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Carryover effects of predation risk on postembryonic life-history stages in a freshwater shrimp

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ABSTRACT

For organisms with complex life histories it is well known that risk experienced early in life, as embryos or larvae, may have effects throughout the life cycle. Although carryover effects have been well documented in invertebrates with different levels of parental care, there are few examples of predator-induced responses in externally brooded embryos. Here, we studied the effects of nonlethal predation risk throughout the embryonic development of newly spawned eggs carried by female shrimp on the timing of egg hatching, hatchling morphology, larval development and juvenile morphology. We also determined maternal body mass at the end of the embryonic period. Exposure to predation risk cues during embryonic development led to larger larvae which also had longer rostra but reached the juvenile stage sooner, at a smaller size and with shorter rostra. There was no difference in hatching timing, but changes in larval morphology and developmental timing showed that the embryos had perceived waterborne substances indicative of predation risk. In addition to carryover effects on larval and juvenile stages, predation threat provoked a decrease of body mass in mothers exposed to predator cues while brooding. Our results suggest that risk-exposed embryos were able to recognize the same infochemicals as their mothers, manifesting a response in the free-living larval stage. Thus, future studies assessing anti-predator phenotypes should include embryonic development, which seems to determine the morphology and developmental time of subsequent life-history stages according to perceived environmental conditions.

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1. Introduction

Many organisms change foraging behavior, morphological traits and the timing of life-history events in response to the risk of predation (see, e.g., Lima, 1998; Benard, 2004). Predator-induced plasticity can reduce predation risk, but it often entails other fitness costs (e.g., slow growth or development; Relyea, 2002; Benard, 2004). Theory developed for organisms with complex life histories predicts that organisms should switch between two life stages when their mortality/growth ratio is lower in the following stage than in the previous one (Benard, 2004 and references therein). For instance, embryonic exposure to egg predators may favor early hatching at a smaller size and less developed stage. Such effects have been widely demonstrated in amphibians, the best-studied taxa with regard to predator-induced hatching plasticity (Warkentin, 2011; Touchon et al., 2013). Plasticity in hatching

can also adjust risks of benthic and planktonic development in benthic marine invertebrates. For instance, if a particular egg mass or brooding parent is frequently attacked, then plasticity at hatching may evolve, because the probability of survival in the benthic zone is greatly reduced and planktonic life may be a relatively better option (Oyarzun and Strathmann, 2011).

Variations in early life experience can lead to delayed effects later in life, which in turn may influence individual fitness, population dynamics, and even the course of evolution (Touchon and Warkentin, 2010 and references therein; Sih, 2011). It is well known that stage-specific effects of predation risk (as well as of other factors such as temperature, food quality and habitat selection) can propagate throughout the life cycle of benthic invertebrates (reviewed in Pechenik, 2006). Predator-induced changes in the defensive morphology of planktonic larvae have been demonstrated in benthic invertebrates that lack parental care: some larvae, for instance, can adjust their size based on experience in the plankton (Vaughn and Allen, 2010 and references therein). Such developmental responses may eventually affect subsequent life-history stages. Species that release eggs (or gametes) directly into

the plankton, however, have less scope for plasticity at hatching (Oyarzun and Strathmann, 2011). Therefore, the carryover effects of hatching plasticity on larval traits are expected to be greater for benthic invertebrates that are able to brood or to encapsulate offspring until hatching (Oyarzun and Strathmann, 2011). Predation risk delays time-to-hatching but does not alter developmental rate in the marine snail *Nucella lamellosa* which lays encapsulated eggs in the benthos (Miner et al., 2010). This form of post-fertilization parental care (egg attendance) differs from egg brooding, a form of parental care where parents carry the eggs after laying (Smiseth et al., 2012). Until now, there have been few documented cases of predator-induced responses and their consequences in brooded embryos. For instance, predation risk experienced by the embryos of the viviparid snail *Bellamya chinensis* affects the phenotypes of the juvenile and adult phases (Prezant et al., 2006). Some other species carry eggs externally. This may render parents and offspring vulnerable to predators due to reduced mobility, increased conspicuousness (Magnhagen, 1991; Reguera and Gomendio, 1999) or prey choice behavior of predators (Li and Jackson, 2003). As far as we know, there have been no documented cases of predator-induced responses (and their consequences) in externally brooded eggs of marine invertebrates.

Predation risk in aquatic ecosystems is indicated by specific forms of chemical information. Predation events entail a series of steps beginning with initial detection leading to attack, capture, and finally prey ingestion (Wisenden, 2000). In this sequence, chemical cues released at each step can be used by prey to assess and avoid predation risk (Wisenden, 2000; reviewed in Ferrari et al., 2010a). Prey may initially detect predators through the predator's signature odor, a kairomone. This is a chemical released by the predator and received by a prey; it is adaptively favorable to the receiver, but not to the source species. A second class of pre-attack chemical signals comes from startled or disturbed prey and usually involves pulses of ammonia in urine; these are referred to as disturbance cues. Alarm cues are a third class of signals that come from damaged prey during attack and handling prior to ingestion; these chemical compounds leak from the prey epidermis and provoke strong behavioral responses from conspecifics. Dietary cues, which are related to alarm cues, may be released from the predator's digestive system during digestion and defecation (Ferrari et al., 2010a and references therein).

In the present study, we address whether predation risk alters the timing of hatching in externally brooded embryos and whether embryonic response has implications for the life cycle by affecting early postembryonic stages. We used freshwater shrimp, *Palaemonetes argentinus* (Crustacea: Decapoda: Palaemonidae), as study animal. As in most decapod crustaceans with indirect development, only the embryos of this shrimp are protected by females, which incubate eggs on the ventral surface of their abdomen prior to their release into the plankton as larvae. Developing embryos are attached to ovigerous setae by the funiculus and egg coat. As they have no vascular or nervous connection with the female, they could be essentially autonomous (e.g., Ituarte et al., 2005). However, a maternal–embryo interaction might be involved in the control of hatching time in *P. argentinus* (Giovagnoli, 2011), as is the case in several other species (Ikeda et al., 2006; Christy, 2011). In the present study we examined the hypothesis that embryos of *P. argentinus* are able to acquire information about potential predators. If embryos are sensitive to substances indicating predation risk, we expect that this information will be propagated throughout the life cycle. We also expect that risk of predation on eggs will alter the timing of hatching and, in turn, risk-induced plasticity in hatching will affect the phenotype of early postembryonic stages. We specifically predict that if risk of predation on eggs causes early hatching, small hatchling size will occur, and as a result, small juveniles, too. Otherwise, if it causes late hatching, we expect large size at hatching and large juveniles.

Our laboratory experiment involved the direct exposure of female *P. argentinus* with newly spawned eggs to the presence/absence of nonlethal predation risk throughout embryonic development. We simulated an imminent but nonlethal threat of predation by allowing developing embryos to be constantly exposed to chemical signals from a predator cichlid (*Australoheros facetus*) and an attacked prey (ovigerous females of the shrimp). Since most predation chemical cues are organic compounds that degrade over time (Forward and Rittschof, 2000; Peacor, 2006; Ferrari et al., 2010a), this experimental design allowed us to ensure that waterborne substances indicative of predation risk were present and active throughout embryonic development. We measured the period of embryonic and larval development as well as larval and juvenile morphology for animals hatched from unexposed and risk-exposed embryos. To determine whether predator-induced responses of embryos affect postembryonic life-history stages, we raised newly hatched larvae in water free of chemical cues, and compared larval period, number of larval instars and juvenile morphology for animals hatched from unexposed and risk-exposed embryos.

2. Materials and methods

2.1. Study species

The freshwater shrimp *P. argentinus* inhabits shallow lakes and streams in southeastern South America, breeds in spring and summer and is found sympatrically with predator fishes such as cichlids, characins, poecilids and cyprinodonts (Ringuelet, 1975; González Sagrario and Balseiro, 2003; Ituarte et al., 2007). This shrimp has a complex life cycle with benthic adult and juvenile stages, and a planktonic phase that includes at least seven larval instars (Menú-Marque, 1973; Magiera, 2009). The newly hatched larvae of *P. argentinus* grow by gradual addition of appendages after every molt, but go through major changes in morphology and swimming behavior when they enter the benthic adult habitat. The last larval stage (decapodid) still has vestiges of larval characters and becomes a juvenile after one or two molts (Anger, 2001; Bauer, 2004). The juvenile is similar to the adult in structure, though without reproductive organs, and has lost all traces of larval morphology.

2.2. Animal collection and maintenance

We collected shrimp and fish from Lake Mogotes (38°03′43″ S/57°32′39″ W), Mar del Plata (Argentina). This small, shallow lake covers an area of 0.0764 km² and has an extended vegetated littoral zone which hosts small to medium-sized fish species, birds and a rich assemblage of predatory macroinvertebrates, as do most of the temperate lakes of central Argentina (González Sagrario et al., 2009). The littoral fish assemblage, generally omnivorous, preys on zooplankton and macroinvertebrates. In turn, the macroinvertebrate assemblage is strongly associated with macrophyte stands and is composed of high abundances of insect larvae, flatworms, water mites and the shrimp *P. argentinus* (González Sagrario and Balseiro, 2003; González Sagrario et al., 2009). Juvenile and adult individuals of *P. argentinus* dwell predominantly in stands of submerged macrophytes associated with the inner parts and/or edges of vegetated patches (González Sagrario et al., 2009).

On October 15, 2011, we collected females (with fully developed ovaries) and males of *P. argentinus*, as well as juveniles of the cichlid *A. facetus*. Specimens were obtained from the littoral zone using a hand net (45 cm width, 30 cm deep and 1 mm mesh size). Shrimp

were kept for one week in a laboratory aquarium (30 cm × 30 cm, 50 cm high), filled with dechlorinated tap water with constant aeration, and were fed daily with freshly hatched nauplii of *Artemia* spp. and fish food (TetraMin Pro; lipids 12%; proteins 46%; Tetra GmbH, Melle, Germany). We examined the aquarium every morning to collect females with newly laid eggs.

Juvenile cichlids (length 6–7 cm) were placed in transparent individual aquaria (32 cm × 27 cm, 22 cm high) with constant aeration and fed one gravid *P. argentinus* daily. Fish were allowed to acclimate to captive conditions for a minimum of 5 days before being included in the experiment.

2.3. Experimental set-up

In addition to kairomones, predator digestion of conspecific tissues (dietary cues), startled prey (disturbance cues) and prey damage (alarm cues) are important for communicating predatory risk to prey species. However, these separate sources of information may or may not induce morphological defenses (Schoeppner and Relyea, 2009; Ferrari et al., 2010a). Due to our interest in the sensitivity of embryos to the presence rather than to the identity of predation cues that trigger a response, we decided to use a combination of all the information sources mentioned above. Thus, shrimp with newly laid eggs were randomly assigned to two treatments: predator-cued (nonlethal exposure to *A. facetus*) and without predator. Twenty experimental units (*n* = 10 with *A. facetus* and *n* = 10 fishless) were used for the experiment. Each experimental unit consisted of a transparent aquarium (32 cm × 27 cm × 22 cm, filled to a depth of 20 cm) where the fish was housed (when applicable), containing a smaller enclosure with the mother shrimp. These smaller enclosures were cylindrical plastic cages (diameter 12 cm, height 10 cm) with perforated walls covered with mosquito netting (2 mm × 1.5 mm mesh size) and a double net bottom. An empty plastic tube (diameter 1.7 cm, length 5 cm) was offered as a refuge for the shrimp. In each experimental unit, enclosures were hung at about 7 cm above the aquarium floor. This experimental design allowed the detection of olfactory cues related to predation risk, but prevented the fish from eating the shrimp. The bottom of each experimental unit was covered with a layer (ca. 5 cm) of pebbles and sand. A thin piece of stone with a concave internal surface (12–15 cm in length) lying on the aquarium floor was offered as refuge for the fish, when applicable.

Experimental units were provided with constant aeration; temperature (22 ± 2 °C) and light (14:10 h L:D cycle) simulated summer conditions. Every morning, we fed shrimp with two or three flakes of fish food (TetraMin Pro), which were carefully placed inside the small enclosure. Approximately 1 h later, fish were fed with a gravid shrimp. Water was partially (70%) changed once a week.

2.4. Response variables

Every morning we observed the mother shrimp in order to determine hatching timing and recover larvae soon after hatching. As embryos from the same brood did not usually hatch simultaneously, we measured hatching time as the period between the spawning of eggs and the onset of larval hatching. Immediately after larval release, we removed the fish from the aquarium and carefully collected the hatchlings using a plastic pipette. We measured adult and larval carapace lengths with an Olympus SZX7 stereo microscope (Olympus, Tokyo, Japan) from the posterior orbital margin to the dorso-posterior edge (Fig. 1A) to the nearest 0.01 mm. We anaesthetized the mothers by chilling (−20 °C), transferred them to pre-weighed capsules of aluminum foil and dried them at 80 °C to a constant weight (at least 48 h). We determined dry weight to the nearest 0.01 mg on a H54 Mettler AR
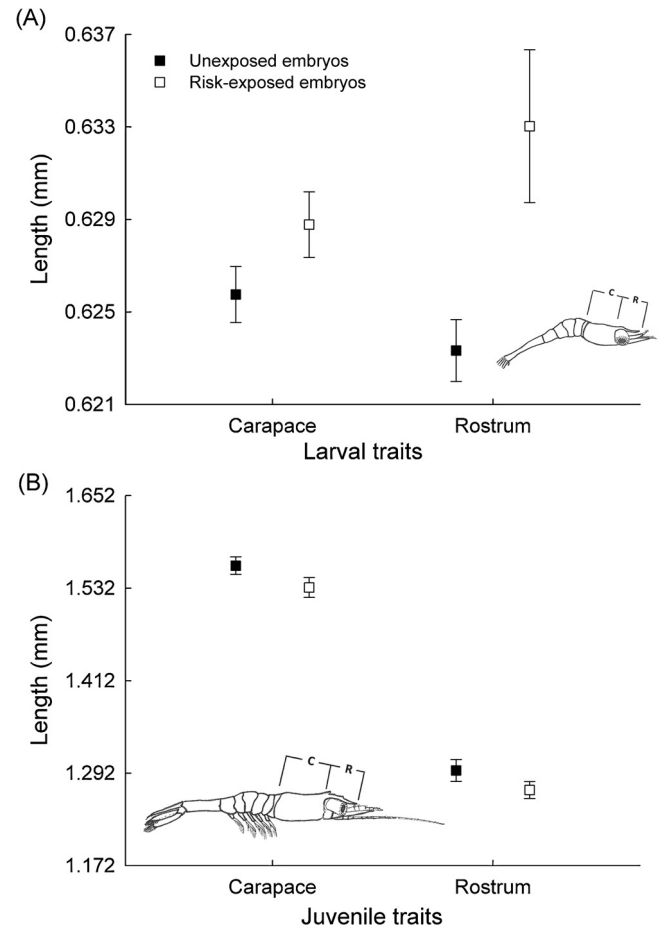
Fig. 1. Carapace and rostrum length in (A) larvae and (B) juveniles of *Palaemonetes argentinus* hatched from unexposed (larvae: n_m = 96; juveniles: n_j = 52) and predation risk-exposed (larvae: n_m = 63; juveniles: n_j = 45) embryos. All comparisons were significant. Data are overall means ± SE across individuals; for further details see Table 1. Insets illustrate the morphometric measurements taken on newly hatched larval and juvenile stages, both drawn without pereiopods and modified from Menú-Marque (1973). Abbreviations: C, carapace length; R, rostrum length.

balance (Mettler-Toledo GmbH, Greifensee, Switzerland). We also measured rostrum length of the newly hatched larvae (n_m = 159; Table 1), from the rostrum tip to the posterior orbital margin (Fig. 1A). We determined carapace and rostrum lengths in subsets of at least 6 larvae per clutch, usually 10 (Table 1). Hatchlings were obtained from 17 out of 20 clutches of eggs (Table 1). Three units of the predator-cued treatment were lost and fewer individuals were obtained from this treatment because hatching occurred mainly at night and *A. facetus* preyed on newly hatched larvae.

Larval period and survival to the juvenile stage were quantified in other subsets of larvae. Each larva (initially n_d = 136; Table 1) was individually reared in a plastic bowl with 30 ml well water at 24 ± 2 °C in a temperature-controlled room. Larvae were fed daily with newly hatched *Artemia* nauplii (Great Salt Lake Artemia cysts, ca. 10–15 ml⁻¹; Artemia International LLC, Fairview, TX, USA). During each water change (every 24 h), culture bowls were individually checked for molts or mortality, and exuviae were removed. Carapace and rostrum lengths of surviving juveniles (Fig. 1B) were measured with an Olympus SZX7 stereo microscope to the nearest 0.01 mm.

2.5. Statistical analysis

We examined whether the presence/absence of a nonlethal predation threat throughout embryonic development affected larval

Table 1

Morphological and developmental responses in larvae and juveniles of *Palaemonetes argentinus* hatched from unexposed (UE, clutches A–J) and predation risk-exposed embryos (EE, clutches K–T). Embryonic developmental time: from spawning of eggs to onset of larval hatching; larval developmental time: from hatching to juvenile stage. Values are means ± SE; n_t = total number of larvae recovered per female (clutch); n_m = number of larvae used for morphological measurements; n_d = number of larvae used for developmental measurements; n_j = number of surviving juveniles measured.

Treatment	Female	Embryos	Larvae						Juveniles			
		Developmental time (days)	n_t	Carapace length (mm)	Rostrum length (mm)	n_m	n_d	Developmental time (days)	Carapace length (mm)	Rostrum length (mm)	n_j
UE	A	24	20	0.626 ± 0.004	0.634 ± 0.003	10	10	23.57 ± 0.57	1.544 ± 0.019	1.345 ± 0.030	7
	B	21	20	0.632 ± 0.004	0.628 ± 0.003	10	10	25.00 ± 1.00	1.594 ± 0.011	1.300 ± 0.030	7
	C	19	20	0.619 ± 0.003	0.621 ± 0.003	10	10	26.22 ± 0.95	1.589 ± 0.037	1.335 ± 0.040	9
	D	21	20	0.623 ± 0.003	0.628 ± 0.003	10	10	24.50 ± 2.45	1.613 ± 0.016	1.339 ± 0.027	8
	E	18	16	0.629 ± 0.002	0.632 ± 0.004	6	10	26.33 ± 1.97	1.585 ± 0.037	1.321 ± 0.031	6
	F	22	20	0.630 ± 0.004	0.610 ± 0.004	10	10	28.14 ± 2.48	1.503 ± 0.023	1.155 ± 0.025	7
	G	19	20	0.634 ± 0.004	0.619 ± 0.003	10	10	25.25 ± 2.71	1.498 ± 0.032	1.264 ± 0.035	8
	H	24	10	0.632 ± 0.003	0.628 ± 0.003	10	–	ND	ND	ND	ND
	I	24	10	0.621 ± 0.004	0.619 ± 0.007	10	–	ND	ND	ND	ND
	J	17	10	0.612 ± 0.004	0.616 ± 0.004	10	–	ND	ND	ND	ND
EE	K	23	20	0.621 ± 0.004	0.653 ± 0.005	10	10	23.12 ± 0.77	1.613 ± 0.017	1.323 ± 0.026	8
	L	21	16	0.623 ± 0.007	0.638 ± 0.010	6	10	24.89 ± 0.54	1.610 ± 0.01	1.300 ± 0.027	9
	M	25	20	0.628 ± 0.002	0.623 ± 0.005	10	10	24.80 ± 0.58	1.458 ± 0.01	1.274 ± 0.012	5
	N	24	20	0.626 ± 0.005	0.626 ± 0.005	10	10	21.78 ± 0.32	1.546 ± 0.024	1.317 ± 0.018	9
	O	20	20	0.634 ± 0.003	0.660 ± 0.009	10	10	23.00 ± 0.82	1.481 ± 0.028	1.250 ± 0.032	7
	P	20	17	0.626 ± 0.000	0.601 ± 0.013	7	10	23.00 ± 1.15	1.427 ± 0.018	1.226 ± 0.053	3
	Q	26	16	0.632 ± 0.003	0.621 ± 0.003	10	6	24.50 ± 1.04	1.434 ± 0.010	1.244 ± 0.024	4
	R	25	WLR								
	S	18	WLR								
	T	22	WLR								

ND, not determined; WLR, without larval recovery.

and juvenile traits. The effects of treatments on the traits of postembryonic stages (carapace and rostrum length, larval developmental time and number of molts to reach the juvenile stage) were analyzed with mixed-model nested ANOVAs with the mother nested within the treatment using JMP version 10.0 (SAS Institute Inc., Cary, NC, USA). The presence/absence of predator threat during embryonic development was a fixed factor and female identity was a random factor in the model. We compared carapace and rostrum lengths of 63 larvae from 7 predator-exposed mothers and 96 larvae from 10 unexposed mothers. Likewise, we tested for differences in larval development time and number of molts to reach the juvenile stage as well as differences in carapace and rostrum lengths of 45 juveniles from 7 predator-exposed mothers and 52 juveniles from 7 unexposed mothers. In addition, survival curves through larval development (from hatching to the juvenile stage) of individuals hatched from predator-exposed and unexposed embryos were analyzed using a nonparametric Log-Rank test, a specific test of the Kaplan–Meier survival analysis.

Hatching time was compared between treatments using a Student's *t*-test. At the end of the incubation period, least-square regression models were fitted to describe the relationships between carapace length (CL) and dry weight of the mother. Dry weight of mothers was compared between treatments performing an analysis of covariance (ANCOVA) using CL as covariate.

3. Results

Exposure to a nonlethal predation threat during embryonic development induced changes in the morphology and development time of postembryonic life-history stages. Morphological changes were recorded for both larvae and juveniles. Larvae from the predator-cued treatment group had longer carapaces and rostra (nested mixed-model ANOVA: $F_{1, 157}$ = 599 and $F_{1, 157}$ = 511, respectively; both $P < 0.0001$; Fig. 1A). Carapace and rostrum lengths of juveniles also differed between treatments (nested mixed-model ANOVA: $F_{1, 96}$ = 101.2 and $F_{1, 96}$ = 113.9, respectively;

both $P < 0.0001$), but juveniles from the predator-cued treatment group were smaller and had shorter rostra (Fig. 1B).

The presence/absence of predation risk had no effect on the time to complete embryogenesis (mean ± SE, exposed eggs: 22.4 ± 0.8 days, unexposed eggs: 20.9 ± 0.8 days; t_{18} = −1.28, $P = 0.216$), but the power of the performed test was below that desired (0.11 vs. 0.8). Embryonic development ranged from 18 to 26 days in the predator-cued treatment group and from 17 to 24 days in unexposed eggs (Table 1). There was no difference in the percentage of larval survival in unexposed and risk-exposed embryos (mean ± SE: 74.29 ± 3.69% and 68.09 ± 8.29%, respectively; $\chi^2_1 = 0.36$, $P = 0.55$). In contrast, the larval period was 2 days shorter in larvae from the predator-cued treatment (nested mixed-model ANOVA: $F_{1, 96}$ = 106.7, $P < 0.0001$; Fig. 2A). There were also differences in the number of molts to reach the juvenile stage (nested mixed-model ANOVA: $F_{1, 96}$ = 108.6, $P < 0.0001$, Fig. 2B). All larvae went through 7–11 molts to reach the juvenile stage, but most individuals that hatched from the predator-cued treatment completed their development earlier, after 8 molts only.

Dry weight of the mothers at the end of the incubation period was linearly and positively related to their carapace length in both treatments (Fig. 3). Non-exposed mothers were 10.6% heavier than those exposed to a predation threat (test of parallelism, $F_{1, 18}$ = 0.09, $P = 0.77$, ANCOVA: $F_{1, 17}$ = 8.32, $P = 0.01$).

4. Discussion

Embryonic experience elicited noticeable differences in larval and juvenile traits, but we did not detect any differences in hatching time between treatments. As far as we know, this appears to be the first direct experimental study to demonstrate carryover effects of predation threat during the embryonic phase on larval/juvenile morphology and larval development in a shrimp. It is also one of only a few studies documenting such alterations in brooded eggs of aquatic invertebrates (Prezant et al., 2006). Our results show that embryos were able to perceive the threat of predation but its effects became visible only after hatching. Such a link between the
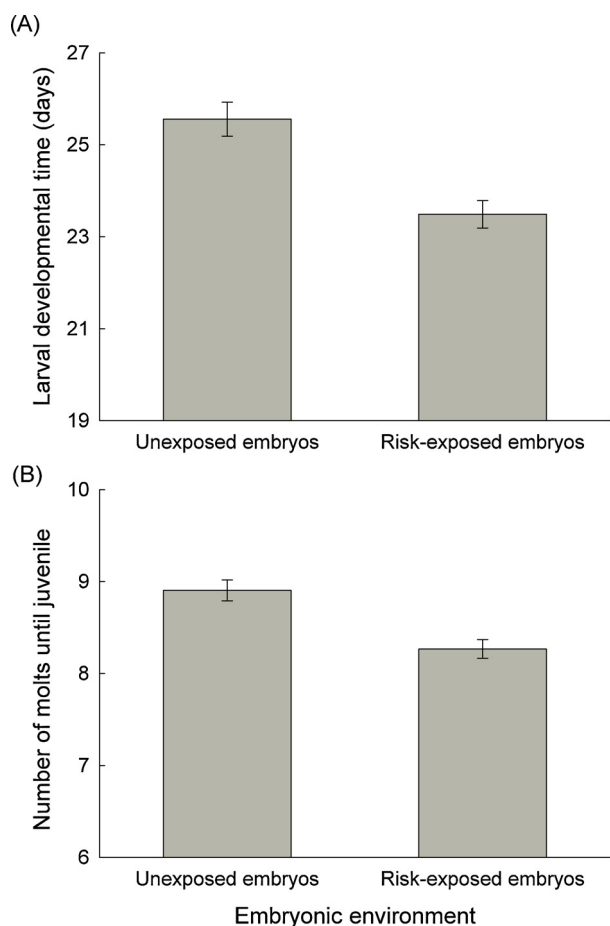
Fig. 2. (A) Developmental time from hatching to juvenile stage and (B) number of molts until reaching juvenile morphology in *Palaemonetes argentinus* larvae hatched from unexposed (n_j = 52) and predation risk-exposed (n_j = 45) embryos. All comparisons were significant. Data are overall means ± SE across individuals; for further details see Table 1.

embryonic, larval and juvenile stages of life history could be important in organisms with complex life cycles.

It is known that planktonic larvae have little possibility of seeking a physical refuge from predators in open waters; instead, morphological and/or behavioral changes may provide protection from predation by preventing capture (Lass and Spaak, 2003). The
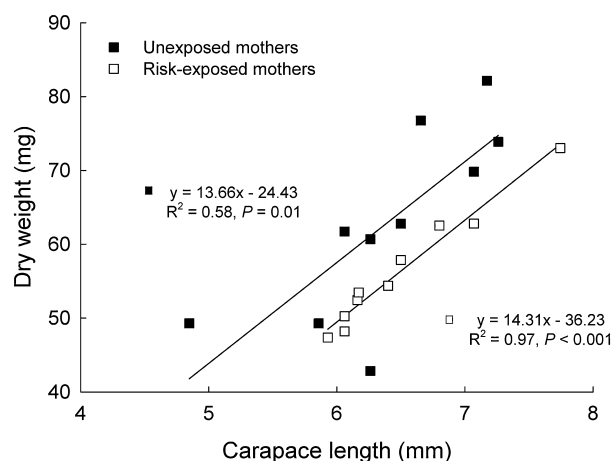
Fig. 3. Relationship between carapace length and dry weight of unexposed and predation-exposed female *Palaemonetes argentinus* at the end of egg incubation.

effectiveness of spination as a defense against small fish predators has been experimentally tested for decapod zoeae (Morgan, 1987, 1989). It has been shown that grass shrimp larvae do not evade fish attack but rely on their spines and armor to survive initial attacks. If zoeae are released after an attack, they either swim very quickly in unpredictable loops or flex their abdomen to quickly traverse short distances (Morgan, 1987). Larger body size of shrimp zoeae, as observed in hatchlings from predator-cued eggs, makes them more conspicuous for visual predators (Morgan, 1987), but it probably has anti-predator benefits by increasing escape speed (Chia et al., 1984). Moreover, large size in combination with an armor exoskeleton and long spines also make zoeae less vulnerable to gape-limited predators (Morgan, 1987). Thus, larger body size, longer rostral spines and flexion response of shrimp larvae may be effective at preventing predation by small fish, because in combination, they increase handling time (Morgan, 1987). The longer rostrum and carapace lengths of larvae of *P. argentinus* may confer protection against small fish predators, but further experimental studies are needed to understand whether or not the carryover effects of embryos on larval morphology are adaptive.

Large larval size in the predator-cued treatment group may have resulted from a relatively longer period until hatching. It is likely that these zoeae hatched later, but we have no statistical power in support of this (see below). Also, predator-cued embryos became juveniles at smaller sizes, in less time and after fewer molts, even though the predation threat was no longer present during larval development. Smaller juvenile size might be associated with an accelerated larval development due to a lower number of molts needed to attain the benthic stage. Rapid development may allow the larvae to leave the hazardous planktonic phase faster (reviewed in Arendt, 1997). However, the smaller juvenile size in shrimps may lead not only to small body size but also to small size at the onset of sexual maturity, and thus to reduced fecundity (Alon and Stancyk, 1982; Ituarte et al., 2007). Smaller juveniles are likely weaker competitors for food and refuge, which should further reduce their chances of survival and growth in the natural habitat (Hines, 1986). Consequently, the effects of predation risk during embryonic development can have far-reaching implications for individuals and probably also for populations.

Predator-induced plasticity in the timing of egg hatching has been identified as a potential anti-predator defense in organisms with complex life cycles (Kusch and Chivers, 2004; reviewed in Warkentin, 2011 for amphibians). We found that non-lethal exposure to predation threat did not to alter the timing of egg hatching but the power of the performed test was below the desired threshold indicating that we might have overseen a difference if it actually existed.

The morphological changes in the newly hatched larvae indicate that the embryos somehow perceived environmental signals indicating predation threat. Future experimental studies should evaluate whether embryos are able to detect predatory cues occurring in a critical period during embryogenesis and should also investigate the development of specific tissues/organs involved in monitoring the surrounding chemical environment (e.g., Mandrillon and Saglio, 2008).

A possible behavioral response to predator cues was also detected in female *P. argentinus*: weight decrease in mother shrimp exposed to a predator threat throughout the incubation period is likely due to a reduction in feeding activity. Prey, even shrimp, can alter foraging behavior in response to the risk of predation (e.g., Turner, 1996; Lima, 1998; Bernot and Whittinghill, 2003; Lammers et al., 2009; Ferrari et al., 2010a). Unexposed mothers actively searched for food whenever it was offered whereas exposed mothers did not show this behavior and spent most of the time within their refuges (R. Ituarte, pers. obs.). The egg mass

makes gravid shrimp more conspicuous to predators and also less able to escape predation since it may mechanically prevent abdominal strokes from reaching maximum efficiency (Berglund and Rosenqvist, 1986). Therefore, hiding should reduce the likelihood of being detected by predators and could potentially increase the survival chances of both mother and offspring. Besides chemical cues, vision might have contributed to behavioral responses (such as reduced feeding) if the mothers had been able to see the predatory fish through the cages. In our system, the mothers could increase both their own and their offspring's (eggs and larvae) chances of survival by changing their behavior rather than by altering the embryonic period.

Our results agree with recent findings which highlight the fact that the earliest time at which an individual can acquire information about potential predators in most aquatic species is when the embryo is still in the egg and is in contact with the aquatic medium (Ferrari and Chivers, 2010; Ferrari et al., 2010b; Warkentin, 2011). In the common frog *Rana temporaria*, for example, there is a critical period during development in which embryos are 'vulnerable' to substances indicating a predation risk. This critical period is most likely associated with the development of specific tissues/organs involved in monitoring their surrounding chemical environment (Mandrillon and Saglio, 2008). In the water flea *Daphnia pulex*, kairomone perception during the late embryonic stage is thought to initiate a series of biological reactions: neuronal signals are converted into endocrine signals that subsequently induce changes in the expression of morphogenetic genes and finally result in defense morph formation (Miyakawa et al., 2010). Yet, how these chemical cues are perceived, transmitted and refined into specific gene/protein regulatory pathways and subsequently into form and function, is still largely unknown (Schoeppner and Relyea, 2009; Ferland-Raymond et al., 2010; Tollrian and Leese, 2010).

Different developmental pathways have to be activated during embryogenesis to effect the observed morphological alterations in larvae. We speculate that they might be triggered by chemical signals released during predation events (kairomones, dietary and/or alarm cues). Moreover, in systems in which females can detect environmental cues that are reliable indicators of a given predatory regimen, natural selection may favor the maternal manipulation of offspring traits to match the expected predatory regimen (Agrawal et al., 1999; Storm and Lima, 2010; Sih, 2011). Thus, we cannot rule out a potential influence of the mothers on the phenotype of newly hatched larvae. Future experimental studies with eggs of *P. argentinus* isolated from their mothers (e.g., Ituarte et al., 2005) should determine whether predation cues are perceived by the embryos themselves and/or whether there is a mother–embryo interaction that contributes to some extent to larval responses. Considering that carryover effects can play a major role in population dynamics, and eventually in the relationships that structure aquatic communities, further studies are required to explore the importance of chemically mediated plasticity during the embryonic process (Mandrillon and Saglio, 2008).

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