183	1617.49	< 0.001
Treatment	5	0.002	1.33	0.364
Aquarium (treatment)	6	0.001	0.505	0.804
Error	165	0.002		

Non-significant covariate × treatment interaction terms ($P > 0.10$) were removed from the final model. Final shell mass originally measured in grams and final shell length originally measured in millimeters

Because an overall thickening of the shell may developmentally constrain soft-tissue growth (Palmer 1981), we suggest that allocating shell material to the apertural lip may be a way for *N. lamellosa* to mount an ‘intermediate’ defense while minimizing soft-tissue growth costs associated with overall shell-thickening (e.g., Bourdeau 2010b). Our finding that apertural lip thickening in response to *Cancer* did not incur any reduction in soft-tissue growth beyond that observed in snails exposed to less threatening, or non-predatory crabs supports this hypothesis. Furthermore, although not statistically significant, there was a trend for snails exposed to *Cancer* to develop shells with relatively lower aspect ratio than snails exposed to chemical cues from other crabs (Online Appendix A); that is, they were shorter and more rotund. Such relatively rotund shells have been hypothesized to spread crushing forces more evenly over a given amount of shell material, increasing crushing resistance without investing in additional shell material (DeWitt et al. 2000). Thus, re-allocating shell material to change the overall shape of the shell to one that is more resistant to crab attacks without adding more shell material and thus limiting somatic growth (i.e., low aspect ratio with thicker apertural lip) may itself be an adaptive, low-cost strategy against crabs like *Cancer* that attack via both shell-peeling and shell-crushing.

Observational and experimental evidences also support a cost-minimization strategy for apertural lip thickening in *Nucella* spp. For example, *N. lapillus* from habitats where predation risk from crabs is high, thicken just one microstructural layer of their shell (the homogeneous layer)—a relatively weak but energetically inexpensive shell material with low organic content (Avery and Etter 2006)—and *Cancer*-induced lip thickening in *N. lamellosa* appears to show a similar pattern (Bourdeau 2010b). Enhancing the weaker shell microstructural layer may be energetically cheaper, reducing the cost to soft-tissue growth and allowing snails to grow rapidly to a size refuge, which may ultimately be more important for reducing the risk of predation than maximizing shell strength. Further experimentation is needed to assess the relative importance of lip thickening, shell shape (i.e., aspect ratio), and shell size for protection from crab predation for *Nucella*.

Two different mechanisms could lead to increased lip thickness in *N. lamellosa* exposed to *Cancer*: (1) increased deposition rate of shell material at the apertural lip with constant linear shell translation (i.e., increase of shell length along the axis of coiling; Brookes and Rochette 2007), or (2) reduced linear translation of the shell in conjunction with constant deposition of shell material (Bourdeau 2010b). Our study was not designed to distinguish between these two mechanisms, but our results are partially consistent with the 'reduced linear translation and constant shell deposition' model. For example, shell length (linear shell growth) did not differ between crab-exposed and control snails and, for a given shell length, the amount of shell material deposited (shell mass) also did not differ. Thus, snails exposed to *Cancer* produced a thicker apertural lip without increasing shell deposition relative to the control or other crab cue treatments. It should be noted, however, that there was also no reduction in linear shell translation in snails exposed to *Cancer* relative to the other treatments. Thus, in this study, *N. lamellosa* appeared to thicken the shell by modifying the geometric allocation of shell material (i.e., changing its shell shape), rather than depositing more shell material (e.g., Appleton and Palmer 1988; Palmer 1990) or simply passively accreting shell at the apertural lip via reduced linear shell growth (Bourdeau 2010b).

Surprisingly, *N. lamellosa* exposed to lower risk and non-predatory crabs exhibited reduced growth similar to those snails exposed to *Cancer*. It is likely that this result is closely linked to snail feeding rates. Although we did not quantitatively document snail feeding behavior (e.g., barnacle drilling), mid-experiment visual scans of experimental snails indicated a marginally significant trend ($F_{5,6}=4.28$, $P=0.053$) for snails in the presence of crab (predatory or otherwise) cues to be on the underside of stones in experimental aquaria, whereas snails in the control treatment were not. Many studies have documented similar increases in refuge use and reduced feeding activity in gastropods exposed to the presence of predatory crab cues (Palmer 1990; Richardson and Brown 1992; Trussell et al. 2003, 2006; Bourdeau 2009, 2013; but see Hooks and Padilla (2014) where some snails exposed to a nonnative predatory crab did not show this response). The use of refugia (e.g., hiding) reduces the risk of encountering predators, but often comes at the cost of reduced foraging and, therefore, reduced somatic growth.

For increased refuge use and reduced feeding to be adaptive for snails when exposed to cues of crabs that do not pose a risk, costs must be balanced by or outweighed by the benefits of the response (Levins 1968; Lively 1986). Snails experience a growth cost with reduced feeding, increasing the time to attain a size refuge from many predators (e.g., Harding 2003) and a smaller overall body size with reduced fecundity (e.g., Harding et al. 2007). Given these opportunity costs, responding to cues from low-risk or non-predatory crabs when predatory crabs are absent would seem maladaptive (Langerhans and DeWitt 2002). Two possible alternative hypotheses could explain these results.

First, the overall abundance of crabs (including both predatory and non-predatory species) on rocky shores may be highly correlated with the abundance of *Cancer.* Both predatory and non-predatory crabs tend to be more abundant on wave-protected than wave-exposed shores, and thus, chemical signals from any crab may indicate a risky environment, stimulating a defensive response. There is precedent for such 'indirect' cues in freshwater systems, where the water flea *Daphnia* undergoes a diel vertical migration as a predator avoidance response to chemical cues released by both planktivorous and piscivorous fish, even though piscivores pose no risk for *Daphnia.* However, because piscivores co-exist with planktivores, their presence indirectly indicates the presence of planktivores (von Elert and Pohnert 2000). Extensive field data will be needed to test the hypothesis that the presence of non-predatory crabs is positively correlated with the presence of predatory crabs across *N. lamellosa's* range.

Alternatively, even low-risk crabs may pose enough risk that reduced feeding in their presence is advantageous for *N. lamellosa*. For example, thicker-lipped shells may not protect snails from attacks by *Pagurus*, *Hemigrapsus*, and *Glebocarcinus*, which are likely to use shell-entry attacks (where crabs insert their claw through the shell aperture and pull out the soft parts of the snails) on snails. Indeed, snails exposed to *Hemigrapsus*, and *Glebocarcinus* showed a trend of developing higher aspect ratio (i.e., longer, narrower) shells relative to control snails and snails exposed to *Cancer* cues (Online Appendix A). Predator-induced increases in shell aspect ratio have been observed in response to other shell-entering predators, and provide more room for snails to withdraw into, better protecting them against shell-entry attacks (e.g., DeWitt et al. 2000; Bourdeau 2009). However, entry-resistant shells can be more susceptible to crushing attacks, indicating an inherent survival trade-off between entry-resistant and crush-resistant shells (e.g., Bourdeau 2009). Thus, a shell elongation response could represent an adaptive response specific to shell-entry attacking crabs that is balanced by a survival trade-off with the shell-thickening response induced by shell-breaking crabs.

Given that snail behavior can track temporal changes in predation risk more rapidly than changes in shell morphology, snails that respond over-cautiously or even inappropriately to a general crab cue could quickly reverse their behavior and compensate for periods of inactivity with increased feeding and growth during periods when cues associated with predation risk are absent (Arendt 1997; Stachowicz and Hay 1999). Thus, while opportunity costs of responding to general cues from crabs might exist over short time scales, long-term costs may be relatively minor and outweighed by the survival benefits of responding cautiously in risky habitats.

Finally, it is also possible that relatives of the non-predatory crab species are predators on juvenile *N. lamellosa.* Snails may then respond to non-predatory species due to cue similarity with closely related predatory species (Sih et al. 2010). For example, although *P. granosimanus* rarely feeds on *Nucella* hatchlings (Gosselin 1997), its congener *P. hirsutiusculus* may be a significant source of mortality for the early juvenile *Nucella* (Gosselin and Chia 1995). If the chemical signature of *P. granosimanus* and *P. hirsutiusculus* are similar enough, *N. lamellosa* may respond to the non-predatory congener as if it was a predatory threat.

Our results indicate that *N. lamellosa* can distinguish between chemical cues released by high-risk predators and low-risk and non-predatory species and respond phenotypically in a risk-sensitive manner. Fully factorial experiments that cross gradients of risk and resources will be necessary to fully determine the precise nature and magnitude of integration among snail feeding activity, soft tissue and shell growth, and changes in shell mass and shell shape in *N. lamellosa* and other marine gastropods capable of modifying their phenotype in response to risk cues from crabs. More information is needed about indirect environmental cues that may signal risky environments to fully understand the role of chemical signaling on inducible responses that we presently interpret as adaptive.

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Author contributions PEB conceived, designed, and performed the experiment, and analyzed the data. PEB and DKP wrote and edited the manuscript.

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Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

Ethical approval All applicable international, national, and institutional guidelines for sampling, care, and experimental use of organisms for the study were followed. Research was completed under permits from the Washington Department of Fish and Wildlife (WDFW).

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