In utero predator-induced responses in the viviparid snail *Bellamya chinensis*

R.S. Prezant, E.J. Chapman, and A. McDougall

Abstract: Predator-induced defenses are well-known adaptive responses found in many marine and freshwater organisms. These responses usually occur in juveniles or actively growing adults and reduce the ability of a predator to consume its prey. We report a predator-induced response occurring in utero in the viviparid snail *Bellamya chinensis* (Gray, 1863) that results in significant changes in offspring number, shell size variability of released juveniles, and juvenile shell organic content. Water-borne cues derived from a predatory crayfish (*Orconectes limosus* (Rafinesque, 1817)) induced a doubling of the number of offspring released; these newly born snails were generally smaller, though more variable in size, and possessed a greater shell organic content than controls. The generally smaller size of juveniles released in the presence of a predator reflects a faster rate of generation and passage through the uterus. These responses to the presence of a predator could be adaptive by decreasing predation pressure within a population or by reducing mortality following failed attacks.

Résumé : Les défenses induites par les prédateurs sont des réactions adaptatives bien connues retrouvées chez de nombreux organismes marins et dulcicoles. Ces réactions existent généralement chez les jeunes et chez les adultes encore en croissance active et ont comme conséquence de réduire la capacité d'un prédateur à consommer sa proie. Nous présentons une réaction induite par les prédateurs qui se manifeste in utero chez le gastéropode vivipare *Bellamyia chinensis* (Gray, 1863) et qui produit des changements significatifs du nombre de rejetons, une variabilité de la taille de la coquille chez les jeunes qui sont relâchés et une variabilité du contenu organique de la coquille des jeunes. Des signaux transmis dans l'eau provenant d'une écrevisse prédatrice (*Orconectes limosus* (Rafinesque, 1817)) induisent une augmentation par un facteur de 2 du nombre de jeunes relâchés; les gastéropodes néonates sont aussi généralement plus petits, mais de taille plus variable, et leurs coquilles possèdent un contenu organique plus important que les témoins. La taille généralement plus petite des jeunes relâchés en présence des prédateurs reflète les taux accélérés de génération et de passage à travers l'utérus. Ces réactions à la présence d'un prédateur pourraient être adaptatives en décroissant la pression de prédation au sein de la population ou en réduisant la mortalité à cause des attaques ratées.

[Traduit par la Rédaction]

Introduction

Inducible defenses could have significant adaptive value. These organismal responses are well known in herbivore–plant interactions and are being increasingly studied in animal predator–prey associations. Inducible defenses can include morphological (Krist 2002; Teplitsky et al. 2003), behavioral (DeWitt et al. 1999; Delgado et al. 2002; Wollerman et al. 2003), and chemical responses (Karban and Baldwin 1997; Toth and Pavia 2000; Rohde et al. 2004) in potential prey. Some induced changes can become fixed if they are adaptive, resulting in constitutive defenses. For instance, an adaptive coevolutionary "race" has existed between predation mechanisms found in some decapod crustaceans and the defensive "responses" in their shelled molluscan prey (Appleton and Palmer 1988; Vermeij 1993; DeWitt et al. 2000; Trussell 2000; Trussell and Nicklin 2002*a*, 2002*b*). Specifically, as some marine molluscs evolved crab-resistant shells and shell structures, predatory decapods developed new means of preying on molluscs, often through modified or strengthened claws. This evolutionary interaction is considered well established based on the marine fossil fauna; however, recent laboratory and field studies have revealed short-term induced plasticity in shell form in extant molluscs over very short periods of developmental time.

Induced morphological responses can be rapid, occurring in a matter of weeks in growing juveniles. Members of the freshwater snail genus *Physa* variably modify shell shape in the presence of different predators (Crowl and Covich 1990; DeWitt 1998; DeWitt et al. 2000). More rotund shells resist the shell-crushing jaws of predatory fish, while more elongated shells with a narrower aperture better resist predation by crayfish, which normally reach the snail's soft tissue through the shell opening. It could be argued that these results reflect selective cropping within a remnant population of variably shaped individuals (predator selection of the most vulnerable prey within a naturally variable population) and thus reflect a post-predation phenotype. However, within laboratory populations, protected snails merely exposed to predators are able to modify shell shape (*Physa* spp., Crowl and Covich 1990; *Elimia* spp., Krist 2002).

Additional reports of variation in adult shell form induced

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Table 1. ANOVA testing the significance of size and mass variation in control and experimental adult snails (*Bellamya chinensis*).

		Sum of squares	df	Mean square	*F*	*P*
Shell length	Between groups	7.806	1	7.806	0.894	0.350
	Within groups	331.769	38	8.731		
	Total	339.575	39			
Shell width	Between groups	0.930	1	0.930	0.190	0.665
	Within groups	185.882	38	4.892		
	Total	186.813	39			
Aperture length	Between groups	2.007	1	2.007	0.954	0.335
	Within groups	79.933	38	2.104		
	Total	81.940	39			
Aperture width	Between groups	2.204	1	2.204	1.701	0.200
	Within groups	49.242	38	1.296		
	Total	51.447	39			
Wet mass	Between groups	0.172	1	0.172	0.018	0.893
	Within groups	356.176	38	9.373		
	Total	356.347	39			

Note: Results show that no significant differences were found among adult snail shell size or mass measurements.

by external factors can be found for a variety of molluscan taxa (see, for example, Kemp and Bertness 1984; Palmer 1990; Gibbs 1993). Environmentally induced morphological variations have been found in the live-bearing gastropod *Viviparus georgianus* (I. Lea, 1834) in Georgia and Florida, USA (Katoh and Foltz 1994). Individuals in populations of adult *Bellamya* (*Cipangopaludina*) *chinensis* (Gray, 1863), another viviparid snail, show distinct shell variations and have been designated as "morphotypes" assumed to reflect variations in allometric shell growth in different environmental regimes (Chiu et al. 2002). Morphological changes induced by environment can also reflect or parallel physiological changes. Molluscan reproduction and reproductive potential, for instance, can be regulated or modified by environmental factors (Beekey and Karlson 2003) including exposure to predators (Chase 2003; Watt and Aiken 2003).

As a member of the mesogastropod family Viviparidae, *B. chinensis* gives birth to fully developed juveniles. In brooding adults, all stages of development are found simultaneously in utero, from newly fertilized ova to large (approximately 5 mm long), fully shelled juveniles. Thus, a single brooding female contains all ontogenetic stages within a uterine sac. This could mean that any changes in the parent's environment, including exposure to a predator and its infochemicals, could be reflected in biochemical, physiological, and (or) morphological changes in developing young. To test for this, we exposed adult specimens of *B. chinensis* to the waters circulated past an isolated predatory crayfish to determine what influence the presence of a potential predator might have on in utero development of juvenile snails in terms of size, number, and shell composition of newly released offspring.

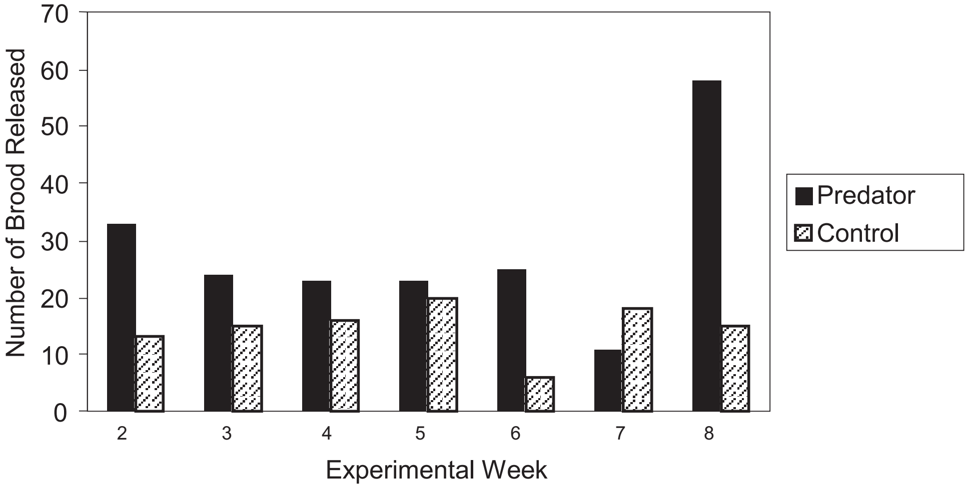
Materials and methods

Specimens of *B. chinensis* were collected from the south side of Lake Ocquitunk, Stokes State Forest, in northern New Jersey, USA, in May 2003. Adult shells were measured using digital calipers accurate to 0.01 mm. Shell length and width and aperture length and width were recorded. Wet masses were also taken to the nearest 0.0001 g. Prior to experimentation, we used a one-way ANOVA (SPSS®, SPSS Inc. 2002) to insure that all adults used in the experimental tanks were statistically similar in size for the five parameters measured (Table 1). The predetermined ratio of male:female snails in the Lake Ocquitunk population is 1:1. We insured that a minimum of 8 females were present in both experimental and control aquaria (described below) by observing the release of juveniles from 16 of the 40 individual snails in isolated aquaria for 10 days prior to experimentation. While males of *B. chinensis* can be identified by the presence of a modified right tentacle that acts as a penis, we chose to minimize handling of test organisms prior to experimentation. Post-experimental dissections indicated that 16 of the 20 experimental snails were female; 14 gave birth during the experiment and 2 retained early embryos in the uterine sac. Of the 20 snails in the control tanks, 14 were female; 10 released juveniles during the experiment and the remainder retained embryos.

Individual adult snails were placed in 945 mL cylindrical plastic containers pierced with numerous 3.9 mm diameter holes. These holes permitted water circulation but prevented escape of newborn snails and predation and contact by crayfish. Eight 76 L aquaria were filled with dechlorinated water. Water was circulated in each aquarium by means of an external filter without filtration medium. Five containers, each holding one adult snail, were placed in each aquarium. Four aquaria received one crayfish (*Orconectes limosus* (Rafinesque, 1817)) with 25–35 mm carapace length. Juveniles born in each container were collected daily and immediately preserved in 70% ethanol.

Changes in the number of juveniles born can arise from either differences in the number of juveniles produced in a brood or differences in the rate at which juveniles move through the uterus (i.e., brood number is constant). To test which case held true for our experiments, adult snails were sacrificed immediately after the 8-week testing period and their brood dissected out of the uterus. The number of shelled young (any stage of development with even minimal

Fig. 1. A comparison of the number of brooded *Bellamya chinensis* juveniles released each week, starting with week 2, between experimental (filled bars; with predatory crayfish, *Orconectes limosus*) and control (hatched bars) tanks.



shell present) was compared between experimental and control groups.

Experimental procedures

Little work has been done on the natural feeding behavior of viviparid snails, so it is difficult to assess their specific nutritional needs. Plinski et al. (1978) found phytoplankton and zooplankton in the stomachs of some Canadian specimens of *Viviparus malleatus* (Reeve, 1863), indicating that this large gastropod is able to filter feed, in addition to being able to graze hard surfaces and soft sediments. To minimize potential anomalies resulting from handling of animals during the course of this experiment, adult snails were removed from experimental aquaria once per week for 8 h and fed ad libitum a generic flake fish food, which they readily consumed. After feeding, the snails were replaced in their individual containers in the experimental aquaria. Crayfish were also removed from the experimental aquaria for the same duration as the snails and fed, once a week, a diet of generic shrimp pellets. Handling and feeding of experimental animals in this manner also prevented food contamination, and thus additional sensory stimuli, in the test aquaria. Our feeding protocol and the nutritive value of the food appeared to be sufficient to maintain healthy and actively brooding snails. The experiment ran for 8 weeks in June and July 2003, with a 12 h light : 12 h dark cycle at ambient laboratory temperatures (22 ± 1 °C). Number of brood released, size of juveniles (shell length and width, aperture length and width), wet and dry masses, and ash-free dry mass of juvenile shells (combusted at 550 °C for 1 h to determine organic content) were recorded. (Juvenile soft tissues (“bodies”) were easily removed from the dried shell prior to combustion using a dental hook.) Similarly, ash-free dry mass of adult snail shells was also taken at the end of the experiment to determine relative (juvenile to adult and experimental to control) shell organic content.

Statistical procedures

This experiment consisted of two treatment (TRT) groups (control and predator), each with four replicate aquaria (TANK) containing five adult snails (PARENT) in individual containers. Since the size measurements (shell length, shell width, aperture length, and aperture width) and the wet mass of the released juveniles were highly correlated, we first employed a principal components analysis of the data (SAS/STAT®, SAS Institute Inc. 2000). This revealed substantially larger variation in the size of the juveniles exposed to the predator crayfish and hence potential problems associated with heterogeneity across groups in an ANOVA model analysis of the data. Owing to this heterogeneity in the predator and, to a lesser extent, the control tanks, and owing to large differences in the number of juveniles released in the two treatments, the most appropriate statistical method for the analysis of these data was the two-sample *t* test (SPSS®, SPSS Inc. 2002). We analyzed four shell measurements (shell length, shell width, aperture length, and aperture width), wet mass of the released juveniles, and shell organic content individually with the two-sample *t* test. Using a two-sample *t* test with unequal variances (see, for example, Moore and McCabe 2005), we examined differences in the average size of juveniles between the two treatments. This *t* test procedure was also employed to examine shell organic content, while a χ^2 test was used to compare the number of juveniles released.

Results

Brood release

Adult *B. chinensis* in the presence of the crayfish *O. limosus* released a combined total of almost twice as many offspring as controls did (197 vs. 103) over the 8 weeks of the experiment. Among the experimental adults, 14 females gave birth to an average of 14.07 juveniles during the course of the experiment. In the control tanks, 10 adult females released an average of 10.3 juveniles during the same period. On a weekly basis during the course of the experiment, control organisms, with one exception in week seven, produced fewer offspring than experimental animals did (Fig. 1).

A χ^2 analysis of the number of juveniles released shows that there was essentially a constant rate of release prior to week 6 for both treatment groups ($P = 0.3461$). Weeks 6, 7,

Table 2. Descriptive statistics and two-sample *t* test results for in utero *B. chinensis* juveniles from experimental (with predator) and control experiments.

	Tank	Mean	SD	*P* value (two-tailed)
Shell length	Predation	5.3729	0.7660	0.097 (ns)
	Control	5.5312	0.5211	
Shell width	Predation	6.0115	0.8604	0.008 (*)
	Control	6.3071	0.6311	
Wet mass	Predation	0.0505	0.0174	0.064 (ns)
	Control	0.0547	0.0137	

Note: Experimental tanks (with predator) had 102 in utero juveniles, whereas control tanks had 84 in utero juveniles. *P* values were obtained using a two-sample *t* test (*, $P < 0.05$; ns, not significant). In utero juveniles were those that had already begun shell development; amorphous, earlier embryos were not counted in this analysis.

and 8, however, showed significant deviations even though, as will be seen, there were no obvious changes in juvenile size.

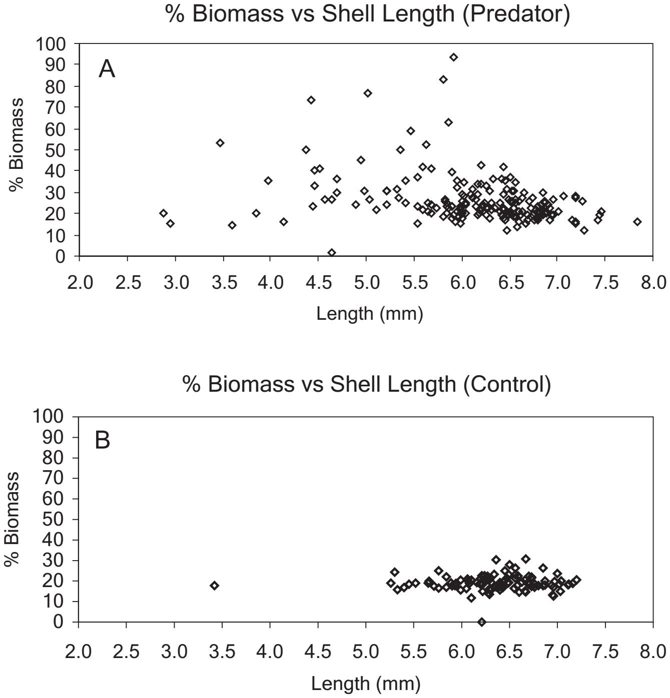
In utero brood

To delineate the effects of the predatory crayfish on in utero brood, we examined uterine contents of both control and experimental adults at the termination of the 8-week experiment. There were no significant differences between treatment groups in the number of embryos or shelled juveniles held in utero ($P = 0.604$). Two-sample *t* tests were conducted on length, width, and wet mass of in utero shelled juveniles; significant differences occurred only in shell width ($P = 0.008$) (Table 2). Thus, compared with controls, experimental adults released almost twice as many juveniles during the experimental period but retained almost the same number of juveniles in utero at the end of the experiment, indicating that the experimental adults were producing and moving juveniles through the uterine system more rapidly than the control adults, rather than producing more juveniles at any moment in time. On the other hand, the variation in soft tissue biomass compared with shell length of in utero brood was much greater in experimental populations (Fig. 2).

Principal components analysis

The five juvenile measurements — shell length and width, aperture length and width, and wet mass — were highly correlated. Therefore, we initially focused on a principal components analysis of the data (Fig. 3). As shown in Table 3, the first two principal components (PCs) account for over 96% of the total correlation. Note that the coefficients of the first PC (eigenvector) essentially represent an average of the five original measurements (shell length, width, wet mass, aperture length, and aperture width). The coefficients of the second PC single out aperture width as the most important variable, which is interesting because the *t* test results (see below) show that the average aperture width is the only variable that did not differ significantly between the two treatment groups. Examining aperture width alone demonstrates that only 4.94% of the total variation is accounted for by this single variable. This information, in concert with the nonsignificant result from the *t* test (Fig. 4E), shows aperture width to be unimportant in affecting overall juvenile variation due to the presence of a predator.

Fig. 2. Scatter plots demonstrating greater variability in soft tissue biomass and shell length in experimental offspring still in uterine brood and exposed to predator effluent. Percent biomass was calculated using dried soft tissue divided by dried shell material.



In Figure 3, the original data are projected onto the space spanned by the first two PCs; 95% density ellipses (based on a bivariate normal distribution) were computed for each treatment group. An obvious feature of this plot is that the density ellipse for the control group is substantially smaller than that for the experimental group in the vertical direction but only slightly smaller in the horizontal direction. Since the vertical axis corresponds to the first PC, we can now see clear evidence of a predator effect in terms of much greater variability in the size of experimental juveniles compared with controls. In contrast, there does not appear to be a meaningful difference in variability between the two treatment groups with respect to the horizontal axis; that is, aperture width does not appear to be affected by the presence of a predatory crayfish.

Juvenile size at release

Juveniles released from adults in the aquaria that held crayfish were significantly smaller than those released in control aquaria in all aspects except aperture width (*t* test with unequal variances). Figs. 4A–4E show 95% error bars for the mean. While experimental juveniles were significantly smaller than control juveniles, it is important to note that experimental juveniles exhibited significantly greater variation in size (Fig. 3).

Shell organic content

Percent organic content ((mass of dried shell – mass of combusted shell) / mass of dried shell) also differed between experimental and control juveniles, demonstrating an increase in variability as a result of predator presence (Fig. 4F). On average, juveniles released in the presence of

Fig. 3. Principal components analysis plot for experimental and control juvenile *B. chinensis*. Ellipses represent 95% confidence intervals and demonstrate wide variation in experimental snails exposed to the effluent of a predatory crayfish during in utero development. The larger outer ellipse represents the experimental tank; the smaller inner ellipse represents the control tank, showing significantly less variation in the measurements taken. Closed circles represent control juveniles; open circles represent experimental juveniles.

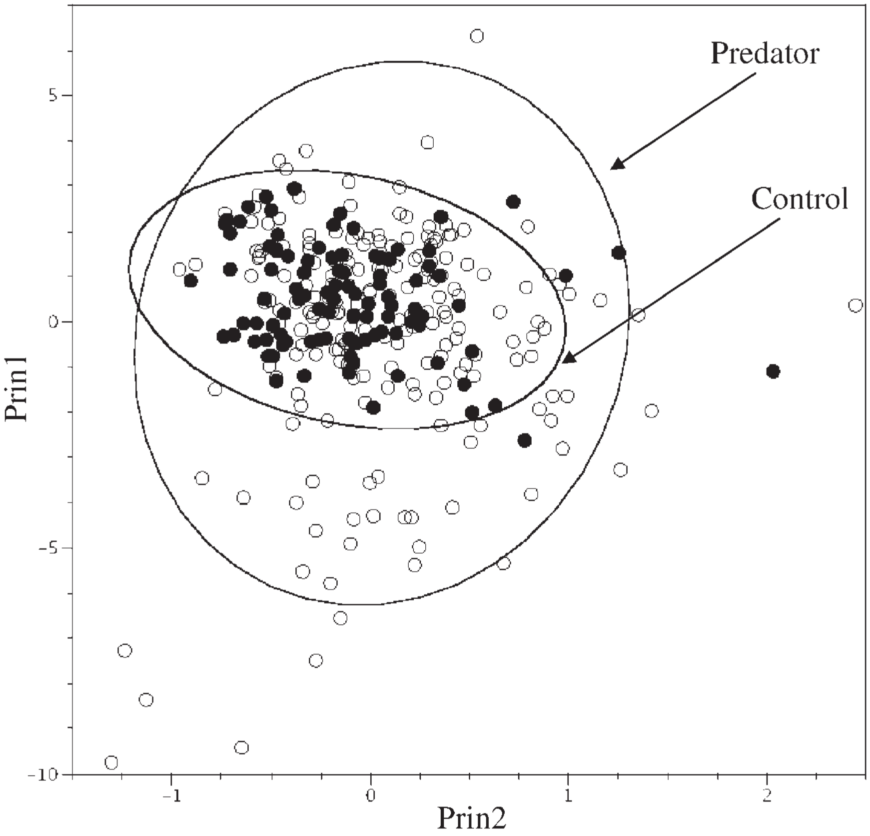


Table 3. Results from principal components analysis evaluating shell length, width, wet mass, aperture length, and aperture width of juvenile *B. chinensis*.

			Coefficient (eigenvector)				
PC	Eigenvalue	Percentage of variation	Shell length	Shell width	Wet mass	Aperture length	Aperture width
1	4.566	91.32	0.456	0.459	0.448	0.453	0.419
2	0.247	4.94	–0.180	–0.123	–0.365	–0.159	0.891

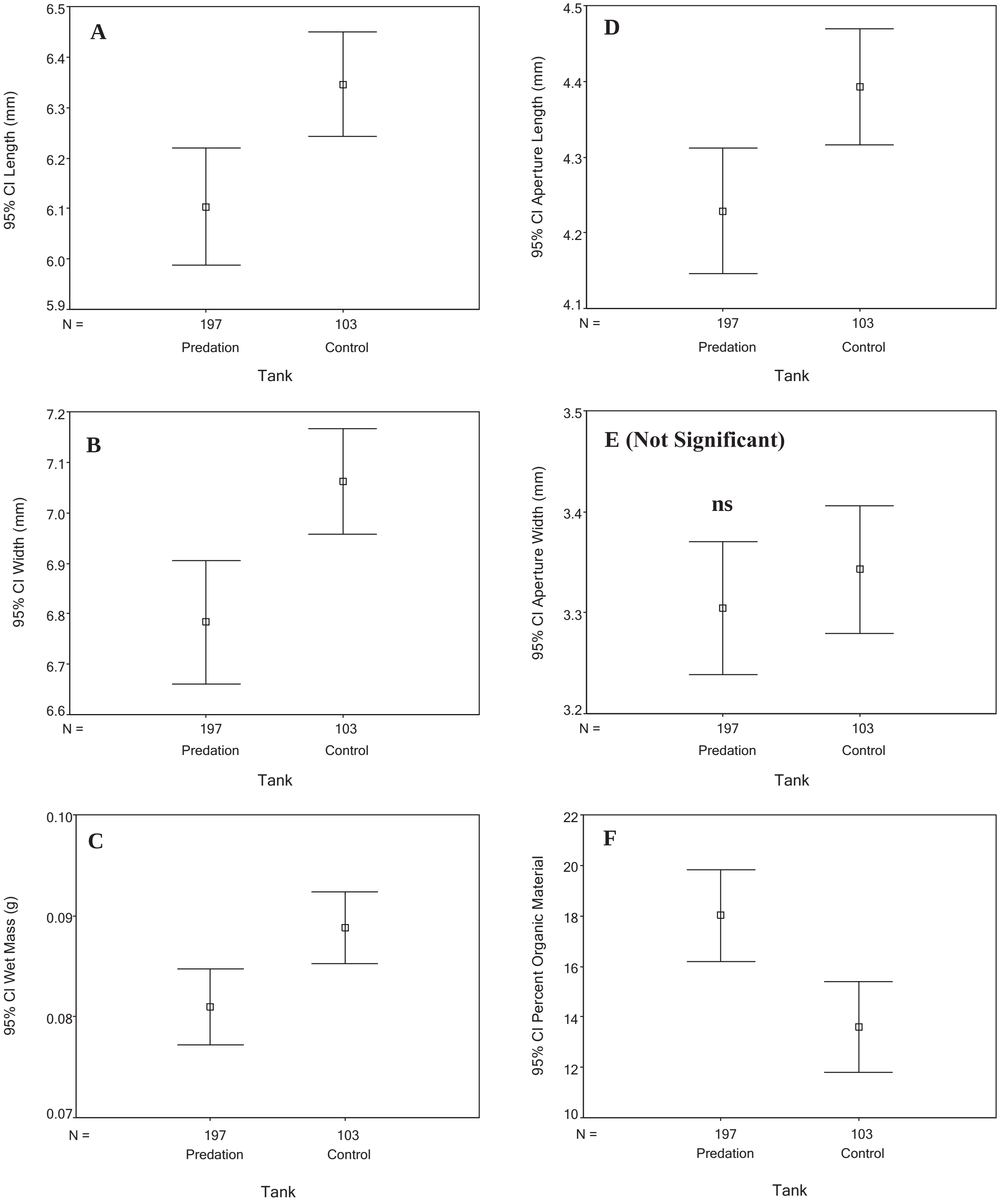
predatory crayfish had a shell composed of 18.0% organics (periostracum and "conchiolin") and 82.0% calcium carbonate, whereas control juveniles had a shell composed of 13.6% organics (Fig. 4F). This variation in organic versus mineral content was found only in juveniles in the two treatments. An independent sample *t* test confirmed that there was no significant difference in shell organic content between experimental and control adults ($P > 0.05$).

Discussion

To our knowledge, this study represents the first report of a predator-induced defensive response in a viviparid mollusc that influences developing young. Our results indicate that (*i*) juveniles of *B. chinensis* born in the presence of crayfish effluent are smaller, (*ii*) more juveniles are released when brooding adults are exposed to predator effluent, (*iii*) variability in the size of released juveniles is more pronounced in the presence of a predator, (*iv*) predators induce a faster rate of release of juveniles, possibly reflected in their smaller size at release, (*v*) juveniles released from adults in the presence of predator effluent have a significantly higher ratio of shell organic to shell mineral content than controls, and (*vi*) all juvenile specimens of *B. chinensis* (control and experimental) have a considerably higher ratio of shell organic to shell mineral content than adults.

Compared with controls, experimental adults released almost twice as many juveniles during the experimental period but retained almost the same number of juveniles in utero at the end of the 8-week experiment, indicating that the experimental adults were producing and moving juveniles through the uterine system at a more rapid rate. Thus, the predator-induced response promotes faster release of fertilized eggs into the uterus, and these eggs move more quickly through the uterus; as a result, released experimental juveniles are, on average, smaller than controls.

Fig. 4. Box plots demonstrating differences between newly born *B. chinensis* in experimental tanks (with predatory crayfish effluent) and those in control tanks. Shell length (A) and width (B): juveniles in experimental tanks were significantly smaller than those in control tanks (shell length, $P < 0.01$; shell width, $P < 0.001$). Wet mass (C): experimental juveniles were significantly lighter than control juveniles ($P < 0.01$). Aperture length (D) and width (E): length differed significantly between experimental and control juveniles ($P < 0.01$), whereas aperture width did not (ns, not significant). Organic content of juvenile shells (F): experimental juveniles had shells with significantly higher organic content ($P < 0.01$). P values were obtained using a two-sample t test with unequal variances.



The smaller size of many of the juveniles released in the presence of predators could mean that more juveniles can find refuge in smaller retreats. Larger populations of juveniles might give predators an increased chance of finding prey (Brodersen and Madsen 2003) but would yield an overall decreased risk of total population loss as refugia unavailable to larger juveniles became accessible to smaller offspring. We can assume that doubling the number of juveniles in the presence of predators is adaptive. As the sheer number of potential prey available to a predator increases, the number of juveniles that might escape predation also increases (a presumptive r-selected strategy). Thus, increased numbers and greater size variability are inextricably linked. Unlike adult *B. chinensis*, which are typically found on surfaces or partially buried in mud or silt (Pace 1973), juveniles are often found nestled in crevices or under rocks. We have found juvenile *B. chinensis* along the eaves of rocks or in rocky crevices and erosion pits at field sites in New York, New Jersey, and Pennsylvania. Similarly, field collections of other viviparids, including *Campeloma decisus* (Say, 1817) and *V. georgianus*, show that their juveniles gravitate towards protected crevice refugia.

The mean number of juveniles released each week remained relatively constant over the course of the experiment (Fig. 1), and there was no apparent trend towards increasing or decreasing juvenile size over the 8-week period. The increased variability in juvenile size in the presence of a predator, however, suggests that potential predators would have a wider range of prey sizes to ‘‘select’’ from. We have not determined whether prey selection based on size occurs among predators of *B. chinensis*. If it does occur for predatory crayfish, then a variably sized array of young snails could promote survival of at least some offspring from a particular age, but not size, cohort. It is possible that, as in some other crayfish, juveniles of *O. limosus* are more uniformly predaceous than adults (becoming omnivorous as they age). It is, however, unlikely that a crayfish small enough to fit into the tight crevices housing juvenile *B. chinensis* would be a challenge to the snail’s relatively large size and well-developed shell. We have not followed a field population from season to season, so we cannot rule out the possibility of a longer term trade-off in ultimate size and reproductive capacity in the smaller snails produced in the presence of predator effluent. In the intertidal brown alga *Fucus vesiculosus* (L., 1753), the production of antiherbivore chemical defenses in response to grazing by the periwinkle snail, *Littorina littorea* (L., 1758), terminates within 2 weeks of cessation of active consumption (Rohde et al. 2004). We have not followed the full growth cycle of *B. chinensis* nor duplicated the experiments with juvenile crayfish as predators to ascertain any differences in growth in our experimental populations after removal of the predator.

In closed predator–prey experiments, it is difficult to accommodate every conceivable variable. In our experiment, crayfish were not able to interact directly with potential prey organisms, but water that passed over the crayfish also passed over the snails. Along with possible cues emanating from the crayfish, fecal material from the crayfish may have affected the snails by increasing the potential for additional food resources. It is possible that the increased fecal nutrients also increased bacterial material in the water and thus increased food available to the snails, since *B. chinensis*, at least in part, is capable of filter feeding. Therefore, it is possible that the predator’s presence influenced our final results by increasing the available food supply. However, it is highly unlikely that even with an enhanced food supply the number of juveniles would double and the average size of offspring would be reduced. One would predict that increased nutrients might yield an increase in the number of offspring, but not a decrease in juvenile size. However unlikely, this is an interesting question that would demand a much more complex experimental protocol to be answered definitively.

The percent organic content of shells of adult *B. chinensis* ranged from 3.2% to 4.0%. This is consistent with published values for adult molluscan shells (1.0%–5.0%) (Kamat et al. 2000; Pereira-Mouriès et al. 2002). We have found no studies reporting the shell organic content of juvenile, non-larval molluscs. The organic content we found in juvenile *B. chinensis* (approximately 13.0%–18.0%) is high compared with that found in adults. The bulk of the organic material could be locked in the setose periostracum of the juveniles. Juvenile Viviparidae often have concentric rings of periostracal setae that ornament the shell. These setae could act as a physical defense against small predators that would otherwise engulf the entire snail (i.e., centrarchid fish), but likely offer little protection from crayfish. The setae are absent in the larger, thicker shelled adults.

It remains equally possible that the large organic component of the juvenile shells is found in the organic matrix composing the shell proper. This organic material is considered the substrate for calcification and surrounds calcareous crystals and individual microstructural units and layers of the shell. The organic shell component is a complex mix of proteins, carbohydrates, proteoglycans, and glycoproteins, but its synthesis is poorly understood, as is the process of shell mineralization. It is certainly under some level of metabolic control (Hasse et al. 2000). There is evidence that higher levels of organic material in molluscan shells can offer increased resistance to shell fracture or might lend additional and protective flexibility to the shell (Taylor et al. 1969; Carter 1980). Thus, a larger organic component in the shell matrix of juvenile viviparids could offer some resistance to a shell-crushing or shell-peeling predator (fish or crayfish, respectively). In the laboratory, adult specimens of *O. limosus* fed on juvenile *B. chinensis* by ‘‘peeling’’ their way to the soft tissues from the external shell lip inward, ultimately consuming the entire shell and the soft tissues. An increase in organic material within the shell proper is unlikely to be of value in warding off a sustained attack by *O. limosus*, based on the specific mode of feeding by this crayfish (aperture peeling). The increased organic component could, however, be of value in retaining shell integrity after a disrupted attack that leaves a damaged, but surviving, juvenile. This kind of shell damage could be caused by juvenile crayfish able to invade the small, protective crevices housing juvenile snails. In a complex community with a range of juvenile specimens available to a predator, a failed attack might leave a shell partially fractured. The organic matrix within the shell can act to dissipate a fracture, allowing it to travel along an inter- and intra-crystalline web of relatively soft material rather than fracturing the entire shell. A thicker periostracum or one that is more setose, reflecting the other sink for shell organics in juvenile *B. chinensis*, could also offer some increase in survival through enhanced external shell integrity but also by acting as a physical protective barrier during an attack.

Organic shell components are considered energetically more expensive to produce than the mineral shell component (Palmer 1992). Thus, the response of *B. chinensis* to a predatory crayfish could reflect at least a reallocation of energy expenditures, leading to a higher organic to mineral ratio in juveniles as well as a significant increase in the number of juveniles born, whose total mass still exceeds the total mass of control juveniles. There is a cost in generating variable morphs because of the developmental instability of maintaining phenotypic plasticity (DeWitt 1998; DeWitt et al. 1998). The relative expense to the brooding adults in the presence of a crayfish must be examined relative to the slightly lower shell organic content in control juveniles, but in a size cohort that contains twice the number of usually smaller but more variably sized juveniles. Thus, we must balance the energy apportioned to the (*i*) number and (*ii*) rate of young produced and released, (*iii*) shell organic to mineral ratio, (*iv*) size of juveniles, and (*v*) potential increased survival and longevity (at the individual and population levels). Most critically, we must determine the long-term ecological and evolutionary implications of increased phenotypic variability of juveniles of this brooding gastropod when the parent is found in the presence of a potential predator. Based on earlier thermal experiments (R.S. Prezant and E.J. Chapman, unpublished results, 1999), we recognize that *B. chinensis* can retain juveniles in utero for extended periods of time. This makes it difficult to know how long any ‘‘mix’’ of juveniles was retained in the uterus before release and the relative growth rates of those juveniles. This also prohibits a specific knowledge of the relative ages of released juveniles in a given brood; however, as shown, it appears that the fertilized eggs and consequent embryos and juveniles pass through the uterus more quickly in the presence of a predator.

It has been suggested and repeated often in the literature that natural selection would favor development of short-term inducible defenses over genetically inherent constitutive defenses only when the risk of predation is short-term and (or) unpredictable, when energetic costs are beneficial or outweigh the costs of no change, and when a reliable stimulus initiates the induction (Lively 1986; Stearns 1989; DeWitt et al. 1998; Tollrian and Harvell 1999; Trussell and Nicklin 2002*a*, 2002*b*). The fairly rapid change in reproductive output in *B. chinensis* in response to the presence of a predatory crayfish must be evaluated from an energetics point of view, but the change in the number of offspring and the percent organic content in the juvenile shell almost certainly reflect a fast response to a potentially dangerous environment for offspring. These induced defensive responses promoted by predatory infochemicals are ecologically and potentially phylogenetically important, but remain poorly studied in all but a few taxa and life stages. To this realm we must add the possibility that predator or other environmental inducements could influence in utero development and that these changes could portend significant morphological shifts and possible impacts in population dynamics of potential prey organisms even before birth.

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