

Whelks induce an effective defense against sea stars

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ABSTRACT: The whelk *Nucella lamellosa* displays phenotypic plasticity in the presence of the sea star *Pisaster ochraceus* by becoming more retractable. In this study, we directly tested whether this response is an inducible defense, and looked for evidence of phenotypic costs associated with the induced phenotype. We found that whelks held in the presence of sea stars consuming conspecific whelks became more retractable, while whelks that were not exposed to sea stars became less retractable — indicating that this is a reversible and symmetric (i.e. similar magnitude of change) response. We did not find changes in aspect ratio of the shell or size of the whelks. Following the induction experiment, whelks were fed to sea stars. In this predation experiment, sea stars were much less likely to consume whelks previously exposed to sea stars compared to whelks not exposed to sea stars. There was a strong relationship between mortality and retractability relative to shell length, and individuals that could retract 50% of their shell length had relatively little chance of being consumed during the predation experiment. These results support the hypothesis that increased retractability is an inducible defense. We also conducted field surveys of *N. lamellosa* populations and found differences in retractability among populations, most of which were more retractable than the whelks in our induction experiment. For these field-collected individuals, we found evidence of phenotypic costs, with a negative relationship between retractability and tenacity. Thus, *N. lamellosa* responds to a sea star predator by becoming more retractable but at the cost of becoming less tenacious.

KEY WORDS: Inducible defenses · *Nucella lamellosa* · Predation · Phenotypic plasticity · *Pisaster ochraceus* · Retractability · Sea star · Whelk

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INTRODUCTION

Many organisms are phenotypically plastic and have the ability to express different phenotypes in response to different environmental conditions (Bradshaw 1965, Pigliucci 2001, DeWitt & Scheiner 2004). Inducible defenses are a specific type of plasticity in which individuals produce a defense against a threat, but only when reliable and predictable cues for the

threat are present (Harvell 1990, Padilla & Adolph 1996, Tollrian & Harvell 1998, Gabriel et al. 2005). For example, the ciliate *Euplotes octocarinatus* produces extended lateral wings when exposed to cues from the predatory ciliate *Lembdion lucens*: this 'winged' morph of *E. octocarinatus* is too wide for *L. lucens* to consume (Kuhlmann & Heckmann 1985). In addition to better protecting an individual from a threat, inducible defenses also have evolutionary and

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ecological consequences. For example, inducible defenses can alter communities by modifying trophic cascades, rates of evolution, and rates of speciation (Harvell 1990, Agrawal 2001, Trussell et al. 2002, Werner & Peacor 2003, Miner et al. 2005, Gibson & Reed 2008, Schlichting 2008).

To test whether a plasticity response is an inducible defense, researchers have induced undefended and putatively defended individuals and then challenged these individuals with a threat (e.g. Harvell 1984, Kuhlmann & Heckmann 1985, Lively 1986a, Van Buskirk et al. 1997, Agrawal 1998). The inducible defense hypothesis is supported when putatively defended individuals perform better when faced with the threat. This direct test of the inducible defense hypothesis can allow researchers to make strong inferences about whether plastic responses are indeed inducible defenses, but is uncommon relative to the number of examples in which authors hypothesize a plastic response in an inducible defense. However, investigators must also carefully design their experiments because patterns that support the inducible defense hypothesis can arise from causes inconsistent with this hypothesis (Woods & Harrison 2002). For example, the undefended organism might perform worse when moved to the environment with the threat, not because it is more susceptible to predation but because it is not acclimated to the new environment. In a different scenario, an organism may devote more resources to altering its phenotype compared to an individual maintained in the same environment, hence decreasing its ability to escape predation. Thus, the strongest support for adaptive plasticity comes from the accumulation of studies with different approaches.

While inducible defenses can protect an organism from a threat, they typically come at a cost, such that an undefended phenotype is fitter when the threat is absent (Tollrian & Harvell 1998). This group of costs, termed phenotypic or phenotype-environment mismatch costs, should not be confused with the costs of plasticity (Callahan et al. 2008, Auld et al. 2010), and can occur in a variety of ways (DeWitt et al. 1998). For example, a defense might require energy to produce or maintain, reduce the ability to gain resources or defend against other threats, or it might negatively affect reproduction by reducing fecundity, mating, or caring for offspring. Identifying phenotypic costs can provide evidence that plasticity is adaptive and help explain why plasticity evolved (e.g. Lively 1986b, Pettersson & Brönmark 1997, Van Buskirk & Relyea 1998, Agrawal et al. 1999). The types and magnitudes of phenotypic costs can also affect the evolu-

tion of inducible defenses and influence the ecology of organisms and communities (e.g. Ramos-Jiliberto 2003, Kopp & Gabriel 2006).

Recently, Bourdeau (2009) demonstrated that the whelk *Nucella lamellosa* exhibits a plastic response in the presence of the sea star *Pisaster ochraceus*. Whelks exposed to water-borne, chemical cues from sea stars or injured conspecifics were able to retract farther into their shells and had more elongated shells compared to whelks that were not exposed to cues from sea stars. This response might protect *N. lamellosa* from sea star predation because *P. ochraceus* everts its stomach into the aperture of the snail's shell to feed (Feder 1959, Paine 1969). In the second part of his study, Bourdeau (2009) estimated the level of threat posed by predatory crabs relative to sea stars, and demonstrated that whelks with elongate shells collected from the field were consumed by sea stars less often than field-caught individuals with rotund shells (although retractability was not measured). It is unknown whether these field-caught whelks were more elongate due to exposure to sea star predators or to some other environmental factor. In another study, Markowitz (1980) manipulated the retractability of *Chlorostoma funebris* (previously *Tegula funebris*) by grinding away part of the shell; snails with reduced retractability were more susceptible to predation by *P. ochraceus*. However, whether *C. funebris* increases retractability in the presence of sea stars is unknown. Thus, although the indirect evidence supports the inducible defense hypothesis, there is no direct evidence that the response is a defense.

The phenotypic costs, if any, of the induced response to *Pisaster ochraceus* in the absence of sea stars are largely unknown. Bourdeau (2009) suggested that crabs can more easily consume whelks previously exposed to sea stars. Additionally, costs associated with changes in morphology of the foot seem likely. To withdraw further into the shell, individuals might reduce the size of their foot, which in turn could affect their ability to attach to or move over the substratum. The strength with which individuals can attach to a surface is especially important for intertidal gastropods (Denny 2006), and is affected by the surface area of the foot in contact with the substratum, properties of the mucus, pressure under the foot, density of conspecifics, and wave exposure (Etter 1988a, Smith 1991, 1992, Trussell et al. 1993, Trussell 1997, Holmes et al. 2002, Coleman et al. 2004). The shape of the foot and properties of the mucus are related to the speed at which individuals can move, and individuals with a long and narrow foot and less viscous mucus are typically faster than

individuals with a round foot (Miller 1974, Branch & Marsh 1978, Denny 1984). Thus, because changes in retractability likely affect changes in the foot, they may also affect the strength at which individuals attach or the speed at which they move.

In this study, we directly tested whether the response in *Nucella lamellosa* to *Pisaster ochraceus* is an inducible defense, and looked for evidence of phenotypic costs associated with the induced phenotype. We first demonstrated that individuals exposed to predation cues from sea stars were more retractable compared to individuals that were not exposed to predation cues from sea stars, confirming the results of Bourdeau (2009). We then tested whether individuals recently exposed to cues from sea stars, and therefore more retractable, were better defended compared to individuals that were not recently exposed to cues from sea stars. Lastly, we collected individuals from the field and tested for relationships between the characteristics associated with the foot (e.g. force required to dislodge an individual, tenacity, and foot shape) and retractability to look for evidence of phenotypic costs.

MATERIALS AND METHODS

Laboratory experiments

Induction experiment

During the summer of 2008, we performed a laboratory experiment at the Shannon Point Marine Center, Anacortes, Washington, USA to verify that the sea star *Pisaster ochraceus* induced the whelk *Nucella lamellosa* to retract further into its shell. We manipulated the presence of *P. ochraceus*—the species used by Bourdeau (2009)—with 2 treatments: sea star present (sea star treatment) and sea star absent (control). Both *N. lamellosa* and *P. ochraceus* were collected from Shannon Point Beach, Washington (48.509° N, 122.685° W) and were transported in separate buckets to the laboratory. Ninety-six whelks were haphazardly chosen and placed into 16 plexiglass aquaria (0.3 m wide × 0.6 m tall × 0.6 m deep), 6 in each aquarium, along with several barnacle-covered rocks for food. Seawater from the seawater system continuously flowed through each aquarium. Each aquarium was divided into 2 compartments by plastic grating—one compartment held the experimental whelks and the other either held a *P. ochraceus*, if that aquarium was assigned to the sea star treatment, or lacked a sea star, if assigned to the con-

trol. The plastic grating allowed water to flow between the 2 compartments but restricted the sea stars from physically contacting the whelks. Each aquarium was randomly assigned a treatment (8 replicates for each of the 2 treatments). Once a week, 2 individuals of *N. lamellosa* were also added to the predator compartment of each of the 16 aquaria. Before adding more whelks to the predator compartment, we removed any living whelks and remains of whelks consumed during the week. We added whelks to the predator compartment because cues from the consumption of conspecifics by predators can induce stronger responses in whelks (Appleton & Palmer 1988, Bourdeau 2010a, Grason & Miner 2012), although this makes the source of cue unclear. The experiment ended after 30 d, and 3 whelks died during the induction experiment (2 from the sea star treatment and 1 from the control).

We measured the following metrics of each whelk at the beginning and end of the experiment. Shell length was measured as the longest dimension along the coiling axis. Shell height was measured as the distance 90° to the plane of the aperture between the aperture and the top of the shell. Wet weight was measured with an electronic balance after blotting an individual with a paper towel. Retractability was measured as the distance the operculum retracted into the shell. We gently poked the foot of the whelk with a blunt probe while it was immersed in water until the whelk fully retracted its foot into its shell. A small strip of plastic was then inserted into the aperture of the shell until it touched the operculum, and the distance was measured with digital calipers. We did not measure retractability as the unoccupied volume of the shell when an individual was retracted into its shell (Palmer 1990, Bourdeau 2009) because we were specifically interested in the distance that an individual could withdraw into its shell. Unoccupied volume can increase even though an individual is less retractable because the unoccupied volume is influenced by size of the aperture as well as retractability. We tagged each whelk at the beginning of the experiment by attaching to the shell with cyanoacrylate glue a number printed on waterproof paper so that initial and final measurements could be paired for each individual.

To determine whether the response of whelks to sea stars was similar to that reported by Bourdeau (2009) we used the following analyses. Because whelks from an aquarium are not independent and our design is nested, we calculated the average of each metric for each aquarium and analyzed these averages. Retractability was analyzed with ANCO-

VA, in which treatment was the fixed factor and initial retractability was a covariate. Aspect ratio (the ratio of shell length to shell height) was analyzed with ANCOVA, in which treatment was the fixed factor and initial aspect ratio was the covariate. Because the analysis of ratios is problematic when there are allometric changes between traits, we also analyzed these data with a MANCOVA with final length and height as the dependent variables, treatment as the fixed factor, and final weight as the covariate. We tested whether treatment affected the growth of individuals by analyzing shell length and wet weight with an ANCOVA. Shell length or wet weight at the end of the experiment was the response variable, treatment was the predictor variable, and shell length or wet weight at the beginning of the experiment was the covariate. The statistical program R was used for all analyses (R Development Core Team 2012).

Predation experiment

We used whelks from the induction experiment to test whether whelks previously exposed to sea stars were better protected from sea stars. Whelks from the induction experiment were returned to their original aquaria and a *Pisaster ochraceus* was randomly assigned and added to the whelk compartment of each of the 16 aquaria so that the stars could consume the whelks—we used different sea stars in the induction and predation experiments. To reduce the possibility that behaviors induced by sea stars during the induction experiment affected mortality, we removed the barnacle-covered rocks and allowed the tanks to overflow, which eliminated any air space at the top of the tanks. We recorded the number of whelks that were dead after 5 d. All mortality was attributed to sea stars because all dead whelks were devoid of tissue and bacterial growth, and their opercula were located away from the empty shell.

Whelk mortality was analyzed with a generalized linear model (GLM). In the first analysis, the proportion of whelks consumed by sea stars was the response variable and treatment was the predictor variable. In the second analysis, we tested whether mortality due to sea stars was related to retractability relative to shell length. The proportion of whelks consumed by sea stars was the response variable and retractability/shell length was the predictor variable. We did not observe changes or differences between treatment in shell length during the experiment (see 'Results'), and therefore we avoid the issues that

arise from analyzing ratios. Because the response variable was a proportion in both analyses (calculated from the number of individuals killed out of the initial number of whelks), we used a binomial error structure and a logit link.

Field surveys

Phenotypic costs

We collected individuals from the field to test for phenotypic costs associated with the sea star-induced morphology of *Nucella lamellosa*, as opposed to costs of simply being phenotypically plastic. We used field-collected individuals because all experimentally induced individuals were used for the predation experiment. To look for evidence of phenotypic costs, we quantified the relationship between tenacity (force per unit area of the foot) and retractability, force of attachment and retractability, and area of the foot and retractability.

We collected and measured 30 ind. of *N. lamellosa* during the summer of 2008 from each of 6 sites around the San Juan Archipelago, Washington (Fig. 1). We chose these sites based on data from a field survey completed the previous summer (see next subsection) so that whelks were from a range of thin-shelled and thick-shelled populations. Whelks were collected by hand from the mid-intertidal zone during low tide.

For each whelk, we measured retractability, shell length, and shell height as described for the laboratory experiments above. In addition, we measured the force required to dislodge an individual from the substratum, and the length, width, and surface area of the foot when attached to the substratum. We first measured the force required to dislodge a whelk from a glass surface with a voltmeter attached to a 40 lb (ca. 18.1 kg) force transducer and converted the output to Newtons (N) and kilonewtons (kN). Each whelk was placed in a small glass bowl filled with seawater, and placed on ice to keep seawater temperature similar to that of ambient seawater during the trial. Once a whelk was attached to the bottom of the bowl, a small clamp was attached to the shell and connected to the transducer. To measure normal stress the whelk was then pulled perpendicular to the surface until it detached. The force transducer recorded the maximum force generated during a trial. The force required to remove an individual is affected by the rate and force applied to it. However, as we had no way to standardize this, we randomly

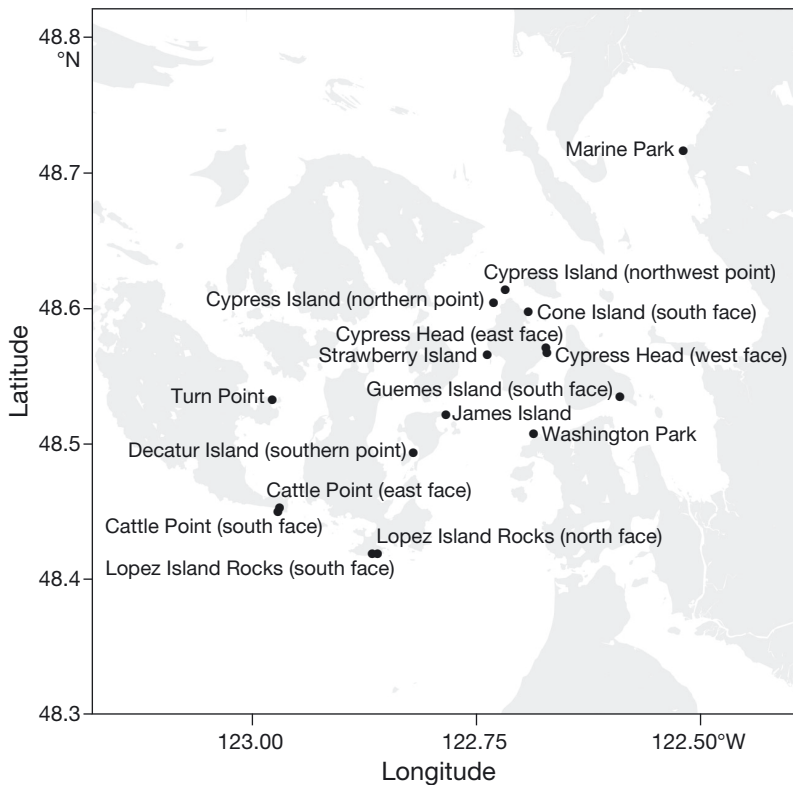


Fig. 1. Field sites in the San Juan Islands, Washington, USA. All 16 sites were sampled during the summer of 2007. During the summer of 2008, we resampled 6 sites (Cypress Head (east face), Cypress Head (west face), Cypress Island (northwest point), James Island, and Strawberry Island) to test for phenotypic costs associated with increased retractability in *Nucella lamellosa*

selected whelks and measured each whelk 3 times with >10 min intervals between each measurement. The 3 measurements for each individual were averaged. In this way, our method likely increased the variance among individuals but did not systematically bias the results. We measured the normal force by pulling perpendicular to the plane of attachment, as opposed to the shear force by pulling horizontal to the plane of attachment, because we felt that the normal stress better represented an attacking predator. However, shear stress might be more important in strong water flow. The length, width, and surface area of the foot of *Nucella lamellosa* were determined through image analysis. Each whelk was placed on a microscope slide immersed in water until it attached. The slide was then inverted and a digital picture was taken of the foot alongside a ruler. The length, width, and surface area of the foot were measured with the image analysis software Image J (NIH, <http://rsbweb.nih.gov/ij>).

These data were analyzed with linear, mixed-effect models. We performed 4 analyses, one for

each of the following response variables: force required to dislodge an individual, tenacity, and area and shape of the foot. Tenacity was calculated by dividing force by surface area of the foot. Shape of the foot was calculated by dividing the length by the width of the foot. In all 4 analyses, shell length and retractability were predictor variables, and site was specified as a random factor to account for the correlation among individuals from a site. For each analysis, we compared the AIC values among 3 models: a null model, a model with only the covariate shell length, and the full model with the covariate shell length and retractability. The model with the lowest AIC value was selected as the best model. To compare the relative effects of retractability and length for predicting each response variable, we also calculated the standardized beta coefficients for each analysis. The statistical program R and the lme4 package were used for all 4 analyses.

Natural variation in the field

To estimate the natural variation of retractability in *Nucella lamellosa*, we collected individuals from 16 sites throughout the San Juan Archipelago, Washington during the summer of 2007 (Fig. 1). Due to time constraints, we did not collect information on sea star densities. Whelks were collected and measured (shell length, shell height, and retractability) with the same procedures as described above. In addition, we used these data to estimate and compare the variation between the field-caught whelks and our laboratory-induced whelks. To compare the variation in retractability among sites and our experiment, we estimated the retractability for a whelk that was the same length as the average length of whelks in the induction experiment (26.2 mm). To do this, we calculated for each site the best fit line for the relationship between retractability and length. Using these equations, the average retractability was determined (for a whelk 26.2 mm in length) for each site (i.e. the marginal means). Whelks from each site spanned the size distribution of whelks from our experiment.

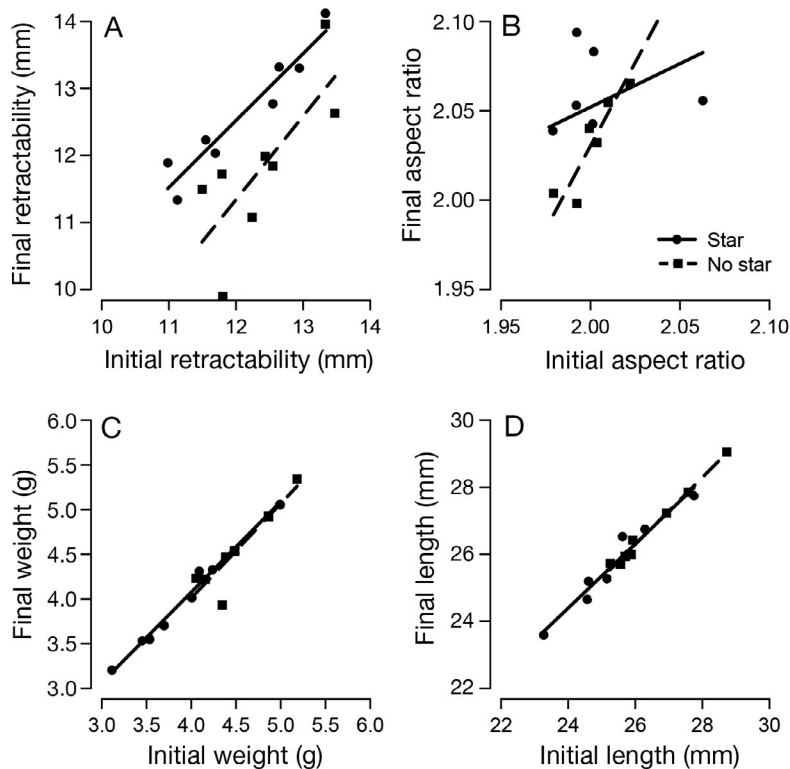


Fig. 2. *Nucella lamellosa*. Changes in morphology of *Nucella lamellosa* held in the presence of the predatory sea star *Pisaster ochraceus* ('star') or in a control treatment ('no star'). (A) Final retractability was significantly different between the 2 treatments, with whelks held in the star treatment increasing in retractability compared to their initial retractability and whelks in the no star treatment decreasing in retractability. (B) Final shell aspect ratio was not different between whelks in the 2 treatments, but whelks from both treatments were narrower than they were initially. There were no significant changes in (C) weight or (D) shell length compared to initial values

RESULTS

Laboratory experiments

Induction experiment

Final retractability was related to the presence of sea stars (treatment) and initial retractability (Fig. 2A), and there was no interaction between treatment and initial retractability ($F_{1,12} = 0.35$, $p = 0.56$). Whelks exposed to cues from sea stars were significantly more retractable than whelks exposed to only seawater (Table S1 in the Supplement at www.int-res.com/articles/suppl/m493p195_supp.pdf). On average, whelks exposed to cues from sea stars could retract 1.1 mm more than whelks from the control regardless of their initial retractability; this corresponds to an average increase of 10% in the ability to retract into the shell. This difference was because

whelks in the control treatment became less retractable during the experiment, whereas whelks in the sea star treatment became more retractable during the experiment (Fig. 2A).

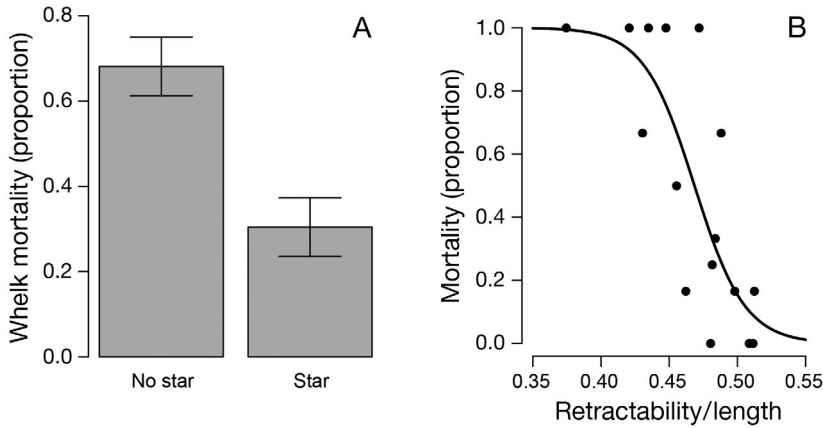
By contrast, the aspect ratio of the shells at the end of the experiment related to the aspect ratio of shells at the beginning of the experiment but not to the presence of sea stars (Fig. 2B). The interaction between treatment and initial aspect ratio of the shell was not significant ($F_{1,12} = 3.49$, $p = 0.09$), though the p -value was close to 0.05, and whelks in the sea star treatment did not significantly differ in aspect ratio compared to whelks in the control whether we included or excluded the interaction (Table S1 in the Supplement). The results from the MANCOVA were very similar. The interaction was not significant but near 0.05 (Wilks' lambda approximate F -value $_{2,11} = 2.37$, $p = 0.14$), and treatment was not significant regardless of whether the interaction was included (Wilks' lambda approximate F -value $_{2,12} = 0.94$, $p = 0.42$; interaction excluded). However, at the end of the experiment the average aspect ratio for all whelks was 1.3% narrower than at the beginning of the experiment (Fig. 2B).

There was no evidence that whelks grew during the experiment (Fig. 2C,D). The wet weight and shell length of whelks at the end of the experiment related to the initial measurements of each metric at the begin-

ning of the experiment, but not to the presence of sea stars (Table S1 in the Supplement). There was no interaction between treatment and initial weight ($F_{1,12} = 0.0015$, $p = 0.80$) or between treatment and initial shell length ($F_{1,12} = 0.15$, $p = 0.70$). Whelks in the sea star treatment did not significantly differ in weight compared to whelks in the control and, in addition, whelks were nearly the same weight or shell length at the beginning and end of the experiment (Fig. 2C,D).

Predation experiment

Recent exposure to sea stars significantly affected the number of whelks consumed by sea stars (Fig. 3A). Whelks recently exposed to sea stars were consumed less often than whelks not recently exposed to sea stars ($Z = -3.54$, $p = 0.0004$). On average,



recent exposure to sea stars reduced the probability of whelk mortality by 77 % (Fig. 3A). There was also a strong relationship between mortality and retractability relative to shell length ($Z = -4.78, p < 0.0001$). Sea stars were much less likely to consume whelks that could retract more than half the length of their shell (Fig. 3B).

Field surveys

Phenotypic costs

Fig. 3. *Nucella lamellosa*. Mortality of *Nucella lamellosa* from the induction experiment in the presence of the sea star *Pisaster ochraceus*. (A) Whelks that had been previously exposed to sea star cue ('star') were significantly less likely to be consumed by sea stars than whelks that had not been exposed ('no star'). Error bars are SE. (B) Mortality was strongly related to relative retractability (retractability per unit length) and whelks that could retract approximately half their shell length (0.5) had little risk of being consumed

The distance individuals could retract into their shells was related to tenacity and surface area of the foot, but not to the force required to dislodge an individual from a glass sur-

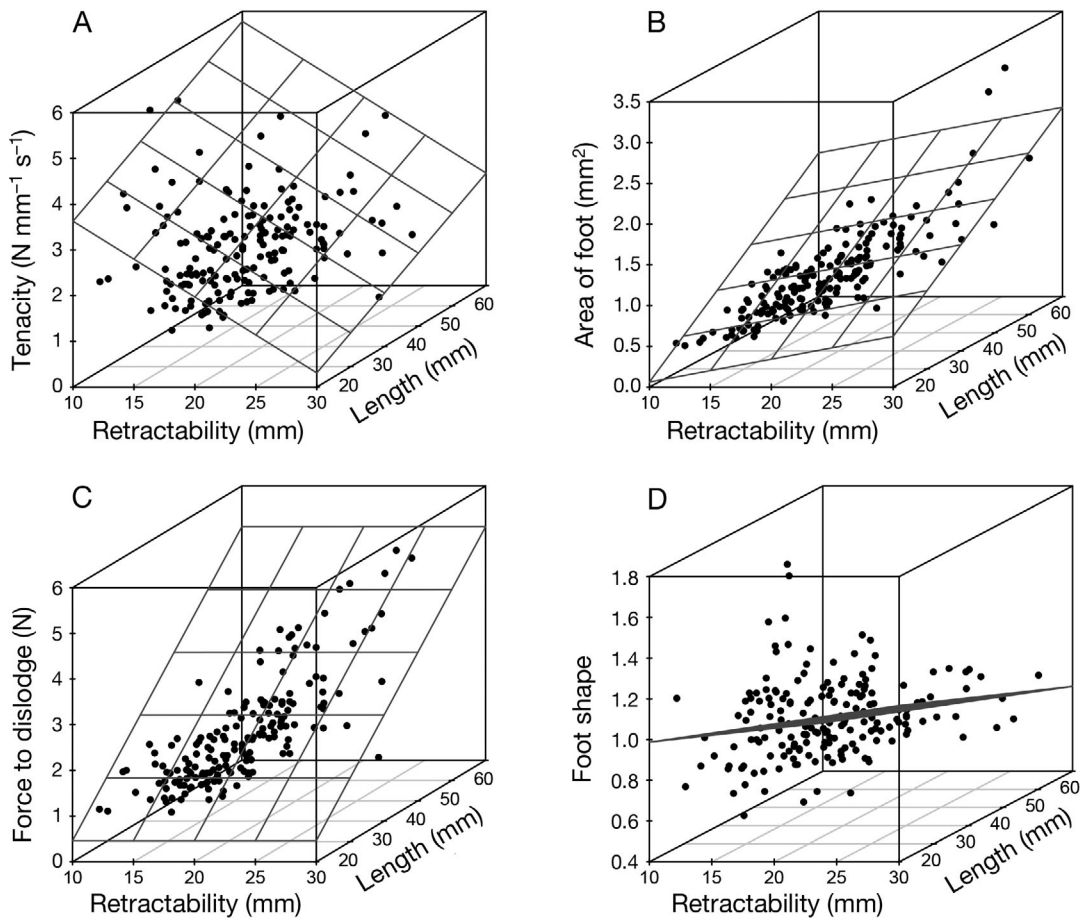


Fig. 4. *Nucella lamellosa*. Survey data relating retractability and shell length to putative costs of retractability: (A) tenacity, (B) area of the foot when attached to the substratum, (C) force required to dislodge an individual from the substratum, and (D) shape of the foot. The planes were predicted from multiple, linear regression, and are helpful for comparing the effect of retractability versus shell length

face or the shape of the foot (Fig. 4; Table S2 in the Supplement). Tenacity was negatively related to retractability and positively related to shell length (Tables S2 & S3 in the Supplement; Fig. 4A). On average, tenacity was 22.8 kN m^{-2} ($\pm 9.3 \text{ kN m}^{-2}$ SE) and decreased 1.0 kN m^{-2} for each additional millimeter of retractability. By contrast, surface area of the foot was positively related to both retractability and shell length (Tables S2 & S3, Fig. 4B). On average, surface area was 0.96 cm^2 ($\pm 0.40 \text{ cm}^2$ SE) and increased 0.03 cm^2 for each additional millimeter of retractability. Interestingly, the force required to dislodge an individual was positively related only to shell length—individuals required the same force to dislodge regardless of their retractability (Tables S2 & S3, Fig. 4C). The shape of the foot was not related to either retractability or shell length (Tables S2 & S3, Fig. 4D).

Natural variation

Average retractability relative to shell length differed among sites and between years (Fig. 5). The average for all sites and years was 0.51, which indicates that the distance the average individual was able to retract into its shell was approximately half of its shell length. Individuals were most retractable relative to shell length at Marine Park and least retractable at Decatur Island. The average retractability relative to shell length at most sites was greater than 0.5, the value from our predation experiment in which individuals were relatively well protected from sea stars (Fig. 3B). In 2008, all but one site sampled both years had a smaller average retractability relative to shell length compared to the previous year. The decrease from 2007 to 2008 was possibly caused by individuals increasing their length relative to their retractability, and not a decrease in the average retractability (Fig. 5), though our field samples were measured by different people in 2007 and 2008, and thus time and measurer are confounded.

The retractability of individuals used in our induction experiment differed from that of individuals collected at field sites (Fig. 6). The average retractability of individuals not exposed to sea stars during our experiment (11.6 mm) was less than the average of each field site (the minimum was 12.3 mm). In contrast, the average retractability of individuals exposed to sea stars during our experiment (12.9 mm) was similar, though typically less than, the average of each of the field sites (13.6 mm).

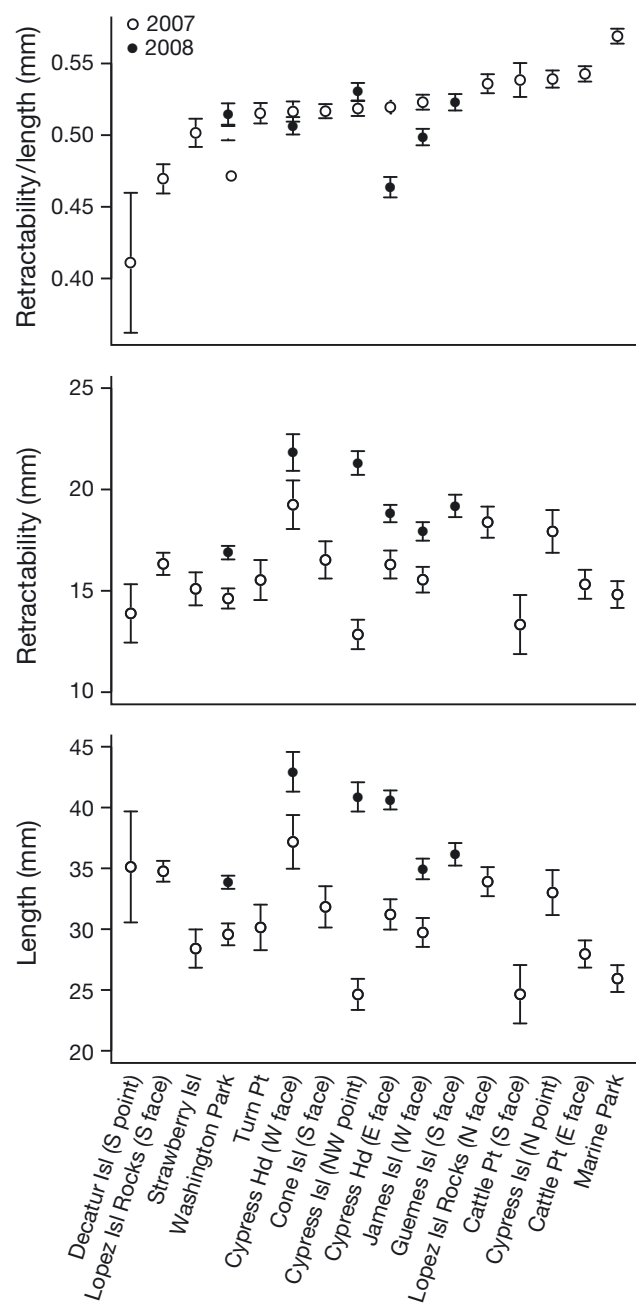


Fig. 5. *Nucella lamellosa*. Mean relative retractability (retractability per length), absolute retractability, and length from the 16 field study sites (for full site names see Fig. 1). (○): sites sampled during the summer of 2007; (●): sites resampled during the summer of 2008. Error bars are SE

DISCUSSION

Laboratory experiments

We found strong evidence that the ability to withdraw further into the shell is an inducible defense.

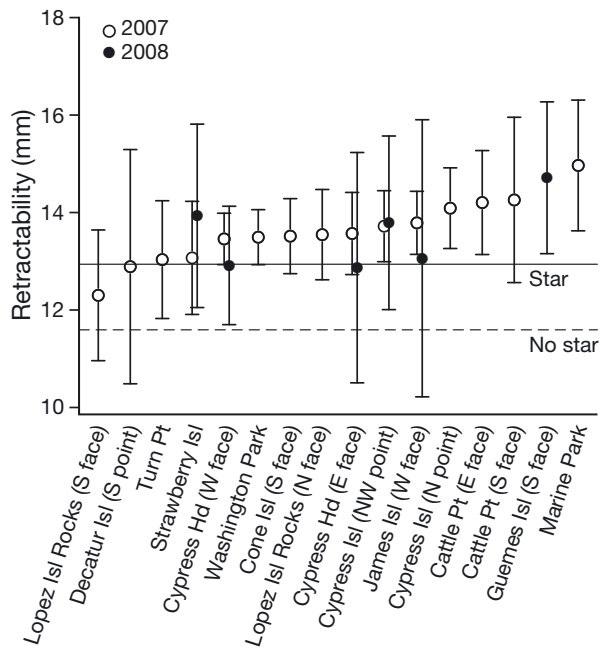


Fig. 6. *Nucella lamellosa*. Mean retractability adjusted to the average shell length of our experiment for whelks collected from the 16 field study sites (for full site names see Fig. 1). The solid and the dashed horizontal line indicate the average retractability for individuals from the sea star treatment ('star') and control ('no star'), respectively. (○): sites sampled during the summer of 2007; (●): sites resampled during the summer of 2008. Error bars are SE

Data from the induction experiment verified the finding of Bourdeau (2009) that individuals of *Nucella lamellosa* are able to withdraw further into their shells after they have been exposed to cues from *Pisaster ochraceus* feeding on conspecifics. Sea stars in our predation experiment were much less likely to consume whelks previously exposed to sea stars during the induction experiment compared to whelks that were not exposed to sea stars. Finally, there was a strong relationship between mortality and retractability relative to shell length, and individuals that could retract 50% of their shell length had relatively little chance of being consumed during the predation experiment.

In contrast to Bourdeau (2009), we did not find evidence that individuals changed the aspect ratio of their shells in response to cues from sea stars. We propose 2 hypotheses for this difference between Bourdeau (2009) and our experiment. It is possible that whelks in our experiment did change the aspect ratio of shell length to shell width because we measured aspect ratio differently than Bourdeau (2009): Bourdeau measured shell width (the widest point of the shell from one side to the other with the aperture

in between), whereas we measured shell height (the greatest distance perpendicular to the plane of the aperture between the aperture and the top of the shell). An alternative explanation is that the aspect ratio of the shell responds more slowly than changes in the soft tissue of the body and foot associated with increased retractability. Our experiment lasted 30 d whereas Bourdeau's experiment lasted 70 d, and the duration of our experiment was shorter than any experiment that has demonstrated predator-induced changes in the shell of *Nucella lamellosa* (Appleton & Palmer 1988, Palmer 1990, Bourdeau 2009, 2010a, 2010b, 2011). Given that snails grew very little in our experiment and the coiling of the shell means that altering width initially will alter height later, the latter hypothesis seems more plausible. Regardless of which hypothesis is best supported, the results of this study suggest that retracting the foot further into the shell, and not changing the shape of the shell, is the primary morphological defense against sea stars.

Whelks grew very little during the experiment, and individuals from both treatments were similar in weight and shell length at the end of the experiment. This suggests that the observed differences in retractability resulted from commensurate changes in visceral tissues, or that relatively large changes in retractability can result from very small changes in muscle tissue. For example, to increase retractability individuals might increase the size of the retractor muscle but decrease the size of their gonads, or very small changes in the size of the retractor muscle can result in large changes in retractability. Furthermore, several studies have found that snails from closely related species or populations, especially in the genus *Nucella*, vary in retractability most likely because of changes in tissues and not shells (Edgell & Miyashita 2009, Edgell et al. 2009). It is unlikely that the statistical power differed between retractability and our metrics of growth. We observed very little difference between the treatments of the induction experiment for the average weight or shell length of whelks and the variances were similar among retractability, shell length, and weight.

Our data demonstrate that retractability is reversible and symmetric. Whelks in the control treatment changed by a similar magnitude but in the opposite direction compared to whelks in the sea star treatment. A symmetrical response suggests that individuals pay a similar cost when they are undefended and the predator is present as when they are defended and a predator is absent (Gabriel 1999). Given that less retractable whelks were at much greater risk of dying, we hypothesize that there is

also a large phenotypic cost for individuals that are very retractable when *Pisaster ochraceus* is absent. Our field data suggest that individuals that are more retractable are also larger-footed, and a larger foot could result in a greater risk of desiccation during low tide (Vermeij 1973, Etter 1988b). Previous studies on marine snails have found asymmetrical responses (Etter 1988a, Trussell 1997), and to our knowledge this is the first example of a symmetrical plastic response in marine snails. The ability of *Nucella lamellosa* to become more or less retractable (i.e. reversible) and change within 30 d suggests that individuals experience changes during their lifetime in the risk of predation from *P. ochraceus* (i.e. fine-grained environmental variation) (Gabriel et al. 2005). The Multi-Agency Rocky Intertidal Monitoring Program measures the abundance of *P. ochraceus* at intertidal sites along the west coast of North America (www.eeb.ucsc.edu/pacificrockyintertidal). From their data, it is clear that *P. ochraceus* density can dramatically fluctuate from year to year at a given site. It is common for the number of *P. ochraceus* in permanent plots to double or halve in a 1 to 2 year time frame, and different plots at the same site can be more than an order of magnitude different. For example, at a site on Saddlebag Island, Washington, which is near the sites of this study, there are 4 years of data, and the number of *P. ochraceus* decreased by 50% 2 years in a row in 1 plot and this plot had more than twice as many individuals as another, nearby plot. The density of *P. ochraceus* also changes seasonally, with increased abundance and individuals higher up the shore during the summer (Markowitz 1980). In addition, voracity of *P. ochraceus* is influenced by water temperature (Sanford 1999), and in our study region the risk of being consumed by sea stars likely increases during summer. These seasonal and annual changes in risk of predation from *P. ochraceus* might have selected for the observed reversibility and timescale of less than 30 d so that individuals can track changes in risk of predation from *P. ochraceus* (Padilla & Adolph 1996, Gabriel et al. 2005).

Though our study supports that retractability is an inducible defense, the mechanism by which whelks are defended is not known. We hypothesize that sea stars might perceive a shell as empty when an individual can retract a certain distance into its shell. In a similar situation, more retractable individuals of the genus *Chlorostoma* (previously *Tegula*) were rejected more often by *Pisaster* spp. compared to less retractable individuals or congeners in laboratory experiments (Markowitz 1980, Watanabe 1983), possibly

because the sea star perceived the shell to be empty although this was not explicitly tested (Watanabe 1983). We also suggest that the level of protection is not the absolute distance an individual can retract, but the distance an individual can retract relative to its size. More precisely, the number of whorls within which an individual can retract might hide a retracted individual from the tube feet of sea stars. This would explain the sharp change in mortality associated with increased retractability relative to shell length (Fig. 3). There are also alternative mechanisms. Sea stars might require more time to consume whelks that retract further into their shells. If true, retractability would affect the time needed for sea stars to consume a whelk. We observed that the time required for sea stars to consume whelks more than doubled between treatments—sea stars consumed whelks from the control aquaria (no sea star exposure) at a speed of 0.8 d whelk⁻¹, but consumed whelks from the sea star treatment (recently exposed to sea stars) at a frequency of 0.35 d whelk⁻¹. Increased handling time might increase the risk of sea stars to predators, and thus have selected for sea stars to choose only whelks that they can quickly consume. It is also possible that there are additional defenses that were not measured. For example, whelks might induce a chemical defense to deter sea stars. Several other species of gastropod chemically defend themselves from *Pisaster ochraceus* (Watanabe 1983, Rice 1985). To distinguish among these hypotheses, researchers could closely observe sea stars as they prey on induced whelks, uninduced whelks, and empty shells. The time sea stars spend inspecting and consuming individuals and shells should provide evidence for one of these 3 hypotheses.

Field surveys

With individuals collected from the field, we found evidence of phenotypic costs. Individuals that were more retractable were also less tenacious. In other words, for a given surface area of foot in contact with the substratum, more retractable individuals were weaker. The average values for tenacity which we measured were similar to previously reported values. Miller (1974) measured the average tenacity for moving individuals of *N. lamellosa* as 1.07 and 4.58 N cm⁻² for stationary individuals. We recorded the average tenacity as 2.04 N cm⁻² (min. = 0.65 N cm⁻² and max. = 6.28 N cm⁻²), though we did not note whether individuals had been crawling or stationary before a measurement. Surprisingly, however, there was no difference in the total amount of force re-

quired to dislodge an individual, because more retractable individuals had a larger foot. Thus, it appears that more retractable individuals invest more energy in building a larger foot to maintain the same attachment strength as less retractable individuals. This was contrary to our expectations, which were that more retractable individuals would have a smaller foot to retract further into the shell, and thus require less force to dislodge. Our data therefore suggest that individuals are reducing the size of visceral mass to retract further into their shells, because they are not reducing the size of their foot or changing their shell. A reduction in visceral mass could result in reduced fecundity and growth.

There was variation in the amount of retractability throughout sites around the San Juan Islands. Often sites very close in proximity had whelks that on average retracted different distances (both absolute and relative distances) into their shell. It also appears that the average retractability of whelks at a site changes from year to year, though our design does confound year with measurer, and that, on average, whelks at most of the sites we surveyed were well protected from *Pisaster ochraceus*. Populations of *Littorina obtusata* from the Atlantic express different levels of plasticity in retractability in response to cues from green crabs, suggesting that selection can affect plasticity in retractability (Edgell et al. 2009). It would be interesting to test different populations of *Nucella lamellosa* throughout the Salish Sea to determine whether populations express different levels of plasticity in response to sea stars and whether there is a correlation with aspects of sea star abundance (e.g. average or variance in density).

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