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Journal of Experimental Marine Biology and Ecology 317 (2005) 25–35

**Journal of  
EXPERIMENTAL  
MARINE BIOLOGY  
AND ECOLOGY**

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# Water-borne cues from a shell-crushing predator induce a more massive shell in experimental populations of an intertidal snail

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Received 19 August 2003; received in revised form 19 September 2004; accepted 3 November 2004

## Abstract

Inducible defenses are important in the life strategies of many taxa. In some species of marine gastropods, water-borne chemical cues from potential predators induce defensive changes in shell form and differences in growth rate. We examined such phenotypic plasticity in the direct-developing snail, *Littorina subrotundata* (Carpenter, 1864). Among experimental field populations of *L. subrotundata* exposed to differing intensities of predation by the purple shore crab, *Hemigrapsus nudus* (Dana, 1851), snails collected from predation-intense environments often had more massive shells than closely related snails from adjacent environments where predation was negligible. Snails collected from both environments were raised in tanks containing cages of *H. nudus* that were feeding on conspecific snails and compared to a control group raised in the absence of this stimulus. Most snails developed significantly more massive shells in the presence of the crabs suggesting that adaptive phenotypic plasticity may account for some of the variation we observed in the field. In one case, snails from a predation-intense environment did not exhibit a statistically significant amount of plasticity, but instead grew a more massive shell irrespective of the laboratory stimulus. We interpret this as evidence for a genetic difference in the plasticity of shell form among experimental populations, caused by intense selection by *H. nudus*. There was no statistical difference in the growth rates of snails among treatments.

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*Keywords:* *Hemigrapsus nudus*; Inducible defenses; *Littorina subrotundata*; Phenotypic plasticity; Reaction norms

## 1. Introduction

Adaptive phenotypic plasticity can be an important factor in the determination of phenotype (e.g., Sandoval, 1994; Schmitt et al., 1995; Agrawal,

1998; Aikio and Markkola, 2002). It is useful to organisms primarily because it allows the phenotype to be tuned to spatially or temporally heterogeneous environments, especially where genetic variability might not allow such local adaptation by evolution alone. Adaptive plasticity is also useful because it allows the temporary adoption of morphologies and behaviors that might be prohibitively expensive, in terms of fitness costs, to maintain continually (e.g.,

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van Buskirk, 2000). Inducible defenses (plastic traits that confer resistance to predators) are an example of adaptive phenotypic plasticity and are prevalent in many taxa (Harvell, 1990). They include the production of toxins in plants (Karban and Carey, 1984), spine production in waterfleas (Agrawal et al., 1999), decreased hatching time in spitting spiders (Li, 2002), changes in body shape in carp (Bronmark and Miner, 1992) and increased shell thickness, byssal thread strength and gonad to flesh ratio in mussels (Reimer and Tedengren, 1997; Leonard et al., 1999; Reimer, 1999). Inducible defenses are also present in marine gastropods and are produced in response to water-borne cues from shell-crushing predators or damaged prey. For example, some species of *Nucella* develop larger apertural teeth and/or thicker shells when raised in the presence of a predatory crab (Appleton and Palmer, 1988; Palmer, 1990). Similarly raised *Littorina obtusata* also develop thicker, more massive shells that are more difficult to break (Trussell and Nicklin, 2002). Increases in shell mass and thickness in *L. obtusata* and *Nucella* spp. are accompanied by reductions in relative body mass and body growth rate, suggesting a possible cost to the development of a more predator resistant shell (Palmer, 1990; Trussell, 2000). Theory suggests that only developing a more heavily armored shell in response to cues from potential predators improves fitness by allowing a more efficient allocation of resources (Trussell, 2000; Trussell and Nicklin, 2002).

Despite their apparent ubiquity, the mechanisms involved in the evolution of plastic traits remain enigmatic. For instance, they may be the product of selection on different mean values of quantitative traits across different environments (Via and Lande, 1985; Via, 1993). Alternatively, selection may act on the plasticity itself, so the ability of an organism to respond phenotypically to a variable environment is under stronger selection than the current incarnation of its traits (Schlichting and Pigliucci, 1993; Schlichting and Pigliucci, 1998). Whatever the source, the presence of adaptive phenotypic variation across different environments suggests that selection acts on the set of phenotypes possible from a particular genotype, rather than on a fixed set of traits. This set of ontogenetic trajectories is called the developmental reaction norm (Pigliucci and Schlichting, 1995; Pigliucci et al., 1996).

The role of reaction norms in shaping phenotypic response to the environment is exemplified in the case of *L. obtusata*. Snails from shores of low crab abundance have thinner, less massive shells than those from shores of high crab abundance, but when raised in the laboratory in the presence of crabs the magnitude of the increase in shell mass and thickness is the same in both populations (Trussell, 1996, 2000). Trussell (2000) suggested that increased crab abundance in the field triggered an evolutionary shift in the intercept of the reaction norm, while conserving its slope.

In this study we examine the effects of water-borne cues released by the purple shore crab, *Hemigrapsus nudus*, on the shell mass and growth rate of the direct-developing intertidal snail *Littorina subrotundata* and assess the evolutionary response of the reaction norm for shell mass to the controlled introduction of *H. nudus* in several field populations of *L. subrotundata*. Both *L. subrotundata* and *H. nudus* are abundant in the mid to high intertidal zones of the Pacific Northwest but their distributions do not overlap much as *H. nudus* has a limited tolerance to wave exposure and cannot inhabit some shores. As a result, some populations of *L. subrotundata* have, for practical purposes, never been exposed to predation by *H. nudus*. By building crab shelters near to two such populations, we hoped to simulate the invasion of a novel shell-crushing predator to a marine gastropod population (details in Boulding et al., in review). The aim of our study was to test predictions from the hypotheses that, at least in our experimental populations, (1) shell mass and growth rate exhibit adaptive phenotypic plasticity in *L. subrotundata* in response to water-borne cues from *H. nudus* and (2) selective pressure by recently introduced *H. nudus* has altered the reaction norm of these experimental field populations of *L. subrotundata*. Testing these hypotheses with shell mass as the response variable implies that a more massive shell confers a decreased risk of predation by *H. nudus*. We defend this assumption below.

## 2. Materials and methods

Between May and August 2002, we performed two separate but related experiments to test our hypoth-

eses. The first of these was comprised of a series of field measurements, where we tested whether micro-geographic variation in the shell mass of *L. subrotundata* was correlated with the intensity of predation by *H. nudus*. Our first hypothesis, that growth rate and shell mass are plastic with respect to predation intensity, predicted that *L. subrotundata* collected from predation-intense areas would have more massive shells for their size than those collected from predation-negligible areas.

We also performed a laboratory experiment where we raised snails collected from both areas in the presence and absence of feeding *H. nudus*. Here our hypotheses predicted that (1) snails raised in the presence of feeding *H. nudus* would grow more slowly and develop more massive shells and (2) within each treatment snails collected from predation-intense areas would develop more massive shells and grow more slowly than their counterparts from predation-negligible areas.

### 2.1. Study sites

The snails used in the experiments were collected from the wave-exposed intertidal zones of Prasiola Pt. and Nudibranch Pt. on the west coast of Vancouver Island, Canada (48°50'N, 125°08'W). These sites are adjacent to one another, separated by approximately 400 m of sandy beach. Between 1993 and 1998, concrete crab shelters were constructed by E.G. Boulding at random locations in the intertidal zones of each site (Boulding et al., in review). Each May and December since then shelters have been stocked with *H. nudus* collected from local wave-sheltered habitats. Since wave energy prohibits crabs from living independently of the shelters and there are few natural refuges, they represent a novel predator to the snail populations at each site. Tethering experiments assessing the fate of individual snails at varying distances from the shelters revealed that mortality from crab predation was higher for thin shelled species such as *L. subrotundata* and that the risk of predation was highest within 2 m the shelters but decreased sharply with distance (Boulding et al., in review). We used the fact that predation decreases rapidly with distance from the shelters to evaluate the degree of local adaptation of *L. subrotundata* to predation by *H. nudus* by comparing the shell mass of

snails collected close to the shelters with those collected farther away.

To this end, we established eight permanent circular quadrats (radius 0.5 m) at Prasiola and Nudibranch Pts., with four quadrats at each site. Two quadrats at each site were centered about crab shelters while the remaining two were located at least 5 m from the shelters. Hence, four of the quadrats were located in environments of intense crab predation and four were located far enough from the shelters so as to make the risk of crab predation negligible. Estimates of migration rates between quadrats suggest that it is possible for a snail to move between predation-intense and predation-negligible environments as we defined them. However, extensive back and forth movement is not likely since most snails appear to migrate between 1.6 m and 5.8 m in their lifetime (Boulding et al., in review). Furthermore it seems reasonable to assume that mixing which might have occurred between predation-intense and predation-negligible quadrats would weaken the phenotypic effect for which we were testing and render our results more conservative.

### 2.2. Differences in shell mass of field populations experiencing different levels of predation

To test whether microgeographic variation in shell mass was correlated with the intensity of predation by shell-crushing predators, we collected snails from each of the eight quadrats on Prasiola and Nudibranch Pts. and compared the shell mass of those collected close to the crab shelters with those collected farther away. Snails were collected twice, once between June 20 and 28, 2002, and once between August 1 and 10, 2002. After sorting the snails into size classes by dry sieving, (using US standard brass sieves), we randomly selected 25 snails per quadrat on each date from those between 2.00 and 3.35 mm (minimum dimension) for use in the experiment. We measured the shell length of those snails selected as the maximum dimension of the shell from the apex perpendicular to the axis of coiling to  $\pm 0.01$  mm with digital calipers. To obtain a mass-related measure of total size, we measured the blotted weight wet of each snail by placing it aperture-down on paper towel for 30 min then weighing it on an analytical balance to  $\pm 0.0001$  g. Upon contact with the paper towel, snails

invariably retreated into their shells, sealing their aperture and trapping a small quantity of water proportional to their size inside their shell. There is a strong correlation between two measurements of blotted wet weight on the same snail, with time allotted between measurements for the snail to emerge from its shell and “rehydrate” ( $r=0.995$ ,  $N=48$ ,  $p<0.001$ , Pakes, 2002).

To measure shell mass, snails were individually submersed in boiling water for approximately 3 s then separated from their shells with forceps. Shells were dried at 55 °C for 72 h then weighed on an analytical balance to  $\pm 0.0001$  g.

### 2.3. Phenotypic plasticity in shell mass and growth rate as a response to the effluent of crabs feeding on conspecific snails

To test whether the effluent released by *H. nudus* while feeding on conspecific snails induces a more massive shell and slower growth rate in *L. subrotundata*, we raised snails collected from each of the previously established quadrats at Prasiola and Nudi-branch Pts. in outdoor tanks containing one of two “risk treatments”: (i) crabs feeding on conspecific

snails, hereafter “crab treatment” or (ii) no crabs, hereafter “control treatment”.

Approximately 150 snails from each quadrat were collected between June 1 and 10, 2002 and sorted into size classes by dry sieving. We randomly selected 90 snails per quadrat from those between 2.00 and 3.35 mm (minimum dimension) for use in the experiment. The selected snails from each respective quadrat were randomly assigned to 30 petri dishes yielding 3 individuals from the same quadrat per dish. The dishes were 4.5 cm in radius and had mesh bottoms and tops to facilitate water flow. Individuals in a dish were marked with a small dot of red, orange or yellow paint to identify them. The weight of the dried paint did not significantly change the weight of the snail.

Dishes were randomly assigned to one of six outdoor tanks so that there were five dishes from each quadrat in each tank. Three of the tanks were assigned at random to the crab treatment. In each of these we placed four plastic sandwich containers with mesh sides, each of which housed one male *H. nudus* (mean carapace width=28.28 cm) (Fig. 1). Every 3 days, the crabs were offered 24 *L. subrotundata* each, at which time snails remaining from the previous feeding period were counted and removed. In the remaining

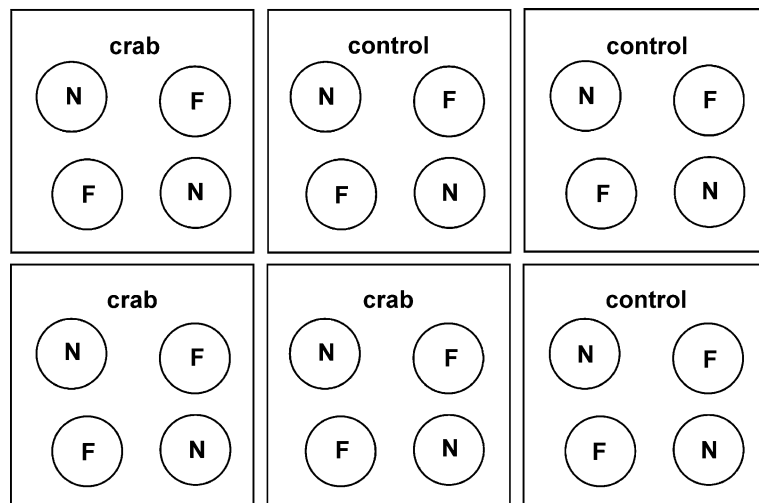


Fig. 1. A depiction of the design for the laboratory experiment. Snails from eight quadrats encompassing two levels of predation intensity by *Hemigrapsus nudus* were assigned in sets of three to modified petri dishes which were then randomly assigned to one of six tanks. Each tank contained the same number of snails from every quadrat, but each dish only contained snails from a single quadrat. To three of the tanks, respectively, four plastic and mesh containers were added, each one of which housed an adult male *H. nudus* that was feeding on conspecific snails. The remaining three tanks had identical plastic and mesh containers but no crabs, and served to control for the effect of laboratory conditions. In the diagram, the circles labeled “N” or “F” represent dishes containing snails from near to the shelters (predation-intense environment) and far from the shelters (predation-negligible environment) respectively. Each tank contained 40 dishes.

three control tanks, identical containers were placed each with 24 conspecific snails, but with no crabs. Food snails were collected on a regular basis from local wave-exposed habitats.

The crab containers were held to the bottom of the tanks with rocks while the petri dishes containing the experimental snails floated above. We assume that the only signal detectable to the snails to alert them to the presence of the crabs or food snails were water-borne chemicals. Each tank contained approximately 200 L of seawater, which was replaced at a rate of  $2 \text{ L} \cdot \text{min}^{-1}$ . While floating in the tanks, experimental snails fed ad libitum on diatoms that grew abundantly on their petri dishes.

In summary, the crab and control treatments were applied separately to three replicate tanks, each of which contained snails from eight quadrats, encompassing two levels of predation (near to and far from the crab shelters) and two locations (Prasiola Pt. and Nudibranch Pt.).

We measured the blotted wet weight and shell length of each snail prior to placing them in the tanks, at 30 days and again at 60 days, at which point we killed the snail and measured shell mass. Growth rate was measured as the change in blotted wet weight and shell length using final minus initial values. After 30 days, we added sunshades made of black plastic mesh (approximate grid size  $2.5 \text{ cm}^2$ ) to the tanks to prevent variation in incident radiation between tanks from differentially affecting the snails.

#### 2.4. Statistical analysis

All analyses were performed on  $\log_e$ -transformed data using analysis of variance by way of the MIXED procedure in SAS (version 8.2, © SAS Institute). Variance components were calculated using Restricted Maximum Likelihood (REML) which prevents negative estimates. For the field experiment, we assessed differences in shell mass between snails collected near to and far from the shelters using a model with date and distance from the shelters as fixed factors and shell length as a covariate. Location was also regarded as fixed, since the experimental sites were selected non-randomly because they are the only two peninsula easily accessible from the Bamfield Marine Sciences Centre in winter. On each peninsula there was only one location with the degree of wave

exposure and suitable substrate for constructing the artificial shelters. Random selection of locations would have allowed more generalized conclusions but was logistically impossible.

Quadrat, a random factor, was crossed with date, since we measured independent snails from each quadrat at each time of year, and this interaction was nested in location crossed with distance from shelters, since each quadrat only appeared in one location but there were predation-intense and predation-negligible quadrats at each location.

We reduced the full model according to the following algorithm. First, random effects estimated to be zero were removed. We then reduced the model step-wise, pooling terms that were not significant at the  $\alpha=0.25$  level one at a time, starting with the highest order terms in the model, each time choosing the term with the largest  $p$  value. For three-way interactions with  $p < 0.25$ , all lower order terms involving their respective components were kept in the model.

The final model used to analyze the field data was:

$$\begin{aligned} \text{shell mass} = & \alpha(\text{shell length}) + \text{location} + \text{distance} \\ & + \text{date} + \text{location} \times \text{date} \\ & + (\text{quadrat} \times \text{date})(\text{location} \times \text{distance}) \\ & + \text{shell length} \times \text{location} \\ & + \text{shell length} \times \text{distance} \\ & + \text{shell length} \times \text{date} \\ & + \text{shell length} \times \text{distance} \times \text{date} \\ & + \text{shell length} \times \text{location} \times \text{date} \quad (1) \end{aligned}$$

where  $\alpha$  was the slope of the relationship between the covariate, shell length, and the dependent variable, shell mass, location was either Prasiola Pt. or Nudibranch Pt., distance was either near to or far from the crab shelters (i.e. intense or negligible predation environments) and date was either June or August. The interaction of shell length with the other factors was included to test the homogeneity of slopes assumption implicit in covariate analysis. These interactions were pooled if not significant as detailed above.

To test for differences in shell mass between groups of snails in the laboratory experiment, we used the MIXED procedure with risk treatment,

Table 1a

Results of analysis of variance on  $\log_e$ (dry shell mass) with shell length as a covariate for two separate samples of *Littorina subrotundata* collected near to and far from the crab shelters at Prasiola Pt. and Nudibranch Pt

Source of variation	df	F	Z	p
Shell length (SL)	1	2238.03		<0.0001
Distance from shelters (Di)	1	9.06		0.0131
Location (Lo)	1	0.00		0.9464
Date (Da)	1	1.86		0.2028
Lo×Da	1	0.67		0.4325
(Quadrat×Da)(Lo×Di)			1.80	0.0356
SL×Di	1	5.15		0.0237
SL×Lo	1	0.58		0.4476
SL×Da	1	1.30		0.2540
SL×Di×Da	1	4.94		0.0268
SL×Lo×Da	1	1.41		0.2355

Independent samples of similar-sized snails were collected in the field and measured immediately in May 2002 and August 2002. The MIXED procedure in SAS gives terms with random effects Z values instead of F ratios.

distance from the shelters and location as fixed factors and shell length as the covariate. Variation due to replicate tank, a random factor, was nested in risk treatment. Variation between the dishes that held the snails was treated as a random factor and nested in the fully crossed combination of location, distance from the shelters, quadrat and tank, because each dish was assigned to a single tank, and contained snails from a single quadrat. Quadrat was nested in distance from the shelters crossed with location. The full model was reduced in the same manner as the field data. The final model was:

$$\begin{aligned} \text{Shell mass} = & \beta(\text{shell length}) + \text{treatment} + \text{distance} \\ & + \text{location} + \text{treatment} \times \text{distance} \\ & + \text{treatment} \times \text{location} \\ & + \text{distance} \times \text{location} \\ & + \text{treatment} \times \text{distance} \times \text{location} \\ & + \text{quadrat} (\text{distance} \times \text{location}) \end{aligned} \quad (2)$$

where  $\beta$  was the slope relationship between shell length and shell mass and treatment was either crab or control.

Growth rate in the laboratory snails was analyzed in a similar manner to shell mass, but without shell length as a covariate. This is because we were interested in the change in size of the snails, rather

than in comparing the attributes of snails of similar size. The final models used to analyze growth rate in the laboratory snails were:

$$\begin{aligned} \text{change in blotted wet weight} \\ = \text{treatment} + \text{distance} + \text{location} \end{aligned} \quad (3)$$

and

$$\begin{aligned} \text{change in shell length} \\ = \text{treatment} + \text{distance} + \text{location} \\ + \text{distance} \times \text{location} \end{aligned} \quad (4)$$

where change was captured by subtracting initial from final measurements for each snail. It was not necessary to account for differences in initial size affecting growth because all snails were of similar size at the beginning of the experiment (see above).

### 3. Results

#### 3.1. Microgeographic differences in shell mass

There was a significant interaction between shell length, distance from the crab shelters and date,

Table 1b

Differences in adjusted shell mass near to and far from the shelters evaluated at each collection date using a Student's *t*-test

Shell length (mm)	Shell mass (mg)			p
	Far	Near	Difference	
<i>June</i>				
3.17	5.42	6.02	0.60	0.0830
3.78	8.24	9.14	0.90	0.0620
4.39	11.78	13.05	1.28	0.0908
<i>August</i>				
3.17	4.86	5.99	1.13	0.0041
3.78	7.86	8.92	1.07	0.0256
4.39	11.83	12.53	0.70	0.2980

The three-way interaction between the shell length (SL), distance from the shelters (Di) and location (Lo) indicates that the slope of the relationship between shell mass and shell length is not equal across groups. As a result we evaluated differences in shell mass for each distance and date at multiple covariate points: at the overall mean shell length (3.78 mm) and one standard deviation to either side.

indicating that the slope of the relationship between shell length and shell mass was not the same for snails collected from different risk environments on different dates (Table 1a). To address this, we compared the adjusted mean shell mass near to and far from the shelters on each date at several covariate points spanning one standard deviation to either side of the overall mean shell length. These comparisons were done using a Student's *t*-test (Table 1b). The most significant difference occurred among average-sized and smaller snails collected in August. Snails of all sizes collected in June showed differences of varying significance, while larger-than-average snails collected in August showed no significant differences. The effect of replicate quadrat was significant, but did not interact significantly with other terms in the model.

### 3.2. Phenotypic plasticity in shell mass and growth rate

Our analyses of differences in shell mass revealed homogeneity of slopes, but significant three-way interaction between the effects of the laboratory treatment, distance from the shelters and location (Table 2). Because of this, we employed pair-wise comparisons of the three-way interaction means, using a Student's *t*-test, to examine the results. Among snails from the same location, in all but one case, *L. subrotundata* subjected to the crab treatment had more massive shells for their size than conspecifics subjected to the control treatment (Fig. 2). The exception to this trend occurred in the response of snails collected from predation-intense quadrats at Prasiola Pt. Although snails from this group that were subjected to the crab treatment developed more massive shells on average, this difference was not statistically significant. However, snails from predation-intense quadrats at Prasiola Pt. were significantly more massive than every other group in the lab experiment.

In contrast to our prediction, snails from predation-intense quadrats did not consistently develop more massive shells than their counterparts from predation-negligible quadrats. Within the control treatment, snails from the predation-intense quadrats at Prasiola Pt. were again the exception to this trend, developing significantly more massive shells than their predation-

negligible counterparts. On average, snails from Prasiola Pt. were more massive than those from Nudibranch Pt. and this difference was statistically significant among snails collected from the predation-intense quadrats.

Table 2

(a) Results of analysis of variance on  $\log_e$  (dry final shell mass) with shell length as the covariate for *Littorina subrotundata* collected near to and far from the crab shelters on Prasiola Pt. and Nudibranch Pt. and raised in the laboratory

Source of variation	df	F	Z	p
Shell length	1	3926.68		<0.0001
Treatment (Tr)	1	69.52		<0.0001
Distance from shelters (Di)	1	1.62		0.2723
Location (Lo)	1	7.93		0.0480
Tr×Di	1	0.99		0.3205
Tr×Lo	1	1.94		0.1642
Di×Lo	1	1.64		0.2698
Tr×Di×Lo	1	8.73		0.0033
Quadrat (Lo×Di)			1.22	0.1119

The two treatments of the laboratory experiment were Crab (raised in the same tank as crabs feeding on conspecific snails) and Control (raised in a tank without crabs). Terms with random effects have Z values instead of F ratios. Table shows the results of a reduced model: see Statistical analysis for details.

(b) Results of comparisons of three-way interaction means using a Student's *t*-test. The effect of Risk Treatment was tested for each location and distance from the shelters

Risk Treatment (Crab–Control)				
Location	Distance from shelters	Difference	Standard Error	p
Prasiola Pt.	Near	−0.03559	0.02338	0.1286
Prasiola Pt.	Far	−0.01239	0.02258	<0.0001
Nudibranch Pt.	Near	−0.01328	0.0223	<0.0001
Nudibranch Pt.	Far	−0.08897	0.0217	<0.0001

Distance from shelters (Near–Far)				
Location	Risk treatment	Difference	Standard Error	p
Prasiola Pt.	Crab	−0.0315	0.04487	0.4830
Prasiola Pt.	Control	−0.1198	0.0453	0.0084
Nudibranch Pt.	Crab	−0.02168	0.0445	0.6264
Nudibranch Pt.	Control	0.02219	0.0446	0.6190

Likewise, the effect of Distance from the shelters was tested for each location and risk treatment. Differences and standard errors are log transformed. See Fig. 2 for a plot of the three-way interaction means.

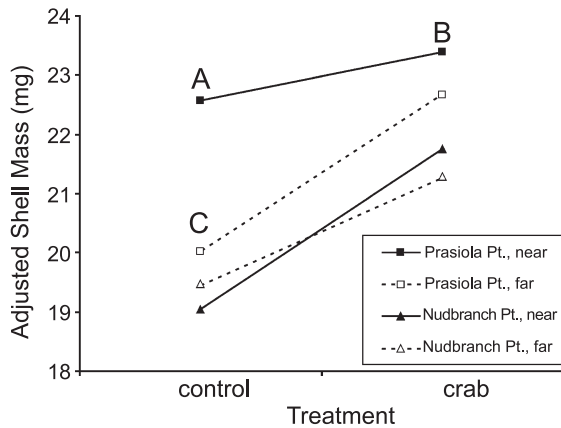


Fig. 2. Adjusted final shell mass for snails collected near to and far from the crab shelters and exposed to either crabs feeding on conspecific snails or a control treatment. Multiple comparisons using a Student's *t*-test gave the following results (see also Table 2b): crab>control and near=far except for labeled points, where A=B and A>C. Shell mass for the laboratory experiment was evaluated at a mean shell length of 5.47 mm.

There was no statistically significant difference in growth rate between groups in the laboratory experiment (Fig. 3, Table 3).

## 4. Discussion

### 4.1. Using shell mass as a surrogate for shell armor

Much of the interpretation of our results hinges on the extent to which increased shell mass for a given size is an indicator of a more predator resistant shell. There is substantial evidence that this is the case. *L. subrotundata* has a very smooth, unornamented shell without a thickened lip. Therefore mass is relatively evenly distributed over the entire shell, as compared to gastropods with more ornamented shells, and comparing dry shell weight among snails of similar size captures differences in shell thickness. *Littorina sitkana*, a close relative of *L. subrotundata* with a significantly more massive shell, requires five times more force to break in a mechanical testing machine (Boulding and Van Alstyne, 1993). Furthermore, Pakes (2002) found that in controlled feeding experiments, *H. nudus* showed a strong preference for ecotypes of *L. subrotundata* with less massive shells.

### 4.2. Phenotypic plasticity in shell mass

The development of more predator-resistant shells in response to cues from shell-crushing predators is well documented in marine gastropods. Defensive shell attributes that are plastic include shell thickness (Palmer, 1990; Trussell, 1996), apertural tooth height (Appleton and Palmer, 1988) and breaking force (Trussell and Nicklin, 2002). In marine gastropods, the origin of phenotypes locally adapted to shell-crushing predation may be the result of natural selection (Seeley, 1986) or of phenotypic plasticity in the form of inducible defenses (Appleton and Palmer, 1988; Palmer, 1990) or of a combination of the two in the form of selection on genetically based reaction norms (Trussell, 2000). Adaptation by natural selection requires that the trait in question be heritable and provide increased fitness in terms of survival and/or reproduction (Futuyama, 1998). Inducible defenses are favored when there is a reliable cue, predation is ephemeral but intense and the fitness costs of employing defenses intermittently are not prohibitively high (Harvell, 1990).

We found that most snails collected from populations experiencing high levels of predation by recently introduced *H. nudus* had slightly heavier shells for their size than those collected from adjacent environments of negligible predation. When raised in the presence of feeding *H. nudus*, snails from each environment developed more massive shells for their size in most cases. For most snails, the magnitude of the increase in shell mass was the same in snails from predation-intense and predation-negligible environments. From this it can be concluded that under certain conditions, inducible defenses alone could account for all of the differences in shell mass we observed in the field.

However, the level of stimulus that snails were exposed to in the laboratory was probably much greater than what may have been present near the crab shelters. In addition, laboratory snails grew much larger than field snails making it statistically difficult and biologically dubious to compare the results of the laboratory and field experiments conclusively. So while the inductive mechanism is present, it is unclear whether the level of stimulus provided by the crabs in the wild would trigger a significant increase in shell mass. Furthermore,

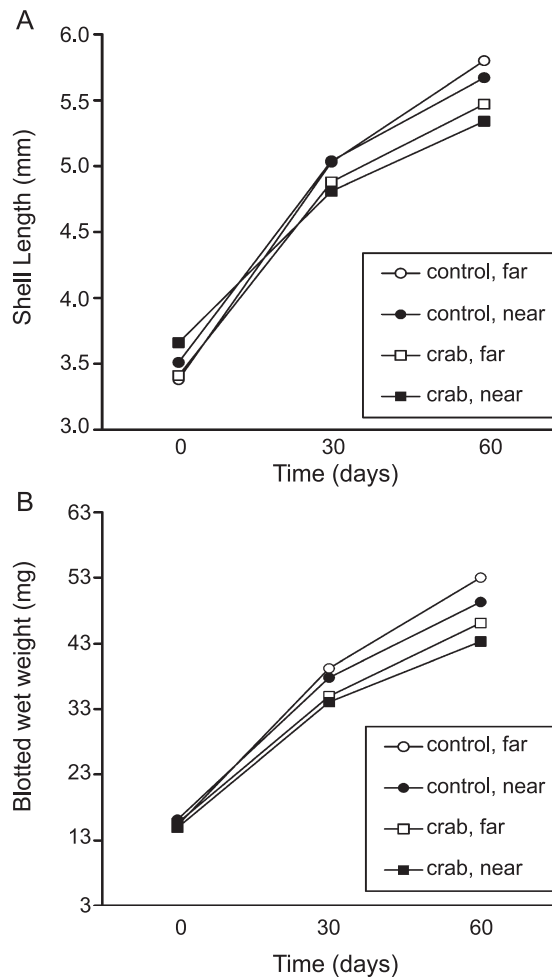


Fig. 3. Change in shell length (A) and change in blotted wet weight (B) as a function of time for snails collected near to or far from the crab shelters and exposed to either crabs feeding on conspecific snails or a control treatment (see also Table 3).

difference in shell mass among field populations was slight and could have resulted from other factors, such as increased mortality for snails with less massive shells living close to the crab shelters or a propensity for snails with less massive shells to migrate away from areas of intense predation.

#### 4.3. Evolution of the reaction norm for shell mass

In terms of our current understanding, the phenotypic variation between predation-intense and predation-negligible environments that we observed in *L. subrotundata* may be explained by two competing hypotheses. First, the variation in shell mass, we

observed could be entirely the product of phenotypic plasticity, either in the form of selection on alternate trait means across an environmental gradient or mediated by so-called “plasticity genes” (Via and Lande, 1985; Via, 1993; Schlichting and Pigliucci, 1993). Alternatively, natural selection may be acting on the genetically based reaction norm for shell mass, causing evolution of its slope and/or intercept, so that the variation we observed is both phenotypic and genotypic.

Evidence from our data supporting the hypothesis of reaction norm evolution comes from snails collected from predation-intense quadrats at Prasiola Pt. These snails did not exhibit a significant increase in shell mass

Table 3

Results of analysis of variance on (a) change in shell length (see Fig. 3A) and (b) change in blotted wet weight in the laboratory experiment (see Fig. 3B)

Source of variation	df	F	p
<i>(a) Change in shell length</i>			
Treatment (Tr)	1	2.68	0.1768
Distance from shelters (Di)	1	0.22	0.7844
Location (Lo)	1	0.01	0.6560
<i>(b) Change in blotted wet weight</i>			
Treatment (Tr)	1	3.21	0.1477
Distance from shelters (Di)	1	0.44	0.4976
Location (Lo)	1	0.53	0.5386
Tr×Di	1	1.42	0.2356

Snails collected near to and far from the crab shelters on Prasiola Pt. and Nudibranch Pt. were subjected to one of two laboratory treatments: either they were raised in the presence of crabs feeding on conspecific snails or they were raised in an identical tank in the absence of this stimulus. Both measures of growth rate were calculated by subtracting initial from final values. Snails were of similar size at the beginning of the laboratory experiment. The table shows the results of a reduced model: see Statistical analysis for details.

in response to the crab treatment, but in the control treatment developed more massive shells than any other group (see Fig. 2). Hence, predation-intense snails from Prasiola Pt. did not respond inductively, but exhibited an increase in shell mass over the course of the laboratory experiment that could not be correlated with the experimental stimulus to which they were exposed.

Shell mass has been found to be moderately heritable in an *L. subrotundata* ( $h^2=0.3$ ) and as a direct developer, *L. subrotundata* may experience less gene flow than similar species with planktonic larvae and may therefore be predisposed to show genetic differentiation on a microgeographic level (Behrens Yamada, 1989; Boulding and Hay, 1993). However, a short period of influence by introduced *H. nudus*, coupled with intermigration between predation-intense and predation-negligible environments, may render selection impotent to produce significant genotypic change in this system. Nonetheless, results of a recently constructed mathematical model, where field-estimated parameters from the study sites used here are employed to estimate magnitudes of demographic and genetic change, suggest that natural selection may retain its potency in this context (Boulding et al., in review).

Alternatively, the anomalous response of predation-intense snails from Prasiola Pt. might be accounted for by variation in the level of effluent among the crab shelters we sampled. Thus snails from this group might have been exposed to a relatively high concentration of risk-stimuli prior to collection which influenced their response to the laboratory treatment by irreversibly altering their ontogenetic trajectory. However, since the snails in the laboratory almost doubled their initial shell length and tripled their initial blotted wet weight, it seems somewhat unlikely that differences in final shell mass were caused by differences in life history prior to collection (Fig. 3). Unfortunately at this time, we do not have enough data on crab abundance or foraging patterns in the shelters to corroborate this hypotheses.

#### 4.4. Conclusions

Geographic variation in gastropod shell armor has been linked in several instances to the abundance of shell-crushing predators, but to our knowledge this is the first observation of this phenomenon occurring over such a short time period (i.e. less than 10 years) and on a microgeographic scale (Kitching et al., 1966; Vermeij, 1978; Seeley, 1986). Our findings provide evidence that, among the experimental populations at the locations we selected, recently introduced *H. nudus* have had a phenotypic effect on the shell mass of *L. subrotundata* and that this effect could be mediated by inducible defenses. We also find some evidence that selection by introduced *H. nudus* has caused a shift in the slope of the reaction norm for shell mass in some populations leading to the development of a more massive shell irrespective of the risk stimulus to which they are exposed.

#### Acknowledgments

We are grateful to O.B. Allen, I. Rajcan and M. Yu of the Ashton Statistics Lab at the University of Guelph for help with the statistical analyses, T. Hay and M. Quinton for statistical programming and M. Lemay, N. Bowes and D. Lombard for assistance in the field. Suggestions by A.R. Palmer, A.J. Underwood and two anonymous referees improved earlier versions of this manuscript. We thank the staff, students and research-

ers at Bamfield Marine Sciences Centre, where the laboratory component of this study was carried out and the Huu-ay-aht First Nations for continually allowing us access to their land. Funding for this project was provided by N.S.E.R.C. and P.R.E.A. grants to E.G. Boulding and a U.R.A. scholarship to B. Dalziel. [AU]

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