

PREDATOR-INDUCED MORPHOLOGICAL DEFENSES IN MARINE ZOOPLANKTON: A LARVAL CASE STUDY

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Abstract. While there are numerous reports of predator-induced morphological defenses for freshwater zooplankton, freshwater larvae, and benthic marine animals, a literature search revealed no reports of predator-induced morphological defenses for marine zooplankton. Rarity of predator-induced morphological defenses in marine zooplankton would imply a difference in predation risks compared to those experienced by freshwater organisms and benthic marine adults, whereas the presence of such plasticity in defenses would imply that risks are modified by developmental responses. This study reports a predator-induced change in defenses and vulnerability of a marine planktonic larva. Specifically, when reared in the presence of zoea larvae of *Cancer* spp., veliger larvae of the intertidal snail *Littorina scutulata* developed significantly smaller shell apertures and rounder shells than did cohort veligers reared in the absence of predator cues. Pairwise predation trials demonstrated that veligers reared with caged zoeas throughout development had greater survival than predator-naïve veligers during short-term exposure to zoeas. The development of predator-induced morphological defenses by some marine larvae introduces a range of testable hypotheses on developmental plasticity that reduces vulnerability of planktonic larvae and other marine zooplankton to predators.

Key words: developmental plasticity; functional morphology; inducible defense; *Littorina scutulata*; marine invertebrate larvae; ontogeny; predator-induced plasticity; zoea.

INTRODUCTION

The variety and ubiquity of predator-induced defensive responses suggests that plasticity is important both ecologically and evolutionarily. Inducible defenses are well documented in varied habitats, to a diverse array of predators, and throughout ontogeny (Tollrian and Harvell 1999). In response to the risk of predation, organisms alter their behavior (Lima and Dill 1990, Neill 1990, Bollens and Frost 1991, DeMeester et al. 1999, Sih 2004), their life histories (Crowl and Covich 1990, Warkentin 1995, Reimer 1999, Tollrian and Dodson 1999, Van Buskirk and Saxer 2001, Benard 2004, Orizaola and Braña 2005), and their defensive morphologies (Havel 1985, Harvell 1986, Young 1987, Palmer 1990, Milewski et al. 1991, Brönmark and Miner 1992, Gowda 1997, McCollum and Leimberger 1997, Kuhlmann et al. 1999, Van Buskirk and Schmidt 2000, Young et al. 2003). Despite the pervasiveness of predator-induced morphologies exhibited by diverse plants and animals in both terrestrial and aquatic environments, at present there is an absence of studies documenting this type of inducible defense in marine zooplankton.

If predator-induced morphologies are truly rare in marine zooplankton, then the marine planktonic envi-

ronment, the largest habitat on earth, contrasts with both freshwater planktonic environments and marine benthic environments, which provide some of the best-documented cases of predator-induced defensive morphologies (Gilbert 1966, Gilbert and Stemberger 1984, Havel 1985, Harvell 1986, Lively 1986, Palmer 1990, Tollrian 1993, Trussell 1996). Also, predator-induced morphologies are extensively documented in freshwater larvae of some frogs (Van Buskirk and Relyea 1998, Relyea 2003, Relyea and Auld 2005) and insects (Arqvist and Johansson 1998, Dahl and Peckarsky 2002), indicating that predator-induced morphological change is not restricted to post-larval stages. Predator-induced morphologies for benthic marine animals include the thickening of gastropod shells (Palmer 1990, Trussell 1996, 2000, Trussell and Smith 2000), the growth of spines along the frontal membrane of bryozoan colonies (Harvell 1986), pronounced allometric changes in barnacles (Lively 1986), and increased spicule concentration in a sponge (Hill and Hill 2002). A predator-induced morphological defense is also reported for marine phytoplankton (Tang 2003). Given that freshwater zooplankton, benthic marine invertebrates, and at least some marine phytoplankton respond to potential predators through induced changes in morphology, inducible morphological defenses might be expected in marine larvae and other marine zooplankton. None, to my knowledge, have been reported.

One possibility is that the diversity of predators encountered by marine zooplankton may render induc-

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ible morphological defenses ineffective. Multiple predators induce morphological defenses in a freshwater cladoceran (LaForsch and Tollrian 2004), but co-occurring marine predators appear to be more diverse than freshwater predators in the manner in which they attack.

Other differences between oceans and lakes do not appear to preclude induced morphological defenses. Marine and freshwater environments differ in size and connectivity, but despite large-scale mixing processes, many marine planktonic predators are abundant only for short periods or in patchy or restricted distributions. Coastal ocean currents can concentrate zooplankton at fronts, increasing the probability of encounters between seasonally variable predators and prey (Shanks et al. 2003).

Marine and freshwater planktonic environments might differ in evolutionary history of predator-prey interactions, but both benthic and pelagic marine animals share a history of escalating defenses from the Paleozoic onward (Vermeij 1977), a trend evident in both adult and larval gastropod shells (Vermeij 1987, Nützel and Fryda 2003). Phenotypically plastic responses to predators in ecological time may be a result of the long-term evolution and escalation of interactions between predators and prey (Agrawal 2001).

There are four conditions under which evolution should favor plastic rather than permanent defensive strategies (Adler and Harvell 1990, Tollrian and Harvell 1999). First, predation should be variable, but occasionally strong. Second, there must be reliable cues to activate the defense. Third, the defense must be effective against predation. Finally, when defenses are induced they must confer a cost-benefit savings over permanent defensive strategies. This assumes a trade-off in terms of overall fitness between the costs and benefits of defensive traits. If there is no trade-off, a trait, defensive or otherwise, should be fixed rather than plastic (Stearns 1992). Each of these conditions may be met in the marine planktonic environment.

To begin, mortality of marine larvae estimated from natural populations is high and variable (Strathmann 1985, Young and Chia 1987, Rumrill 1990, Morgan 1995), although the causes of larval mortality are poorly understood. While transport in currents accounts for some apparent losses, these processes cannot explain the estimated mortality rates of cohorts of larvae in enclosed bays and lagoons (Landry 1978, Lamare and Barker 1999). Predation is a likely cause of this elevated but variable mortality. In addition, the abundance of predators and intensity of predation varies over small spatial scales (Bullard and Hay 2002). Thus the first condition favoring the evolution of inducible defenses, occasionally strong but variable predation, is expected in marine planktonic environments.

Secondly, widespread predator-induced changes in morphology in both marine and aquatic organisms indicate that water is a favorable medium for transmit-

ting chemical signals (Lass and Spaak 2003), such as predator-produced "kairomones" (Brown et al. 1970) or cues emitted by damaged conspecifics. Thus the second condition is met.

The third condition is met by the presence of defensive morphological characters in marine planktonic animals. In the late 1920s, Walter Garstang noted that larval gastropods respond to planktonic predators by withdrawing into their shell and shutting the apertural opening with an opercular door (Garstang 1929). Garstang's observations suggested that induced defensive behavior coupled with the armor of a larval shell disrupts predation. More recently, research with marine predators has demonstrated that larval shells and spines are defensive (Morgan 1995). Larval shells not only function as the first line of defense against predators, but shells damaged by sublethal predation can be repaired (Hickman 2001). Polychaete trochophores experience lower mortality against multiple predators once long setae have developed (Pennington and Chia 1994). Thus, defensive structures in marine larvae appear to be effective against some planktonic predators.

The fourth condition, trade-offs in production of defensive structures, has not, to my knowledge, been examined for marine zooplankton, but shells and spines affect mass and swimming and their production requires energy and materials. Given the presumed trade-offs associated with inducible defenses, some marine larvae, especially those characterized by extended planktonic development, and therefore prolonged but variable exposure to predation, may have evolved inducible defenses.

The molluscan shell is particularly well suited to the analysis of predator-induced changes during larval development as it serves a defensive function in both adults and larvae. Only a few studies have analyzed the protective properties of the larval gastropod shell (Garstang 1929, Hickman 2001, 2004), but many have examined the manner in which predation drives the diversity of adult shell forms (Vermeij 1978, 1979, 1987, Palmer 1979, West and Cohen 1996). Multiple predators of gastropods use different means of extracting the soft-bodied snail from the protective shell (Vermeij 1987, DeWitt et al. 2000). While durophagous predators crush the molluscan armor to expose the vulnerable soft body, others access their prey through the "front door," peeling away the shell at the apertural lip. The numerous and varied predator-induced changes in shell structures of adult gastropods, including the addition of shell surface sculpture, a reduction in the size of the apertural opening, and thickening of shell walls and apertural lips, mirror this predator diversity. Larval gastropods are also confronted by shell-breaking and entering planktonic predators (Hickman 2001), which suggests that similar predator-induced changes may occur in larval shell morphology.

To test the ability of larval gastropods to respond to potential predators through induced changes in shell

morphology, I experimentally reared gastropod veligers in the presence and absence of zoea larvae of crabs. These zoeas, of *Cancer* spp., are abundant during part of the reproductive season of the gastropod *Littorina scutulata* (Strathmann 1987). I tested the following hypotheses: (1) stimuli from a predetermined planktonic predator induce developmental changes in the larval shell and (2) larvae exhibiting predator-induced differences in development have enhanced survival during subsequent interactions with predators.

METHODS

Preliminary predation trials pairing *Littorina scutulata* veligers against numerous spatially and temporally co-occurring planktonic predators demonstrated that *Cancer* spp. zoea larvae were appropriate predators for the experiments. *Cancer* spp. zoeas were aggressive and occasionally successful predators on the veligers. The occasional success of these predators was relevant to the evolution of inducible defenses. If zoeas always attacked successfully, there would be no opportunity or demand for an induced response to be manifested in the larval gastropods. If zoeas were never successful or never attacked, there would be no need for an enhanced defense.

Collection of prey and predators

I collected adult *Littorina scutulata* from the rocky shore at Friday Harbor Laboratories (FHL), San Juan Island, Washington, USA. Four to six adult littorines were placed in mesh-lined plastic containers and floated in flow-through aquaria. After 48 h, several hundred disk-shaped egg capsules containing an average of four embryos were collected and maintained in 0.45- μ m filtered seawater (FSW) at 14°C for 1 wk. I then assigned hatched, actively swimming veligers to experimental treatments. Flow-through aquaria, near ambient sea temperature, provided temperature control for all experiments.

Late-stage *Cancer* spp. zoeas (~3.3 mm from the tip of rostral spine to the tip of the dorsal spine) were collected at the FHL dock at night by submersing a light just below the water's surface. Zoeas swarmed to the light from spring through mid-summer, were dipped from the water, sorted to genus and stage, and maintained overnight (~12 h) in flow-through aquaria prior to being assigned to experimental treatments. Zoeas were offered cohort prey veligers during this holding period.

Experimental set-up

In experiment 1 in 2004, veligers were maintained in 59.1-mL shot glasses (Hot Shots, Bangkok, Thailand) at a density of 5 veligers in 40 mL of 0.45- μ m FSW. Shot glasses were large enough to accommodate actively swimming *L. scutulata* veligers and two *Cancer* spp. zoeas yet small enough to ensure contact between predator and prey and allow multiple replicates per

treatment within a single aquarium. Also, the tapered walls of the shot glass permit clear, unobstructed microscopy.

Veligers not assigned to a particular treatment (cohort prey) were transferred to 1000-mL beakers at an initial density of 100 larvae in 800 mL of FSW. Cohort prey served as food for field-captured zoeas in the "crushed cohort" treatment described below.

In experiment 2 in 2005, veligers were maintained in 1000-mL glass beakers at a density of 50 veligers in 800 mL of FSW. The larger experimental containers accommodated 56 \times 43 mm plastic predator cages lined with 244- μ m mesh netting (tea infusers; Toby TeaBoy, Aldridge, UK) without compromising the activity, growth, and development of the experimental veligers.

In all experimental treatments, water was changed every 3 d, at which time veligers were fed a mixture of microalgae (*Rhodomonas* sp., *Nanochloropsis* sp., *Isochrysis galbana*) ad libitum, and their condition and activity level were noted.

Description of experimental treatments

In experiment 1 in 2004 veligers were reared in shot glasses in: (1) temporally limited but physically direct contact with *Cancer* spp. zoeas (direct contact); (2) FSW containing predator kairomones and cues from injured cohort veligers (crushed cohort); and (3) FSW (control 1). Thus, experiment 1 included two experimental treatments and an experimental control.

Veligers reared in direct contact with predators were in 40 mL of 0.45- μ m FSW at a density of five veligers per shot glass, without predators for the first week. All veligers in this group were then transferred into one of two shot glasses, each of which contained two active, field-captured zoea larvae (*Cancer* spp.). The veligers and zoeas interacted for a period of 4–6 h. Following this interaction, zoeas were removed, veliger survival and condition were recorded, and damaged veligers were photographed. Surviving veligers were transferred into clean shot glasses (at a density of 3–5 veligers in 40 mL of FSW) and maintained in predator-free seawater for the remainder of the week. Interactions with predators took place weekly for a period of 4–6 h over the course of the four-week study. This treatment was not fully replicated because the veligers were pooled for weekly encounters with zoeas and subsequently redistributed into shot glasses for comparable rearing conditions. True replication was not practical because variation in the behavior of wild-caught zoeas (some feeding, some molting), would have produced large differences in veliger mortality between replicates.

In the second treatment in experiment 1, experimental veligers were reared in shot glasses at a density of five veligers in 40 mL of filtered effluent resulting from the interaction between *Cancer* spp. zoea and cohort prey veligers. To produce the effluent, two field-captured zoeas were placed into shot glasses with 10 cohort prey veligers and 40 mL of 0.45- μ m FSW. The zoeas and prey

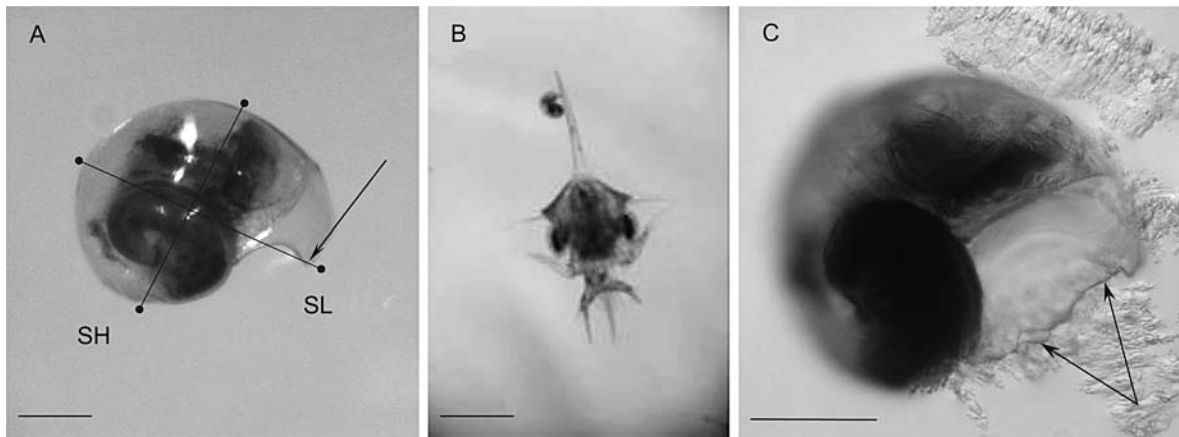


FIG. 1. (A) A veliger larva of the checkered periwinkle, *Littorina scutulata*. Shell length is the maximum dimension across the shell from the tip of the growing edge (indicated by the arrow). Shell height is the longest dimension perpendicular to length. Scale bar = 0.1 mm. (B) A zoea larva of *Cancer* spp. prior to capturing its prey, a veliger larva of the checkered periwinkle, *Littorina scutulata*, near its dorsal spine. Scale bar = 1 mm. (C) Arrows indicate sublethal damage along the edge of the aperture of a littorine veliger reared in direct contact with zoea predators. Scale bar = 0.1 mm.

veligers interacted for 3 d, at which point the water and any damaged or dead veligers from each of the 10 shot glasses were combined, resulting in ~400 mL of effluent. The combined effluent was then passed through a 0.45- μ m filter to allow the transfer of predator kairomones and stimuli from injured cohort veligers while excluding large particles (i.e., crushed veligers, broken shell). This filtrate served as the rearing medium for experimental veligers in this treatment. This experimental treatment was replicated 10 times.

In experiment 2 in 2005, littorine veligers were exposed to caged zoea predators. The experimental veligers were maintained in 1000-mL beakers at a density of 50 veligers in 800 mL of FSW. Zoeas were field-captured as in experiment 1 and offered cohort prey veligers in the laboratory prior to confinement in a cage. A single 56 \times 43 mm mesh-lined cage containing five field-caught *Cancer* spp. zoeas floated in each beaker. The cage permitted the transfer of predator kairomones but prevented direct contact between predator and prey. Caged zoeas were replaced every 3 d during the water changes and feedings. There was no mortality of zoeas prior to or during confinement in cages. Experimental controls (control 2) had an empty cage and the same initial density of veligers. The experimental treatment and control were each replicated five times. At termination after 35 d, approximately two-thirds of the veligers had developed to the pediveliger stage and were therefore competent for settlement and metamorphosis.

Behavioral observations

Video clips documented the manner in which zoeas captured and subsequently accessed the soft bodies of larval gastropods and provided estimates of the handling time of veligers by zoeas.

Analysis of the larval shell

For measurements of length and height of each larval shell at the conclusion of experiments, water was withdrawn from each live veliger at the center of a depression slide to restrict its movement. Linear dimensions were measured ($\pm 10 \mu$ m) with an ocular micrometer. Veligers were positioned aperture down in repose with length defined as the maximum distance across the shell and height defined as the longest dimension perpendicular to length (Fig. 1A). The shape of the larval shell was expressed as the aspect ratio (shell length:shell height). The aspect ratio provided a two-, rather than three-, dimensional measure of form.

In 2004, to examine larval shell surface microsculpture and aperture size, I prepared an unbiased haphazard subset of veligers ($n = 10$ from each treatment in experiment 1) for scanning electron microscopy (SEM). Veligers transferred from seawater to freshwater and subsequently to 90% ethanol fully retracted into their shells. After removal of salt crystals, shells were air-dried, mounted with the aperture up, coated with gold/palladium alloy, and examined at 320 \times magnification with the stub tilted until the image yielded the largest measurable apertural area. Measurements of length and width of the aperture were conducted with Image J (version 1.32j; National Institutes of Health, Bethesda, Maryland, USA) from SEM micrographs. Area of apertural opening was estimated as the area of an ellipse [(aperture length \times aperture width \times pi)/4]. To account for differences in apertural area resulting from differences in overall size (Relyea 2002), the area of the aperture was analyzed with regards to the overall length and height of the shell of each individual veliger.

In experiment 2 in 2005, I used a light microscope with ocular micrometer ($\pm 10 \mu$ m) to measure the aperture of

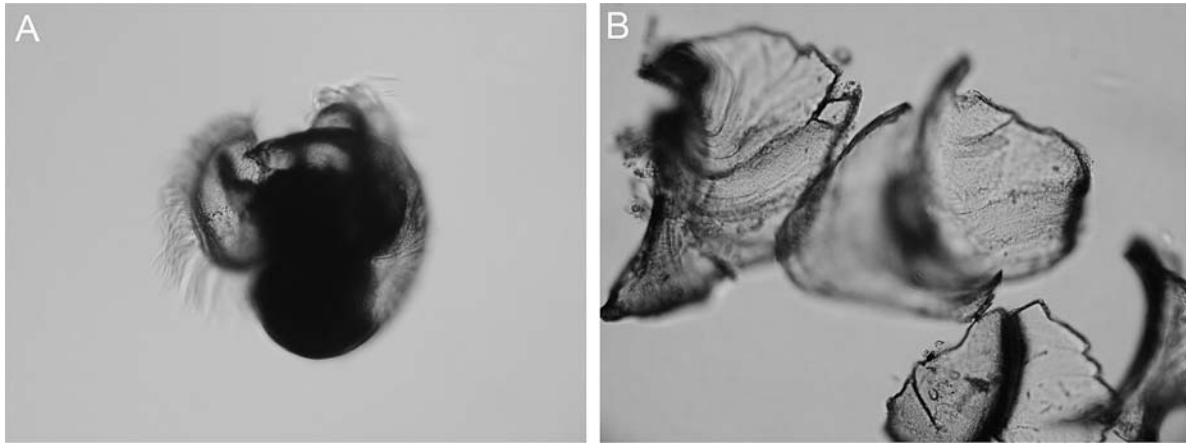


PLATE 1. (A) *Littorina scutulata* veliger swimming with ciliated lobes that extend from the shell through the apertural opening. The shell of the veliger is approximately 325 microns in length. (B) Shell debris indicating a successful attack by a zoea predator. Photo credits: D. Vaughn.

veligers reared in the presence and absence of caged zoea predators. Veligers were placed aperture down on a slide and immobilized by a cover slip supported by clay feet. The slide was then inverted to orient the aperture upwards for viewing at 200 \times magnification. Apertural area was then estimated as in 2004.

Pairwise predation trials

At the conclusion of experiment 2 in 2005, pairwise predation trials determined whether there were differences in survivorship of experimental and control veligers exposed directly to zoeas. An unbiased sample of 10 veligers from each treatment was placed into a shot glass with two field-captured zoeas. Staining veligers with neutral red distinguished the experimental veligers from the controls. The trials were replicated six times. Control veligers were marked with the dye in three trials, and experimental veligers were marked with the dye in the other three. The number of survivors from each treatment in each trial was recorded every half hour over the course of 3.5 h. Photographs of shell debris confirmed predation on veligers by zoeas.

Statistical analyses

I analyzed four morphological traits of the larval shell of experimental and control veligers: shell length, shell height, shell aspect ratio, and cross-sectional area of the shell's aperture. The data in experiment 1 in 2004 were heteroscedastic and therefore analyzed with a nonparametric Kruskal-Wallis single factor analysis of variance by ranks, followed by Tukey-Kramer-type post hoc tests, to compare the mean rank of each morphological trait exhibited by veligers in the direct contact, crushed cohort, and control treatments. Because of varying activity of zoeas, one treatment (direct exposure to zoeas) was pseudoreplicated in that veligers were periodically pooled for each exposure to predators and then reassigned to separate containers for rearing. This

treatment was included for comparison with the replicated treatments.

In experiment 2 in 2005, I compared mean morphological trait values of veligers in the caged predator and control treatments with Student *t* tests. Differential survival of experimental and control veligers in the paired predation trials were analyzed with two-tailed paired *t* tests.

RESULTS

Observations of predator-prey interactions

Observations of interactions between *Littorina scutulata* veligers and *Cancer* spp. zoeas did not reveal a consistent protective behavioral response in the veligers. While some veligers ceased swimming, retracted their velum, and sank to the bottom of the shot glass, others continued to swim, often colliding with and occasionally coming to rest on or near zoeas (Fig. 1B). Only when captured did the veligers consistently withdraw into their shells. Although zoeas occasionally attacked veligers that had retracted into their shells, most attacks took place when the larval snails were actively swimming (see Plate 1). Once captured, zoeas rotated the larval snail while actively chipping away the edge of the aperture, thereby peeling back the larval shell from the aperture's lip. Veligers that survived attacks by zoeas usually bore scars in the form of chipped apertural edges (Fig. 1C), while successful predation resulted from more-extensive fracturing of the larval shell at the aperture (see Plate 1). Handling time was \sim 3 min in each observed predation event.

Differential mortality across experimental treatments

In experiment 1 in 2004, veligers exposed directly to zoeas had 40% mortality during the four-week study in contrast to \sim 10% mortality in the other treatments (crushed cohort and control group 1). The mortality rate of veligers in the crushed cohort treatment and the

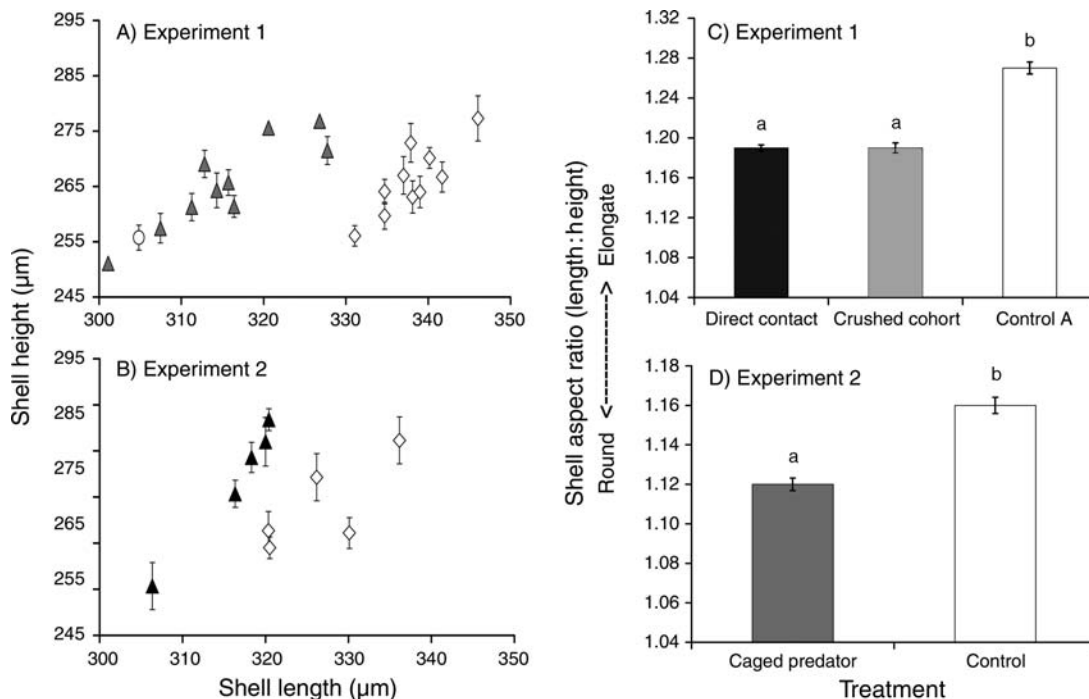


FIG. 2. (A) Experiment 1: The relationship between height and length of the larval shell of veligers in direct contact with *Cancer* spp. zoeas (open circle), in filtrate containing both predator kairomones and stimuli from damaged cohort prey (shaded triangles), and the corresponding control group (open diamonds). The small variance in three replicates in the treatment containing both predator kairomones and stimuli from damaged cohorts (shaded triangles) obscures the error bars. (B) Experiment 2: The relationship between length and height of the larval shells of veligers in the caged predator treatment (solid triangles) and in the cage control (open diamonds). (C) Experiment 1: Shell aspect ratio (round to elongate) of veligers reared in direct contact with zoeas (black bar), in filtrate containing predator kairomones and stimuli from damaged cohort prey (shaded bar), and in the control treatment (open bar). (D) Experiment 2: Shell aspect ratio of veligers in the caged predator treatment (shaded bar) and the control (open bar). Data are mean shell aspect ratios (shell length to shell height) \pm SE. Shared letters above bars within panels indicate means that are not significantly different.

corresponding control was unrelated to predation, whereas the elevated rates of mortality and the shell debris in the direct contact treatment reflects successful predation on veligers by zoeas.

Analyses of the larval shell

In both 2004 and 2005, the experimental treatments resulted in significant differences in veliger shell form. Univariate tests indicate that the shape of the larval shell (aspect ratio) and in some cases the size of the shell's apertural opening were affected (Appendix). Shells of veligers with no exposure to predators (controls) were longer at a given shell height even though there was overlap in overall size (shell lengths and heights) in different treatments (Fig. 2A, B). Veligers exposed to predators (direct contact, crushed cohort, and caged predators) had rounder shells, as indicated by the ratio between the length and height of the larval shells (aspect ratio) (Fig. 2C, D). The overall form of the shells of experimental veligers was significantly different from the corresponding controls in both experiment 1 (Kruskal-Wallis test, $H = 15$, $P < 0.001$) and experiment 2 (Student t test, $t = 8.03$, $P < 0.0001$).

Veligers reared in direct contact with zoeas as well as those exposed to stimuli from caged predators developed significantly smaller apertures than veligers in the crushed cohort treatment and either of the control groups (Appendix: Tables A1 and A2; Kruskal-Wallis test, $H = 19.52$, $P < 0.001$; Student t test, $t = 2.57$, $P = 0.03$). Comparisons were of the cross-sectional area of the aperture relative to overall shell length and shell height, based on measurements obtained from SEM micrographs in 2004 (Fig. 3A) and measurements taken with an ocular micrometer in 2005 (Fig. 3B).

The amount and type of shell surface sculpture or ornamentation were not detectably different across experimental treatments in the SEM micrographs.

Pairwise predation trials

Veligers reared in water with caged predators had greater survival than predator-naïve control veligers when both were exposed directly to zoea predators (two-tailed t test, $t = 6.869$, $P < 0.001$; Fig. 4).

DISCUSSION

The experiments support the hypotheses that stimuli from *Cancer* spp. zoeas induce developmental changes in

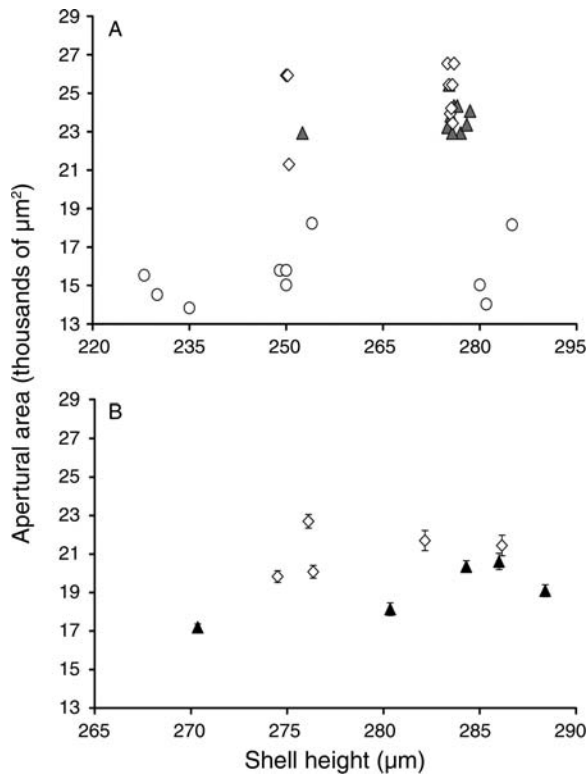


FIG. 3. (A) Experiment 1: The relationship between cross-sectional apertural area and shell height of veligers reared in direct contact with zoeas (open circles), in the crushed cohort treatment (shaded triangles) and in the control (open diamonds). Each datum represents one veliger. (B) Experiment 2: Apertural area in relation to shell height of veligers reared with caged predators (solid triangles) and in the control group (open diamonds). Each datum represents one replicate. Data are means \pm SE.

the form of the larval shell of *Littorina scutulata* veligers and that exposure to zoeas during development enhances the survival of the veligers during subsequent encounters with these predatory larval crabs. Veligers reared with brief, periodic, direct contact with the zoeas and those reared in the presence of caged zoeas developed rounder larval shells with reduced apertural openings. Although the direct contact treatment in experiment 1 in 2004 was pseudoreplicated and the changes in shell form may have resulted from actual selection, not phenotypic plasticity, the significant differences in shell morphology led to experiment 2 in 2005 in which direct contact between predator and prey was prevented and treatments were fully replicated. The results from experiment 2 confirmed those obtained in experiment 1 and indicate that predator kairomones are sufficient to induce morphological changes and reduce vulnerability in these veligers without direct contact between predator and prey and without a failed attack.

Zoea predators access their prey by chipping the shell at the aperture and subsequently peeling back the larval shell from the apertural edge. Veligers that were handled

but not killed by zoeas were damaged along the edge of the shell's aperture. Previous research describes similar apertural chipping in wild-caught veligers, which were under repeated attack by zoeas during collection (Hickman 2001). Moreover, this type of apertural damage occurs under natural conditions as evident by growth lines on earlier portions of the larval shells showing past instances of marginal breakage and repair (Hickman 2001). The method of attack noted in this study and others suggests that the development of a smaller apertural opening makes predation by zoeas more difficult. This induced response is well-documented for adult gastropods who, in the presence of shell-peeling crustaceans, grow smaller apertural openings (Appleton and Palmer 1988, Palmer 1990, DeWitt et al. 2000). The induction of smaller apertures by veligers exposed to zoeas, either directly or with mesh separating predator and prey, was similar in this study.

Rounder shells might also aid defense. Research on predator-induced changes in shells of adult gastropods indicate that round shells are protective against some crustacean and fish predators (DeWitt et al. 2000, Langerhans and DeWitt 2002). It is also possible that roundness was correlated with other features. Aperture form might affect orientation of measured shells and hence aspect ratio.

The shells of veligers reared with caged predators were rounder with smaller apertures than the shells of the control veligers. Their greater survival may have resulted from these induced morphological changes, though induced behavioral changes cannot be ruled out.

Morphological responses of prey may depend upon the concentration of predator stimulus as an indication of the proximity of threat (Havel 1985, Harvell 1990, Tollrian 1993, Relyea 2002, Trussell and Nicklin 2002, Van Buskirk and Arioli 2002). The degree of morphological response by the experimental veligers in the different treatments may have been "dose-dependent" and correlated with differences in the strength of predator stimulus. Predator-derived kairomones alone and in combination with stimuli from injured prey induced development of rounder larval shells relative to control veligers. However, only veligers reared in direct contact with zoeas and those exposed continuously to caged zoeas developed smaller apertures. Induction of a smaller aperture (in addition to a rounder shell) may have resulted from exposure to a stronger predator stimulus. While the morphological changes and increased survival of veligers exposed to caged predators indicate an adaptive response to predation, it is as yet uncertain how these results apply to natural conditions in which the strength and variety of stimuli are unknown.

Reduced vulnerability after exposure to predators has implications for experimental predation studies with larvae or other marine zooplankton that are reared in the laboratory without predators or whose history of exposure to predators is unknown. For example,

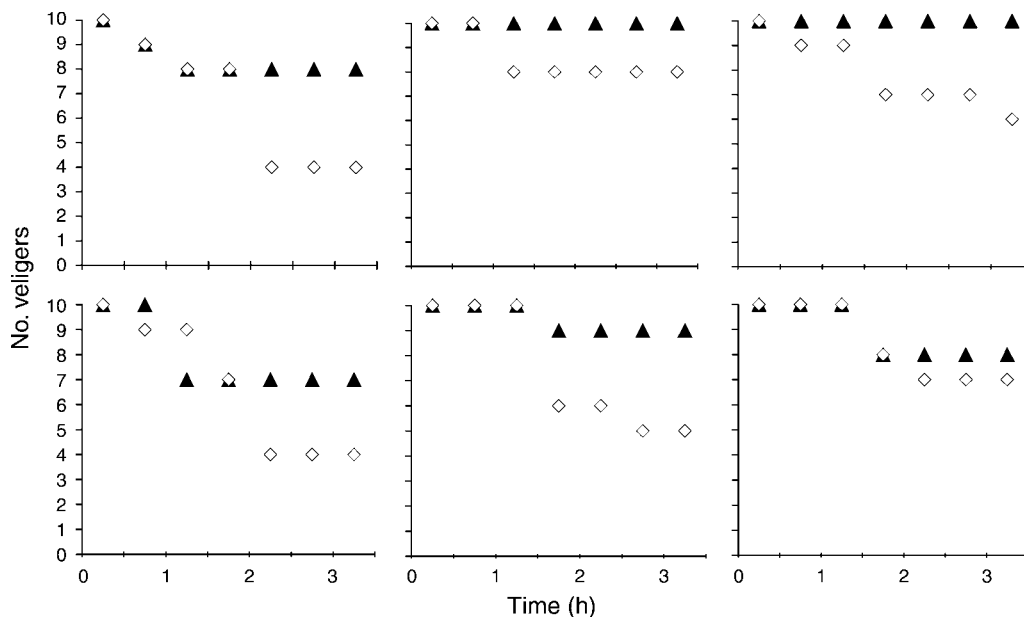


FIG. 4. Plots of survival curves from six terminal predation trials pairing veligers reared in the presence of caged zoeas (solid triangles) and control veligers (open diamonds) that were predator-naïve. Survival of veligers was recorded at half-hour intervals in each of the six pairwise trials. There was a significant difference in survival between experimental and control veligers by 2.5 h ($t = 6.869$, $P < 0.01$). A significant difference in survival continued for the duration of the trials (paired two-tailed t tests, $n = 6$ pairs; 1.5 h, $P = 0.7711$; 2 h, $P = 0.0822$; 2.5 h, $P = 0.0015$; 3.5 h, $P < 0.001$).

different experience might account for the disparity in the behavioral response of field-captured zoea predators and their laboratory-reared veliger prey, which was striking in this study. Initially, the veligers did not protect themselves by withdrawing into the larval shell upon the direct threat of predation by zoeas, although they did withdraw into their shells after capture. All veligers used for behavioral observations were predator-naïve. Conversely the zoeas had survived in the plankton for several weeks prior to observations of attacks. They were proficient at capturing prey, as was evident in their response to the veligers.

These experiments with veligers provide one instance of predator-induced change in morphology and vulnerability in marine zooplankton. Although it is possible that the first species tested was an exceptional case, there are reasons to expect similar developmental plasticity in a variety of marine zooplankters. Marine planktonic environments are highly variable with changing abundances of both holoplanktonic and meroplanktonic predators and prey. The induced morphological changes of the larval gastropods and their decreased vulnerability to predatory crab larvae resembles the plastic responses documented for freshwater zooplankton and larvae.

Predator-induced changes in morphology would seem appropriate for other marine zooplankton. However, costs of the induced changes are unknown. For the veligers, these might include delayed metamorphosis, decreased size at metamorphosis, or increased vulnerability to other planktonic predators. Also, still unknown

are the specificity of the stimuli from predators and the effectiveness of induced defenses for the diverse kinds of predators on marine plankton. For the veligers, would an effective defense against a small crustacean be ineffective against a small fish?

This study demonstrates that plasticity of morphological defenses occurs in marine zooplankton, but it remains unclear how common predator-induced morphological changes are in marine zooplankton. If predator-induced morphological defenses are widespread among marine zooplankton, the results have implications for the evolution of plasticity in marine larval development and complex life histories and also for trade-offs affecting predation on marine zooplankton.

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APPENDIX

Tables presenting differences in larval shell form of experimental veligers (*Ecological Archives* E088-064-A1).