

# Predator-induced morphologies and cue specificity in veliger larvae of *Littorina scutulata*

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**ABSTRACT:** Examples of phenotypic plasticity are well documented, especially in the context of predator–prey interactions. However, evidence of inducible defenses is scarce in marine larvae. In order to better understand morphological plasticity in the larvae of the gastropod *Littorina scutulata*, we exposed veligers to predatory zoeae of *Hemigrapsus nudus*. The treatments included growing veliger larvae in the presence of predators and growing veliger larvae in the presence of predators consuming conspecific veliger larvae. When compared to controls in seawater, veligers developed rounder shells, smaller apertures, and thicker apertural margins in response to the presence of predators. In the presence of predators consuming conspecific veliger larvae, veligers formed smaller apertures and the thickest shell margins, but the shell shape was not significantly different from control veligers. The different responses to different treatments with planktonic predators indicate that larvae can vary shell characteristics and may indicate a trade-off reflective of cue-specific defenses indicative of risk. The induced defenses resulted in enhanced survival of veligers exposed to predators. The use of a different species of predatory crab zoeae and measurement of an additional shell response variable validates and broadens an earlier report of inducible phenotypic plasticity in gastropod veliger larvae (Vaughn 2007; Ecology 88:1030–1039).

**KEY WORDS:** Inducible defense · Shell morphology · Veliger larvae · *Hemigrapsus nudus* · Zoea larvae · Cue specificity

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## INTRODUCTION

Many environmental stimuli induce phenotypic responses, including competitors (e.g. Relyea 2002, Todd 2008), conspecific density (e.g. Kemp & Bertness 1984), nutrition (e.g. Strathmann et al. 1993, Walls et al. 1993), light (e.g. Sultan 2000, Todd 2008), temperature (e.g. Stelzer 2002, Atkinson et al. 2003), and wave exposure/water flow (e.g. Trussell 1997, Marchinko 2003, Todd 2008). Studies on predator–prey interactions abound with examples of phenotypic plasticity where predatory cues such as chemical, visual, auditory, or mechanical stimuli induce behavioral, morphological, physiological, and life history changes in prey and which often result in decreased vulnerability to predation (reviewed by Kats

& Dill 1998, Tollrian & Harvell 1999, Lass & Spaak 2003, Benard 2004). Tollrian & Harvell (1999) outlined the requisite conditions for the evolution of an inducible (as opposed to constitutive) defense: exposure to the response-inducing cue needs to be variable, the cue must be a reliable indication of danger, the induced phenotype must be effective in lessening predation risk, and there should be a trade-off in costs and benefits of the response that warrants conditional implementation.

Predator-induced phenotypic plasticity in freshwater planktonic environments is well documented, with behavioral and morphological responses demonstrated in algae, rotifers, ciliates, and crustaceans (e.g. Lass & Spaak 2003). In contrast, examples of predator-induced plasticities in marine planktonic

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environments are scarce, with limited examples including behavioral responses in crustaceans (e.g. Bollens & Frost 1989, Neill 1990, Cieri & Stearns 1999), larval cloning in echinoderms (Vaughn 2010), colony formation and cell wall thickening in phytoplankton (reviewed by Van Donk et al. 2011), modification of shell morphology in gastropod veligers (Vaughn 2007), and spine length in crab zoeae (Charpentier et al. 2017). The studies by Vaughn (2007, 2010) and Charpentier et al. (2017) are the only examples of predator-induced morphological changes in marine larvae, the former determining that veligers of *Littorina scutulata* developed smaller apertures and rounder shells when exposed to cues from larval decapod predators (zoeae of *Cancer* spp.) and that these alterations enhanced survival.

The occurrence of smaller apertures and changes in shell shape are common examples of predator-induced defenses for adult gastropods (e.g. DeWitt et al. 2000, Krist 2002, Brönmark et al. 2011, Moody & Aronson 2012, Hoverman et al. 2014). Shell thickening as a defense is particularly prevalent in marine snails (Appleton & Palmer 1988, Trussell 1996, Brookes & Rochette 2007, Moody & Aronson 2012, and references therein). The thickened shell reduces vulnerability to both shell-breaking and shell-entering predators (e.g. Vermeij 1974, Hughes & Elner 1979, Palmer 1985, Covich 2010, Moody & Aronson 2012). Gastropod veliger larvae are able to survive predation attempts utilizing other altered features such as spiral sculpturing or changes in shell shape (Hickman 1999, Vaughn 2007); thus an inducibly-thickened shell may benefit both life stages of the snail in resisting mechanical damage. However, to avoid a heavier shell in a planktonic environment, reinforcement by thickening might be limited to regions of the apertural opening, such as the apertural beak or velar notches, as observed in a variety of field-caught veligers (Hickman 1999).

The composition of environmental cues can influence the nature of the induced response, as has been investigated extensively in the inducible traits of adult snails (e.g. Appleton & Palmer 1988, Palmer 1990, Trussell & Nicklin 2002, Bourdeau 2010). Many behavioral plasticities occur in response to injured conspecifics (alarm cues; e.g. Alexander & Covich 1991, Jacobsen & Stabell 1999, McCarthy & Fisher 2000, Grason & Miner 2012). Isolated predator kairomones also elicit behavioral and morphological modifications (Palmer 1990, Marko & Palmer 1991, McCarthy & Fisher 2000, Trussell & Nicklin 2002, Grason & Miner 2012). The greatest degree of morphological and behavioral change commonly occurs

in response to predators consuming conspecific snails (e.g. Appleton & Palmer 1988, Alexander & Covich 1991, Trussell & Nicklin 2002, Dalesman et al. 2006, Bourdeau 2010). This combined signal of predators and injured conspecifics may 'label' the predator as dangerous, and this information may persist in predator excretions and elicit responses by prey (diet cues; reviewed by Chivers & Smith 1998, Ferrari et al. 2010).

In this study, we expanded the number of predator treatments (and by inference the composition of the inducing cues) relative to Vaughn (2007) and examined resultant shell characteristics of veliger larvae of *L. scutulata* Gould, 1849 including the prospect of shell reinforcement. Veliger larvae experienced 1 of 3 treatments: (1) exposure to predators that had been raised on food other than veligers, (2) exposure to predators consuming conspecific veliger larvae, or (3) a seawater control. Although Vaughn (2007) tested the response to predators, it is important to note that her study used zoal predators that were fed veligers of *L. scutulata* prior to being placed in the experimental cages; therefore, it is possible the experimental veligers were exposed not just to predator kairomones, but to post-digestive diet cues as well. Our choice of treatments allows us to distinguish the specificity of responses to different types of predatory cues. As predators, we used zoea larvae of *Hemigrapsus nudus* because zoea larvae of *Cancer* spp. (used by Vaughn 2007) do not co-occur temporally with veligers of *Littorina* spp. in Oregon (USA) waters. Zoea larvae of *H. nudus* are likely to be encountered by the veligers in Oregon plankton, are easily obtainable, are of similar size to those used by Vaughn (2007), and prey on veligers of *L. scutulata* in a laboratory setting via aperture-chipping typical of many zoea larvae (Hickman 2001, Vaughn 2007, J. Valley pers. obs.).

If the larvae respond in the greatest degree to the treatment of predators feeding on conspecifics, this will indicate the usage of combinatory information such as is often seen in adults that would likely alert the larvae to both conspecific injury as well as provide important information about the predator and its pertinent diet.

Although we expected to see veligers develop rounder shells, smaller apertures, and thicker margins in response to predators, we expected these changes to occur to a greater extent in response to predators consuming conspecific veliger larvae. We also expected the morphological alterations resulting from the latter treatment to enhance survival when subjected to direct predator contact.

## MATERIALS AND METHODS

### Predator collection and rearing

Adult *Hemigrapsus nudus* (Dana, 1851) bearing eggs at different stages of development were collected locally from the boulder fields at the south side of Sunset Bay (43° 20.033' N, 124° 22.623' W) or from the rocky shores lining Charleston mudflats, Oregon (43° 20.372' N, 124° 19.098' W or 43° 20.613' N, 124° 19.483' W). The crabs were kept submerged in ~1 l filtered seawater (FSW; 0.45  $\mu\text{m}$ ) in 2 l glass jars equipped with an air stone; these jars were kept in a sea table at ambient seawater temperature (~13°C). Upon hatching (usually early morning), approximately 200 zoeae from a single clutch were placed in each of up to 4 large finger bowls depending on the clutch size. The fingerbowls were stacked in 1 of 2 incubators kept at either 18°C or 13°C to hasten or slow development, thus ensuring a steady supply of zoeae of the desired stages for the duration of the study. Every other day, the zoeae were moved to fresh FSW of the appropriate temperature and were fed newly-hatched *Artemia*, a tripartite algal mixture (*Isochrysis galbana*, *Dunaliella tertiolecta*, *Chaeto-*

*ceros gracilis*), and a diluted solution of artificial plankton rotifer (APR; Ocean Star) filtered through 100  $\mu\text{m}$  mesh. At this time, dead zoeae were counted, molts were noted, and a portion of the zoeae was staged.

### Prey adult and veliger collection

To obtain veligers, ~20 adult *Littorina scutulata* collected beside the jetty at the Oregon Institute of Marine Biology beach (Charleston, Oregon; 43° 20.981' N, 124° 19.825' W) were placed together in 3 screen-bottomed, tricorner plastic beakers suspended in FSW overnight. The following morning, the water was filtered through 350  $\mu\text{m}$  mesh and egg capsules were collected and distributed into 3.8 l glass jars filled with 3 l of FSW for a concentration of ~200 veligers  $\text{l}^{-1}$  upon hatching. During and following the ~8 d required for the veligers to develop and hatch out of the capsules, the ~13°C water was changed every 3 d and gently stirred every 3 s by paddles suspended from a motorized stir rack (Strathmann 1987).

### Experimental set-up

Three replicates of each of the 3 treatments (isolated predators, P; predators feeding on conspecific veligers, PV; and control) were randomly distributed in each of 2 sea tables, for a total of 6 replicates treatment<sup>-1</sup>. The daily temperature of each sea table was ~13°C. Each replicate consisted of a glass 1000 ml beaker containing 800 ml of FSW and 50 newly hatched veligers. Floating in each replicate container was a predator cage fashioned out of a 50 ml falcon tube with 100  $\mu\text{m}$  mesh openings on the sides and bottom (Fig. 1a). Embedded in the lid of the cage was an open-bottomed 1.5 ml Eppendorf tube with a sealable lid. With this lid closed, water was retained in the cage and zoeae remained submerged whenever the cage was moved. Just beneath the lid of the tube, the cage was outfitted with a circle of foam sheeting (4 mm thick) to allow the cage to float. Five stage 4 (~2.6 mm total length) or stage 5 (~3.3 mm) zoeae of *H. nudus* (capable of consuming veligers up to ~300  $\mu\text{m}$  or veligers of all sizes, respectively; J. Valley pers. obs.) were placed in the cages of the 6 P and PV treatment replicates. Twenty food veligers (~1 wk old, ~200  $\mu\text{m}$  shell length) were also added to the cages of PV replicates for the zoeae to consume. Every 3 d, the water was changed, the veligers were

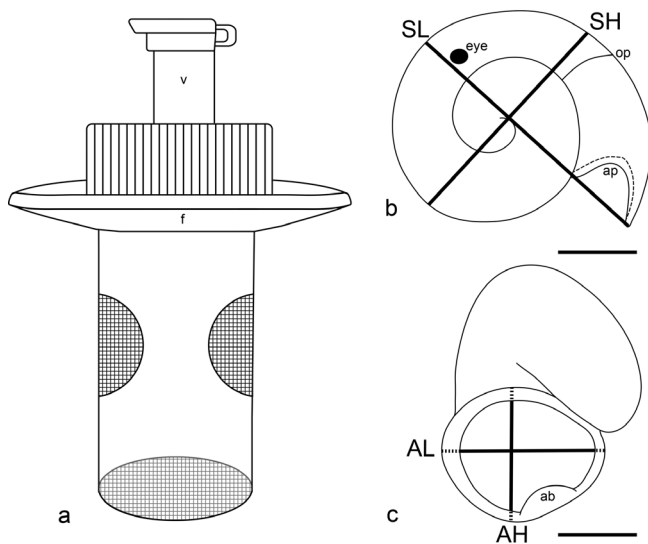


Fig. 1. (a) Cage used to contain the treatment cues had 100  $\mu\text{m}$  mesh, a float (f), and a sealable vent (v) to contain water when removed. (b) Shell aspect ratio (SAR) of *Littorina scutulata* veligers was calculated by dividing the shell length (SL) by the shell height (SH). (c) The elliptical area of the aperture was estimated using the aperture length (AL) and aperture height (AH) (solid lines), and the margin thickness of the aperture was calculated as the average shell thickness from measurements at the periphery of the aperture length and height (dotted lines). Scale bars = 100  $\mu\text{m}$ . ab: apertural beak, ap: aperture, op: operculum

numbers of cells at a combined concentration of 10 000 cells ml<sup>-1</sup>. At the same time, the treatments were renewed: the predators were removed (dead zoeae and molts were noted) and replaced with 5 new stage 4 or stage 5 zoeae. In the PV replicates, any remaining food veligers were counted to confirm prey consumption and replaced with 20 new food veligers (~1 wk old). After 4 wk, the experimental veligers were collected, counted, and fixed in 80% ethanol buffered with sodium glycerophosphate (Turner 1976) except for those used in the predation trials.

### Measurement methods

To measure shell length, height, and aperture area, 15 haphazardly selected, fixed veligers from each replicate were individually placed in the tapered base of a severed Eppendorf tube, which enabled easy manipulation of the veligers into a profile position that minimized the angular tilting seen when placed on a flat surface and allowed the veliger to also be easily positioned aperture-side up. The veligers were photographed at 70× magnification on an Olympus SZH-10 dissecting microscope; measurements from these images were later collected using ImageJ software (Schneider et al. 2012) and were measured to the nearest micron. Measurements of veliger length, height, shell aspect ratio (SAR), and aperture area were modeled after Vaughn (2007). Veliger length was measured as the largest distance from the tip of the apertural beak to the opposite side of the shell, and shell height was measured as the greatest distance perpendicular to shell length (Fig. 1b). SAR was calculated for each veliger as an indication of overall shell shape (length/height). Aperture area was estimated using the formula for elliptical area: [(aperture length × aperture height × π)/4] (Fig. 1c). Margin thickness at the aperture was the average of measurements of shell thickness at each end of the aperture length and height measurements (see Fig. 1c).

### Predation trials

The greatest response was expected from the veligers in the PV treatment; because of this and the number of control veligers that would have been needed to test both PV and P treatments, the predation trials only used veligers from the PV treatment. The methods for the predation trials were modeled

after Vaughn (2007). Five veligers from each of the PV and control replicates were haphazardly selected to be used in predation/survival trials. Half of these 30 PV veligers and half of the 30 control veligers were haphazardly chosen to be stained for 1 h in a 0.01% solution of Neutral Red. The veligers were then haphazardly assigned to 1 of 6 replicate trials, 3 for each of 2 combinations. The first combination consisted of 5 dyed control veligers + 5 undyed PV veligers. The second combination consisted of 5 dyed PV veligers + 5 undyed control veligers. Each replicate combination, along with 2 stage 5 zoeae of *H. nudus*, was placed in a randomly assigned (using a random number generator) well containing 12 ml FSW in a 6-well plate. Well plates were held in an incubator at 14°C; fluorescent lights were on in the incubator during the experiment. Every 30 min for 3.5 h, the number of dyed vs. undyed veligers in each well was counted.

### Statistical methods

Separate ANOVAs in SPSS (v. 22.0; IBM) were used to test the effects of treatment (control, predator cues [P], and predator + injured conspecific + diet cues [PV]) on shell height and on shell shape (SAR). Separate ANCOVAs in SPSS were used to test the effects of treatment on each of the following response variables: shell length, aperture area, and margin thickness. To account for variance due to size, shell height was included as a covariate as it was not affected by treatment (see Table 1). Tests were considered significant at  $p < 0.05$ . There was no interaction between treatment and the covariate for any of the dependent variables ( $p = 0.56, 0.08, 0.64$  for shell length, aperture area, and thickness, respectively); therefore, the interaction term was removed from the models. Subsequently, graphical inspection and data analysis indicated no statistical effect of replicate beaker ( $p \geq 0.362$ ), so it was removed from the models and all individuals within each treatment were pooled (Quinn & Keough 2002). Normality of the standardized residuals for each treatment was demonstrated by Shapiro-Wilk's test, homogeneity of variances was established by Levene's test, and all residuals were homoscedastic. Potential outliers were determined by looking for standardized residuals greater than  $\pm 3$  SD. One outlier was identified in the data for margin thickness but was left in because its removal did not change the outcome of the model. Post hoc analyses were performed with a Bonferroni adjustment.

Survival of veligers from the control and from the PV treatment in the paired-predation trials was analyzed in SPSS using a life tables survival analysis, which is a form of nonparametric analysis that generates and compares time-to-event (in this case, the event is death) distributions for each level of an independent variable and allows the comparison of survival curves between groups (Garson 2012).

## RESULTS

There was a significant effect of the covariate (shell height) on all 3 measured variables ( $p < 0.0005$ ), with increases in shell length, aperture area, and margin thickness as shell height increased, regardless of treatment (see Figs. 2–4). Post hoc analyses showed that veligers from the P treatment had significantly shorter shell lengths than those of veligers from the control or PV treatment, while the shell lengths of veligers from the control and PV treatment were not significantly different from one another (Table 1). These differences translate into differences in shell shape (SAR), which was smaller for veligers from the P treatment than for veligers from either the control or PV treatment (Tables 1 & 2). In other words,

Table 1. Summary of statistical results including ANOVAs (shell height and shell aspect ratio [SAR] of veligers of *Littorina scutulata*), and ANCOVAs with post hoc tests (shell length, aperture area, margin thickness), and life tables survival analysis. C: control, P: in the presence of predators, PV: in the presence of predators consuming conspecific veligers. \* $p < 0.05$

Variable	df	Test statistic	p
Height	2,267	$F = 0.907$	0.405
SAR	2,267	$F = 9.57$	<0.0005*
C vs. P			<0.0005*
C vs. PV			1.000
P vs. PV			0.003*
Length	2,266	$F = 20.741$	<0.0005*
C vs. P			<0.0005*
C vs. PV			0.092
P vs. PV			<0.0005*
Aperture area	2,266	$F = 24.303$	<0.0005*
C vs. P			<0.0005*
C vs. PV			<0.0005*
P vs. PV			1.000
Margin thickness	2,266	$F = 52.868$	<0.0005*
C vs. P			<0.0005*
C vs. PV			<0.0005*
P vs. PV			<0.0005*
Survival (C vs. PV)	1	$W = 4.104$	0.043*

veligers raised in the presence of predators alone developed shells that were significantly rounder than those raised in the presence of predators consuming conspecifics or those from the control, which were not significantly different from each other (Tables 1 & 2, Fig. 2).

Table 2. Summary of results from Vaughn (2007) and the present study on the exposure of veligers of *Littorina scutulata* to predators. Shell aspect ratio (SAR) data are shell length/shell height ( $\mu\text{m}$ )  $\pm$  SE. Data for aperture area and margin thickness are given in  $\mu\text{m}^2$  and  $\mu\text{m}$ , respectively,  $\pm$  SE. P: in the presence of predators, PV: in the presence of predators consuming conspecific veligers, na: treatment and variable were not included in Vaughn (2007)

Variable and treatments	Vaughn (2007)	This study
<b>SAR</b>		
Control	$1.16 \pm 0.41$	$1.192 \pm 0.004$
Predator kairomones (P)	$1.12 \pm 0.32^a$	$1.167 \pm 0.004$
Kairomones, alarm, diet (PV)	na	$1.188 \pm 0.004$
<b>Aperture area</b>		
Control	$21151 \pm 409.4$	$18179 \pm 127.1$
Predator kairomones (P)	$19075 \pm 288.0^a$	$17042 \pm 162.3$
Kairomones, alarm, diet (PV)	na	$16969 \pm 184.6$
<b>Margin thickness</b>		
Control		$15.8 \pm 0.2$
Predator kairomones (P)	na	$16.7 \pm 0.2$
Kairomones, alarm, diet (PV)		$17.8 \pm 0.2$

<sup>a</sup>The predator kairomone treatment used by Vaughn (2007) may have also included diet cues (see 'Introduction')

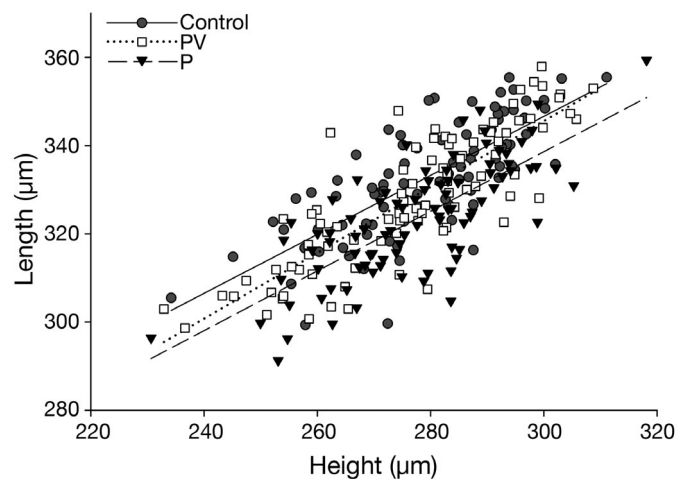


Fig. 2. Relationship between shell length and shell height in veligers of *Littorina scutulata* reared in a control environment (C), in the presence of predators (P), and in the presence of predators consuming conspecifics (PV). This relationship translates into differences in shell shape (shell aspect ratio, SAR: shell length/shell height). As SAR decreases, the shell becomes rounder.  $P < PV = C$  for both shell length and SAR



Veligers from the control had significantly larger aperture areas than those raised in the presence of predators consuming conspecifics and those raised in the presence of predators alone, which were not significantly different from each other (Tables 1 & 2, Fig. 3).

When raised in the presence of predators consuming conspecifics, veligers had significantly thicker margins than those of veligers raised in the presence of predators alone, and both of these groups had significantly thicker margins than those of veligers from the control (Tables 1 & 2, Fig. 4).

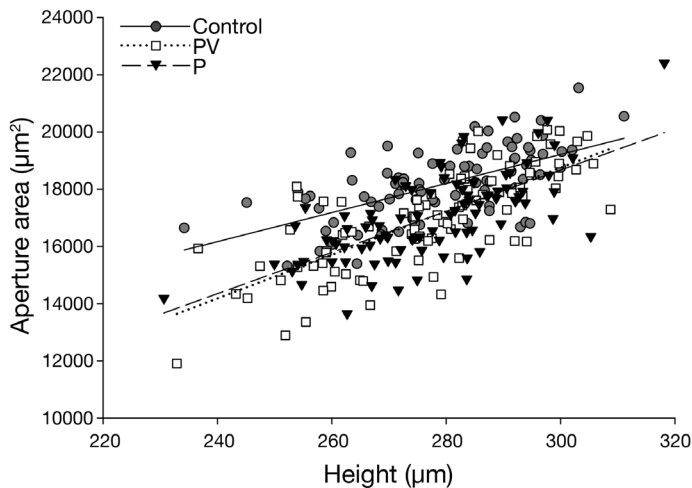


Fig. 3. Relationship between aperture area and shell height in veligers of *Littorina scutulata* reared in a control environment (C), in the presence of predators (P), and in the presence of predators consuming conspecifics (PV).  $PV = P < C$

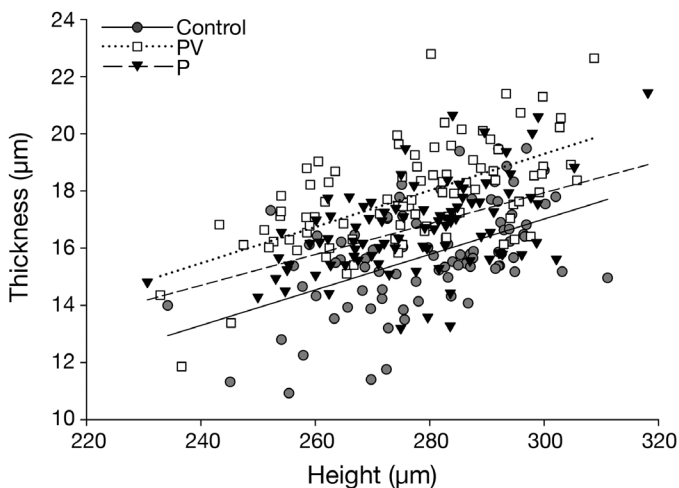


Fig. 4. Relationship between margin thickness at the aperture and shell height in veligers of *Littorina scutulata* reared in a control environment (C), in the presence of predators (P), and in the presence of predators consuming conspecifics (PV).  $C < P < PV$

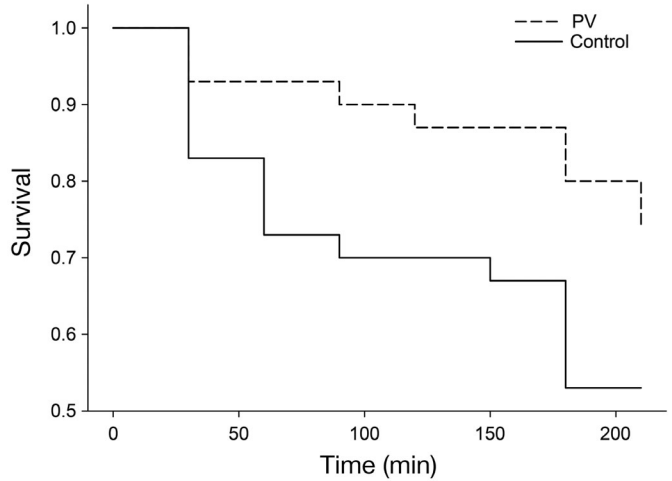


Fig. 5. Proportional survival of veligers of *Littorina scutulata* reared in the presence of predators consuming conspecifics (PV) and veligers reared in a control environment (C) over time when directly exposed to zoea predators.  $C < PV$ . The mean ( $\pm$ SE) number of survivors at the end of the study for the 6 replicate trials was  $2.7 \pm 0.3$  and  $3.8 \pm 0.3$  for the C and PV treatment groups, respectively

Veligers from the PV treatment survived better than those from the control. At 30, 120, and 210 min following the start of the experiment, the survival of veligers that had been raised in the presence of predators consuming conspecifics was 93, 87, and 74 %, while survival for control veligers was 83, 70, and 53 %. The results of the Wilcoxon test used in the survival analysis confirmed that experimental veligers from the PV treatment survived significantly better than those from the control (Table 1, Fig. 5).

## DISCUSSION

### Shell plasticity

Larval shells were altered in veligers grown in the presence of caged predators, and these larvae had increased survival (relative to control larvae) when exposed to uncaged predators. This is in agreement with findings by Vaughn (2007). Because we used a different species of crab as the predator than the one used by Vaughn (2007), our results show that more than one species of zoeal predator can induce phenotypic responses. We measured an additional morphological response (margin thickness) and found differences in the responses dependent on the type of experimental treatment. While the predator-only treatment induced changes in all 3 measured variables (SAR, aperture area, and margin thickness),

the response to the treatment of predators consuming conspecifics consisted of alterations in only 2 variables (SAR and margin thickness).

The experimental design in this study is most similar to the second experiment of Vaughn (2007), and it is to this study that results will be compared. Differences between the 2 studies to keep in mind when applying juxtapositional interpretations include, first, the potentially intermediary intensity of cues used by Vaughn (2007) that likely contained both predator kairomones and diet cues but without alarm cues, and second, the predators used are from different brachyuran crab families and may have affected the veligers differently. Veligers developed smaller apertures and rounder shells in response to the presence of zoeae of *Cancer* spp. (Vaughn 2007) and of *Hemigrapsus nudus* (Table 2; P treatment). Interestingly, when raised in the presence of predators consuming conspecifics (providing all 3 risk cues: predator kairomones, alarm cues, diet cues; PV treatment), the veligers also developed smaller apertures, but shell length (and thus SAR) was not significantly different from those in the control. These 2 features appear to be responses that can occur independently depending on the predatory cues present. Similarly, although both P and PV treatments resulted in changes in both aperture size and margin thickness, there still appears to be some autonomy of the 2 responses since veligers with similar aperture areas from the P and PV treatments had significantly different margin thicknesses.

When confronted with cues resulting from predators feeding on conspecifics or predator cues combined with crushed conspecifics, the measured response in adult gastropods (whether it be behavioral or morphological) is more pronounced than with isolated cues, indicative of an additive effect that correlates to the intensity of predation risk (e.g. Appleton & Palmer 1988, Alexander & Covich 1991, Trussell & Nicklin 2002, Dalesman et al. 2006, Bourdeau 2010). This was clearly the case for margin thickness in the experimental veligers. This larger degree of strengthening coupled with a reduction in aperture area may become the preferential response over changes in shell shape, which was not significantly different between veligers from the PV treatment and control, when the information provided indicates a riskier environment where the predator in question is actually consuming conspecifics. Although roundness might increase predator mishandling, a thicker margin and smaller entryway are likely to be most effective when confronted with a predator such as a zoea that enters via the aperture.

The adult gastropod shell has long been considered a fundamental defense against hungry predators with evidence coming from the fossil record (e.g. Vermeij et al. 1981) and studies on predator-prey interactions in extant species (e.g. Palmer 1979, Bertness & Cunningham 1981, Quensen & Woodruff 1997, Rosin et al. 2013). In addition to serving as an innate protective refuge, damage from non-fatal encounters is repairable and certain features of the shell (e.g. thickness, aperture size, apertural teeth, sculptural reinforcement) can be altered to enhance its effectiveness. The putative purpose of the larval shell is also protective, although definitive evidence confirming this has been scant. Hickman (1999, 2001) explored multiple mechanically defensive features of larval gastropod shells, most of which are restricted to marginal structures such as reinforced apertural beaks, velar notches, and rapid repair of broken apertural margins. It was only around the apertural margins that evidence of reinforcement was observed in this study, although the extent of thickening throughout the shell is unknown. Hickman (1999) proposed that reinforced apertural margins would have to be resorbed continually and re-secreted during growth to minimize the mass of a planktonic organism that relies on swimming and vertical adjustment for multiple aspects of larval ontogeny (e.g. Kingsford et al. 2002, Fuchs et al. 2004). However, added weight could be potentially beneficial, as it might cause the retracted veliger to sink faster and thus aid in escape from predation.

### Costs and trade-offs

Although the fitness benefit of the alterations found in the veligers is clear, with enhanced survival in the presence of predators, the potential costs are unknown as has been common in the study of inducible defenses (Tollrian & Harvell 1999). In adult gastropods, an observable and assumed cost of thickening or change in shell shape is reduced body mass, a feature not feasibly measured in larval veligers, and reduced linear growth (e.g. Appleton & Palmer 1988, Palmer 1990, DeWitt 1998, Trussell 2000, Trussell & Nicklin, 2002, Brookes & Rochette 2007). Although the rounder shells developed by veligers exposed to predators alone were due to a reduction in shell length compared to veligers from the control, this is unlikely to be a cost of thickening considering that veligers from the PV treatment developed the thickest margins with no difference in shell length compared to control veligers. Other potential costs

depend on the extent of shell thickening, its added weight, potential thinning in areas of the shell away from the apertural margins to minimize weight gain, and the method by which the thickening is implemented. One difficulty in the identification of costs of defensive morphologies is that many have latent effects that are not evident until later in ontogeny such as delayed metamorphosis, reduced size at metamorphosis, or lowered juvenile growth rate/survival (reviewed by Pechenik 2006).

### Cue considerations and future directions

The comparability of our results and those of Vaughn (2007) to the larval response *in situ* is unknown. In marine systems, the measure of importance of predation on planktonic larvae remains unclear in part because the estimation of natural encounter rates is complicated by factors such as plankton patchiness, background plankton, and a lack of knowledge of the likely concentrations of predators, meroplanktonic prey, and signals indicative of predation risk (e.g. Vaughn & Allen 2010, White et al. 2014).

In this study veliger larvae experienced predators or predators consuming conspecifics for the 4 wk duration of the experiment. Both frequency and concentration are known to impact the intensity of predatory cues and prey response. Despite the detectable effect of zoea predator cues on gastropod veligers, the induced defenses might have been exaggerated under unnatural predator densities and exposure. Many inducible defenses are positively correlated with predator density, size, or cue concentration (e.g. Wiackowski & Starońska 1999, Van Buskirk & Arioli 2002, Ferrari et al. 2010), although some reach a point where continued increases in stimulus concentration result in a lessened or lack of further morphological change (e.g. Palmer 1990, Duquette et al. 2005) or the production of exaggerated phenotypic responses (Trussell 1996). Most studies examining effects of predators on prey also provide constant exposure to predatory cues that can result in a more pronounced response than if the cues were temporally varied (e.g. Trussell 1996, Sih & McCarthy 2002, Chivers et al. 2008). Vaughn's (2007) study also included a separate experiment (Expt 1) with a treatment where larvae experienced transient exposure to predators (exposure to zoeae for 4 to 6 h on 1 day each week for 4 wk). This treatment was not fully replicated but showed that veligers developed smaller apertures and rounder shells than control lar-

vae. This result was similar to her second experiment and the present study where larvae had prolonged exposure to predators. The consistency of the results in Vaughn's (2007) 2 experiments and our own suggests that prolonged exposure to predators did not yield erroneous results.

In addition to expanding the number of studies demonstrating inducible morphological defenses in veliger larvae, an area worth exploring next is the potential for veligers to perceive differences between predatory and non-predatory zoeae (strictly herbivorous zoeae), native vs. non-native zoeae, or between other types of planktonic predators. Adult snails can distinguish between the effluents of predatory and non-predatory crabs (e.g. Marko & Palmer 1991), between native and introduced predators (Edgell & Neufeld 2008), and between effluents of damaged conspecifics vs. heterospecific or allopatric species in their responses (e.g. Jacobsen & Stabell 2004, Dalesman et al. 2007, Bourdeau 2010). The aperture-entry method of some zooplankton predators is the only implicated, potentially survivable threat that could select for the observed defenses (Hickman 2001, Vaughn 2007, this study). Other types of predatory threats to planktonic veligers relative to adult snails remain unclear but could lead to a fruitful area of study in the ability of veligers to distinguish between predators and adjust their responses accordingly, as is common in adult snails (e.g. DeWitt et al. 2000, Lakowitz et al. 2008, Bourdeau 2009, Hoverman et al. 2014).

Larval defenses in marine organisms continue to be an underexplored topic both in the discovery of novel examples and in further understanding of identified cases and their impacts in planktonic assemblages. Generalities concerning predator-prey interactions in adult organisms and in freshwater systems have generated a strong foundation with integrative potential in a marine planktonic environment. As we continue to better understand population dynamics and post-metamorphic plasticities in marine organisms, the capacity of these larvae to respond to their own set of environmental challenges should be an equally important consideration.

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## LITERATURE CITED

- ✦ Alexander JE, Covich AP (1991) Predator avoidance by the freshwater snail *Physella virgata* in response to the crayfish *Procambarus simulans*. *Oecologia* 87:435–442
- ✦ Appleton RD, Palmer AR (1988) Water-borne stimuli released by predatory crabs and damaged prey induce more predator-resistant shells in a marine gastropod. *Proc Natl Acad Sci USA* 85:4387–4391
- ✦ Atkinson D, Ciotti BJ, Montagnes DJS (2003) Protists decrease in size linearly with temperature: ca. 2.5% °C<sup>-1</sup>. *Proc R Soc B* 270:2605–2611
- ✦ Benard MF (2004) Predator-induced phenotypic plasticity in organisms with complex life histories. *Annu Rev Ecol Evol Syst* 35:651–673
- ✦ Bertness MD, Cunningham C (1981) Crab shell-crushing predation and gastropod architectural defense. *J Exp Mar Biol Ecol* 50:213–230
- ✦ Bollens SM, Frost BW (1989) Predator-induced diel vertical migration in a planktonic copepod. *J Plankton Res* 11:1047–1065
- ✦ Bourdeau PE (2009) Prioritized phenotypic responses to combined predators in a marine snail. *Ecology* 90:1659–1669
- ✦ Bourdeau PE (2010) Cue reliability, risk sensitivity and inducible morphological defense in a marine snail. *Oecologia* 162:987–994
- ✦ Brönmark C, Lakowitz T, Hollander J (2011) Predator-induced morphological plasticity across local populations of a freshwater snail. *PLOS ONE* 6:e21773
- ✦ Brookes JI, Rochette R (2007) Mechanism of a plastic phenotypic response: predator-induced shell thickening in the intertidal gastropod *Littorina obtusata*. *J Evol Biol* 20:1015–1027
- ✦ Charpentier CL, Wright AJ, Cohen JH (2017) Fish kairomones induce spine elongation and reduce predation in marine crab larvae. *Ecology* 98:1989–1995
- ✦ Chivers DP, Smith RJF (1998) Chemical alarm signalling in aquatic predator-prey systems: a review and prospectus. *Ecoscience* 5:338–352
- ✦ Chivers DP, Zhao X, Brown GE, Marchant TA, Ferrari MCO (2008) Predator-induced changes in morphology of a prey fish: the effects of food level and temporal frequency of predation risk. *Evol Ecol* 22:561–574
- ✦ Cieri MD, Stearns DE (1999) Reduction of grazing activity of two estuarine copepods in response to the exudate of a visual predator. *Mar Ecol Prog Ser* 177:157–163
- ✦ Covich AP (2010) Winning the biodiversity arms race among freshwater gastropods: competition and coexistence through shell variability and predator avoidance. *Hydrobiologia* 653:191–215
- ✦ Dalesman S, Rundle SD, Coleman RA, Cotton PA (2006) Cue association and antipredator behaviour in a pulmonate snail, *Lymnaea stagnalis*. *Anim Behav* 71:789–797
- ✦ Dalesman S, Rundle SD, Bilton DT, Cotton PA (2007) Phylogenetic relatedness and ecological interactions determine antipredator behavior. *Ecology* 88:2462–2467
- ✦ DeWitt TJ (1998) Costs and limits of phenotypic plasticity: tests with predator-induced morphology and life history in a freshwater snail. *J Evol Biol* 11:465–480
- DeWitt TJ, Robinson BW, Wilson DS (2000) Functional diversity among predators of a freshwater snail imposes an adaptive trade-off for shell morphology. *Evol Ecol Res* 2:129–148
- ✦ Duquette SL, Altwegg R, Anholt BR (2005) Factors affecting the expression of inducible defenses in *Euplotes*: genotype, predator density and experience. *Funct Ecol* 19:648–655
- ✦ Edgell TC, Neufeld CJ (2008) Experimental evidence for latent developmental plasticity: intertidal whelks respond to a native but not an introduced predator. *Biol Lett* 4:385–387
- ✦ Ferrari MCO, Wisenden BD, Chivers DP (2010) Chemical ecology of predator-prey interactions in aquatic ecosystems: a review and prospectus. *Can J Zool* 88:698–724
- ✦ Fuchs HL, Mullineaux LS, Solow AR (2004) Sinking behavior of gastropod larvae (*Ilyanassa obsoleta*) in turbulence. *Limnol Oceanogr* 49:1937–1948
- Garson GD (2012) Life tables and Kaplan-Meier analysis. Statistical Associates Publishers, Asheboro, NC
- ✦ Grason EW, Miner BG (2012) Behavioral plasticity in an invaded system: non-native whelks recognize risk from native crabs. *Oecologia* 169:105–115
- ✦ Hickman CS (1999) Adaptive function of gastropod larval shell features. *Invertebr Biol* 118:346–356
- ✦ Hickman CS (2001) Evolution and development of gastropod larval shell morphology: experimental evidence for mechanical defense and repair. *Evol Dev* 3:18–23
- ✦ Hoverman JT, Cothran RD, Relyea RA (2014) Generalist versus specialist strategies of plasticity: snail responses to predators with different foraging modes. *Freshw Biol* 59:1101–1112
- ✦ Hughes RN, Elner RW (1979) Tactics of a predator, *Carcinus maenas*, and morphological responses of the prey, *Nucella lapillus*. *J Anim Ecol* 48:65–78
- ✦ Jacobsen HP, Stabell OB (1999) Predator-induced alarm responses in the common periwinkle, *Littorina littorea*: dependence on season, light conditions, and chemical labeling of predators. *Mar Biol* 134:551–557
- ✦ Jacobsen HP, Stabell OB (2004) Antipredator behaviour mediated by chemical cues: the role of conspecific alarm signaling and predator labeling in the avoidance response of a marine gastropod. *Oikos* 104:43–50
- ✦ Kats LB, Dill LM (1998) The scent of death: chemosensory assessment of predation risk by prey animals. *Ecoscience* 5:361–394
- ✦ Kemp P, Bertness MD (1984) Snail shape and growth rates: evidence for plastic shell allometry in *Littorina littorea*. *Proc Natl Acad Sci USA* 81:811–813
- Kingsford MJ, Leis JM, Shanks A, Lindeman KC, Morgan SG, Pineda J (2002) Sensory environments, larval abilities and local self-recruitment. *Bull Mar Sci* 70:s309–s340
- ✦ Krist AC (2002) Crayfish induce a defensive shell shape in a freshwater snail. *Invertebr Biol* 121:235–242
- Lakowitz T, Brönmark C, Nyström P (2008) Tuning in to multiple predators: conflicting demands for shell morphology in a freshwater snail. *Freshw Biol* 53:2184–2191
- ✦ Lass S, Spaak P (2003) Chemically induced anti-predator defenses in plankton: a review. *Hydrobiologia* 491:221–239
- ✦ Marchinko KB (2003) Dramatic phenotypic plasticity in barnacle legs (*Balanus glandula* Darwin): magnitude, age dependence, and speed of response. *Evolution* 57:1281–1290
- ✦ Marko PB, Palmer R (1991) Responses of a rocky shore gastropod to the effluents of predatory and non-predatory crabs: avoidance and attraction. *Biol Bull (Woods Hole)* 181:363–370
- ✦ McCarthy TM, Fisher WA (2000) Multiple predator-avoidance behaviours of the freshwater snail *Physella heter*

- erostropha pomila*: responses vary with risk. *Freshw Biol* 44:387–397
- Moody RM, Aronson RB (2012) Predator-induced defenses in a salt-marsh gastropod. *J Exp Mar Biol Ecol* 413:78–86
- Neill WE (1990) Induced vertical migration in copepods as a defense against invertebrate predation. *Nature* 345:524–526
- Palmer AR (1979) Fish predation and the evolution of gastropod shell sculpture: experimental and geographic evidence. *Evolution* 33:697–713
- Palmer AR (1985) Adaptive value of shell variation in *Thais lamellosa*: effect of thick shells on vulnerability to and preference by crabs. *Veliger* 27:349–356
- Palmer AR (1990) Effect of crab effluent and scent of damaged conspecifics on feeding, growth, and shell morphology of the Atlantic dogwhelk *Nucella lapillus* (L.). *Hydrobiologia* 193:155–182
- Pechenik JA (2006) Larval experience and latent effects: metamorphosis is not a new beginning. *Integr Comp Biol* 46:323–333
- Quensen JF III, Woodruff DS (1997) Associations between shell morphology and land crab predation in the land snail *Cerion*. *Funct Ecol* 11:464–471
- Quinn GP, Keough MJ (2002) *Experimental design and data analysis for biologists*. Cambridge University Press, Cambridge
- Relyea RA (2002) Competitor-induced plasticity in tadpoles: consequences, cues, and connections to predator-induced plasticity. *Ecol Monogr* 72:523–540
- Rosin ZM, Kobak J, Lesicki A (2013) Differential shell strength of *Cepaea nemoralis* colour morphs — implications for their anti-predator defence. *Naturwissenschaften* 100:843–851
- Schneider CA, Rasband WS, Eliceiri KW (2012) NIH Image to ImageJ: 25 years of image analysis. *Nat Methods* 9:671–675
- Sih A, McCarthy TM (2002) Prey responses to pulses of risk and safety: testing the risk allocation hypothesis. *Anim Behav* 63:437–443
- Stelzer CP (2002) Phenotypic plasticity of body size at different temperatures in a planktonic rotifer: mechanisms and adaptive significance. *Funct Ecol* 16:835–841
- Strathmann MF (1987) *Reproduction and development of marine invertebrates of the northern Pacific coast*. University of Washington Press, Seattle, WA
- Strathmann RR, Fenaux L, Sewell AT, Strathmann MF (1993) Abundance of food affects relative size of larval and postlarval structures of a molluscan veliger. *Biol Bull (Woods Hole)* 185:232–239
- Sultan SE (2000) Phenotypic plasticity for plant development, function and life history. *Trends Plant Sci* 5:537–542
- Todd PA (2008) Morphological plasticity in scleractinian corals. *Biol Rev Camb Philos Soc* 83:315–337
- Tollrian R, Harvell CD (1999) *The ecology and evolution of inducible defenses*. Princeton University Press, Princeton, NJ
- Trussell GC (1996) Phenotypic plasticity in an intertidal snail: the role of a common crab predator. *Evolution* 50:448–454
- Trussell GC (1997) Phenotypic plasticity in the foot size of an intertidal snail. *Ecology* 78:1033–1048
- Trussell GC (2000) Predator-induced plasticity and morphological trade-offs in latitudinally separated populations of *Littorina obtusata*. *Evol Ecol Res* 2:803–822
- Trussell GC, Nicklin MO (2002) Cue sensitivity, inducible defense, and trade-offs in a marine snail. *Ecology* 83:1635–1647
- Turner RD (1976) Fixation and preservation of molluscan zooplankton. In: Steedman HF (ed) *Zooplankton fixation and preservation*. UNESCO Press, Paris, p 290–300
- Van Buskirk J, Arioli M (2002) Dosage response of an induced defense: How sensitive are tadpoles to predation risk? *Ecology* 83:1580–1585
- Van Donk E, Ianora A, Vos M (2011) Induced defences in marine and freshwater phytoplankton: a review. *Hydrobiologia* 668:3–19
- Vaughn D (2007) Predator-induced morphological defenses in marine zooplankton: a larval case study. *Ecology* 88:1030–1039
- Vaughn D (2010) Why run and hide when you can divide? Evidence for larval cloning and reduced larval size as an adaptive inducible defense. *Mar Biol* 157:1301–1312
- Vaughn D, Allen JD (2010) The peril of the plankton. *Integr Comp Biol* 50:552–570
- Vermeij GJ (1974) Marine faunal dominance and molluscan shell form. *Evolution* 28:656–664
- Vermeij GJ, Schindel DE, Zipser E (1981) Predation through geological time: evidence from gastropod shell repair. *Science* 214:1024–1026
- Walls SC, Belanger SS, Blaustein AR (1993) Morphological variation in a larval salamander: dietary induction of plasticity in head shape. *Oecologia* 96:162–168
- White JW, Morgan SG, Fisher JL (2014) Planktonic larval mortality rates are lower than widely expected. *Ecology* 95:3344–3353
- Wiackowski K, Starońska A (1999) The effect of predator and prey density on the induced defence of a ciliate. *Funct Ecol* 13:59–65

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