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Determinants in the timing of hatching in egg capsules of a gastropod with maternal care: the case of *Crepipatella peruviana*

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Research Article

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Abstract

In many species with encapsulated larval development, the larvae play an active role in hatching. However, the factors that control when the larvae hatch from each egg-capsule within an egg-mass are largely unknown. Advanced egg-masses of the gastropod *Crepipatella peruviana* were used to determine the hatching time of capsules from each egg-mass. After each female was detached, the egg-mass was also removed from the substrate and all capsules were then counted and measured. All capsules were examined to determine the time of hatching and the order in which capsules hatched from each egg-mass. Larvae were collected from each hatched egg-capsule and the number, size and weight of larvae from each capsule were determined. After 50–60% of the capsules from each egg-mass had hatched, the same characteristics of the remaining unhatched larvae from sister capsules were documented. Larvae were found to have hatched when they reached a size of 354 ± 22 µm ($n = 245$). Larvae from capsules within the same egg-mass hatched over a period of up to 12-days. The order of hatching in capsules from the same egg-mass was determined by larval content: capsules with fewer larvae and smaller capsules with heavier larvae hatched first. The hatching from one capsule in any given egg-mass did not induce the hatching of its sister capsules. Furthermore, hatching also occurred successfully in the mother absence, suggesting that this process is largely or completely controlled by the encapsulated larvae, although a possible maternal role in synchronizing hatching cannot be excluded.

Introduction

The early developmental stages of many marine invertebrates are enclosed within gelatinous masses or capsules that may or may not protected by the mother. These structures promote survival during the period of encapsulation by providing protection against fluctuations in environmental factors such as salinity, desiccation, UV radiation, oxygen levels, and predation (Pechenik, 1978, 1982; Rawlings, 1994, 1996; Fernández *et al.*, 2006). In some species, in addition to containing developing embryos, the capsules also contain extraembryonic nutritional material that has been contributed by the mothers, e.g. intracapsular fluid and nutritional ('nurse') eggs (Penchaszadeh and Miloslavich, 2001; Güler and Lök, 2014; Büchner-Miranda *et al.*, 2018; Chaparro *et al.*, 2019). In addition, the encapsulated embryos of some species also receive protective care from the mothers (e.g. calyptraeid gastropods; Collin, 2003; Lesoway *et al.*, 2014; Segura *et al.*, 2016; Pechenik *et al.*, 2017), which provides additional protection to the encapsulated larvae, especially in species exposed to highly variable environmental conditions (e.g. estuarine or intertidal environments, Chaparro *et al.*, 2008; Pechenik *et al.*, 2017, 2019).

The calyptraeid gastropod *Crepipatella peruviana* deposits egg masses onto the substrate to which the females are attached. The egg masses typically contain between 16 and 69 capsules. All of the eggs within the capsules develop into embryos. There are no nurse eggs available; the vitellum and dissolved organic matter in the intracapsular fluid are the embryo's only external energy sources (Ojeda and Chaparro, 2004; Büchner-Miranda *et al.*, 2018; Han *et al.*, 2022). The capsules are laid on the substrate and incubated beneath the mother's shell, for 4–5 weeks (Gallardo, 1979; Chaparro *et al.*, 2005; Mardones *et al.*, 2013) before the planktonic veliger larvae hatch out of the egg capsules and continue their development in the water column (Gallardo, 1977; Chaparro and Flores, 2002; Chaparro *et al.*, 2005). During these early encapsulated stages, the offspring development depends initially on the energy reserves present in the eggs, and, during the subsequent planktonic period, on exogenous resources obtained through planktotrophic larval feeding (Bertram and Strathmann, 1998). In *C. peruviana*, a suture runs along the upper-middle part of each egg capsule (Gallardo, 1979); at hatching, this area is 'unzipped,' allowing the veliger larvae to escape (Andrade-Villagrán *et al.*,

2018a). The walls of the egg capsule are transparent, making it easy to identify the developmental stage of the encapsulated embryos without having to open the capsules (Gallardo, 1979; Chaparro *et al.*, 2005). In this species, veligers hatch at shell lengths (SL) between 320 and 365 µm (Ojeda and Chaparro, 2004; Chaparro *et al.*, 2005). The hatching mechanism has been identified to be osmotic in origin, with water entering the interior of the capsule, causing the capsule to open through increased internal pressure (Andrade-Villagrán *et al.*, 2018a). In some marine gastropod species, regardless of the hatching mechanism, capsule opening appears to be influenced by the encapsulated embryos (e.g. enzymatic activity, Andrade-Villagrán *et al.*, 2018a, degradation of the internal capsule layers, Ojeda and Chaparro, 2004; Büchner-Miranda *et al.*, 2018), especially in species where the mother plays no role in the hatching process or in species in which the mothers abandon the capsules after oviposition (Andrade-Villagrán *et al.*, 2018a).

Surprisingly, little is known about the conditions that determine the moment of hatching in encapsulated species with mixed development (encapsulated embryos followed by a pelagic larval phase, Pechenik, 1979) and physical parental care, such as in *C. peruviana*, where encapsulated larvae hatch into the water column and continue their pelagic development for a couple weeks before settling. In those species, the timing of hatching may be related to the egg capsule's age. Few studies have specifically examined the extent to which variation in the timing of hatching among egg capsules within an egg mass can be explained by variation in when each capsule within an egg mass was deposited by the mother. The time spent laying an egg mass is generally less than the time spent during hatching from capsules within the same egg mass (Vasconcelos *et al.*, 2004; Smith and Thatje, 2013; Lesoway *et al.*, 2014; Andrade-Villagrán *et al.*, 2018b).

In some species, hatching must be triggered from within the encapsulating structure by the developing embryos themselves (Pechenik, 1975; Sullivan and Bonar, 1984; Hawkins and Hutchinson, 1988; Andrade-Villagrán *et al.*, 2018b). The timing of hatching appears to be related to the embryos having developed the physiological and morphological traits necessary to function in the open environment (Warkentin, 2011). However, we still don't know the characteristics of the encapsulated content for *C. peruviana* that would allow us to accurately predict the timing of hatching in capsules found within the same egg mass. In consequence, the present research was undertaken to discover the existence of intracapsular conditions that might allow us to identify the extent to which the timing of hatching is determined by the encapsulated veliger larvae, and to what extent the mothers play a role in this process.

Material and Methods

Collection and maintenance of biological material

Sessile females of the suspension-feeding gastropod *Crepipatella peruviana* (Chaparro *et al.*, 2001, 2005) measuring between 31 and 58 mm in shell length were collected from Pelluco Beach, Puerto Montt (41°28′S; 72°56′W) during the Southern hemisphere spring. Specimens were collected from the middle intertidal zone and then transported to the laboratory, where they were maintained for 5 days in aquaria with unfiltered circulating seawater (salinity 32 psu), at 14 °C and with constant aeration. In addition, the diet was supplemented with daily additions of pure cultures of the microalgae *Isochrysis galbana*.

To obtain the egg masses, *C. peruviana* females were detached from the original substrate to which they were attached. The capsular masses were then carefully removed from the substrate and the developmental stage of the encapsulated embryos was identified for each capsule (Chaparro *et al.*, 2005). Only egg masses containing embryos at an advanced pre-hatching stage were used for our experiments (Chaparro *et al.*, 2005, see Figure 3F). The hatching process was recorded for 21 egg masses, 19 of which had at least 50% of their capsules hatched. Egg masses used in the analysis contained between 16 and 58 capsules.

Experimental design

The capsules from each egg mass were carefully separated from the base of adhesion to the substrate. Each capsule was individualized and photographed by using a camera coupled with an Olympus SZ61 (25× magnification) stereomicroscope. These images were analyzed by using Image Pro-Plus 5.0 software, to obtain the capsule area (one capsule side). Subsequently capsules were placed in an individual plastic container containing 30 ml of seawater (salinity: 30–32 psu, temperature: 17–18 °C), previously filtered (0.5 µm) and UV sterilized. Seawater was changed daily. Capsules were observed in the morning and afternoon of each day and the timing of the onset of embryonic hatching was recorded. A capsule was considered to have hatched if at least one larva had escaped from the capsule (Figure 1A, B). The first hatched capsule was considered as day 1 and used as reference time for hatchings of the rest of capsules from the same

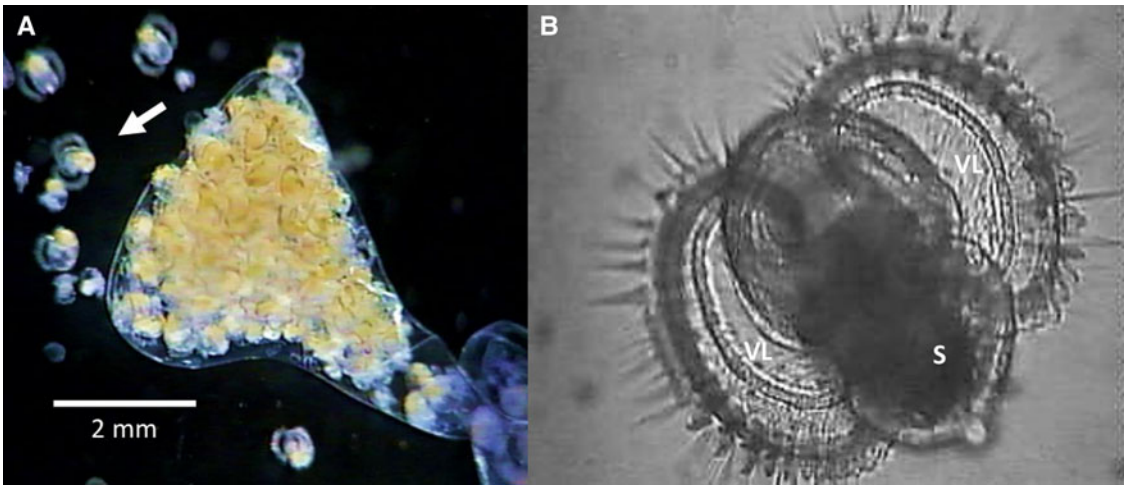

Figure 1. *Crepipatella peruviana* (A) Capsule with hatching larvae. Arrow = hatched larva. (B) Newly hatched larva. S = shell larva. VL = velar lobe.

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egg mass. We observed hatchings from the remaining sister capsules until at least 50% of the capsules of each egg mass had hatched. Then, for the remaining capsules that had not yet hatched, the same variables (larval SL, number of larvae, weight of all larvae in each capsule, individual larval weight obtained by dividing the total larvae weight by its number of larvae) were quantified as in the hatched sister capsules, except, of course, for size at hatching.

Capsule processing and timing of hatching

The released larvae were all removed from the respective aquaria and counted. All hatched larvae were then photographed using the same equipment as described above in the experimental design section. These images were analyzed using Image Pro-Plus 5.0 software, to determine the shell lengths. The larvae from each capsule were then deposited on a fiberglass filter, which was previously washed, dried, and weighed. The filters loaded with a known number of larvae were quickly rinsed with distilled water to remove salt crystals and then dried in a Memmert oven at 60 °C for 48 h. Each of these filters was then weighed to determine the dry tissue weight of the hatched larvae; from this information the average dry weight per larva was estimated.

When approximately 50% of the capsules in each egg mass had hatched, the unhatched capsules were carefully opened manually and the larvae were counted and measured. The measurements performed on the encapsulated larvae (larval SL, number of larvae, weight of all larvae in each capsule, individual larval weight) were the same as those performed on the hatched larvae. This information made it possible to compare the characteristics of the contents of the hatched capsules with those of the sister capsules that had not yet hatched. Embryos from manually opened capsules were photographed as described above, the number of larvae in each capsule was quantified, and shell lengths were measured. The larvae were then placed on a pre-treated fiberglass filter, dried in an oven, and weighed.

Influence of the hatching capsules on the hatching of the sister capsules

Females of *C. peruviana* were detached from their substrate and 10 egg masses containing advanced embryos were collected. Each egg mass was placed in a 30 ml container filled with filtered (to 5 µm) seawater at 32.2 ± 0.3 psu and maintained at 16.5 ± 0.5 °C. Seawater was changed daily. Before each water change, the containers were checked for the presence of hatched larvae. When newly hatched larvae were identified, all sister capsules in the egg mass were checked during the current day and the following day to determine how many capsules had hatched or begun to hatch in order to identify whether the hatching of one capsule induced the hatching in the remaining sister capsules.

Statistical analysis

Since the assumptions of homogeneity of variance (Levene test) and normality of the data (Shapiro–Wilk test) were not met, both larval biomass and mean larval size were analyzed using the nonparametric Mann–Whitney test to identify differences between larvae that hatched and those that did not After evaluating these differences, linear regressions were performed specifically for hatching individuals to establish the relationship between hatching days and variables such as number of larvae per capsule, hatching size, capsule area, capsule biomass, and larval biomass.

A non-linear regression with a polynomial equation was used for determining the relationship between number of days until hatching and female dry weight, using as reference the day of the first hatched capsules from each egg mass. Finally, a multiple regression analysis was performed to examine the dependence of hatching time on the variables of larval density per capsule and hatching size. For all analyses, a significance level of 0.05 was used and the analyses were performed in the Sigmaplot 12.0 statistical program.

Results

Larvae from each isolated capsule were able to hatch successfully in the absence of the mother. On the other hand, in an intact egg mass, the hatching of the first capsules in an egg mass did not stimulate hatching of neighboring sister capsules in the same egg mass.

Larval shell length at hatching

Hatched larvae had a significantly greater mean shell length (353.8 ± 21.4 µm, $n = 188$) than was found for the unhatched larvae (343.1 ± 19 µm, $n = 57$) (Mann-Whitney rank sum test: $P = 0.007$, Figure 2A).

Larval biomass

There was no significant difference between the total larval biomass of *C. peruviana* contained in the hatched and unhatched sister egg capsules (Mann-Whitney rank sum test: $P = 0.222$, Figure 2B).

However, the mean biomass per larva of *C. peruviana* showed significant differences between newly hatched larvae and those taken from unhatched sister capsules (Mann-Whitney rank sum test: $P = 0.047$). Hatched larvae were approximately 14% heavier (mean 8.93×10^{-6} g larva^{-1}) compared to unhatched larvae (7.78×10^{-6} g larve^{-1}) (Figure 2C).

In general, egg capsules of *C. peruviana* containing larger, heavier, and fewer larvae hatched earlier than the sister capsules that did not meet these criteria (Figure 3).

Hatching time of the capsule

Larvae per capsule vs hatching time

The timing of hatching of the *C. peruviana* capsules taken from the same egg mass was related to larval content, with capsules containing fewer embryos hatching sooner than those with more embryos (linear regression: $y = 348.064 + (15.911x)$, $R^2 = 0.1416$; $F_{(1,174)} = 28.29$, $P < 0.001$) (Figure 4A).

Capsular surface area vs hatching time

A positive and significant linear relationship between hatching time (compare to the first hatched capsule from the same egg mass) and capsular surface area was recorded for the hatched capsules (linear regression: $y = 11.795 + (0.575x)$, $R^2 = 0.1777$; $F_{(1,174)} = 37.33$, $P < 0.001$) (Figure 4B).

Hatching size vs hatching time

Length of encapsulated larvae at hatching was not related to capsule hatching time (linear regression: $y = 350.134 + (0.599x)$, $R^2 = 0.008$; $F_{(1,173)} = 1.492$, $P = 0.224$) (Figure 4C).

Individual larval weight vs hatching time

Larvae with greater biomass generally hatched sooner than those with lower biomass (linear regression: $y = -4e^{-7}x + 1e^{-5}$, $R^2 = 0.0509$; $F_{(1,171)} = 9.124$, $P = 0.03$) (Figure 5A).

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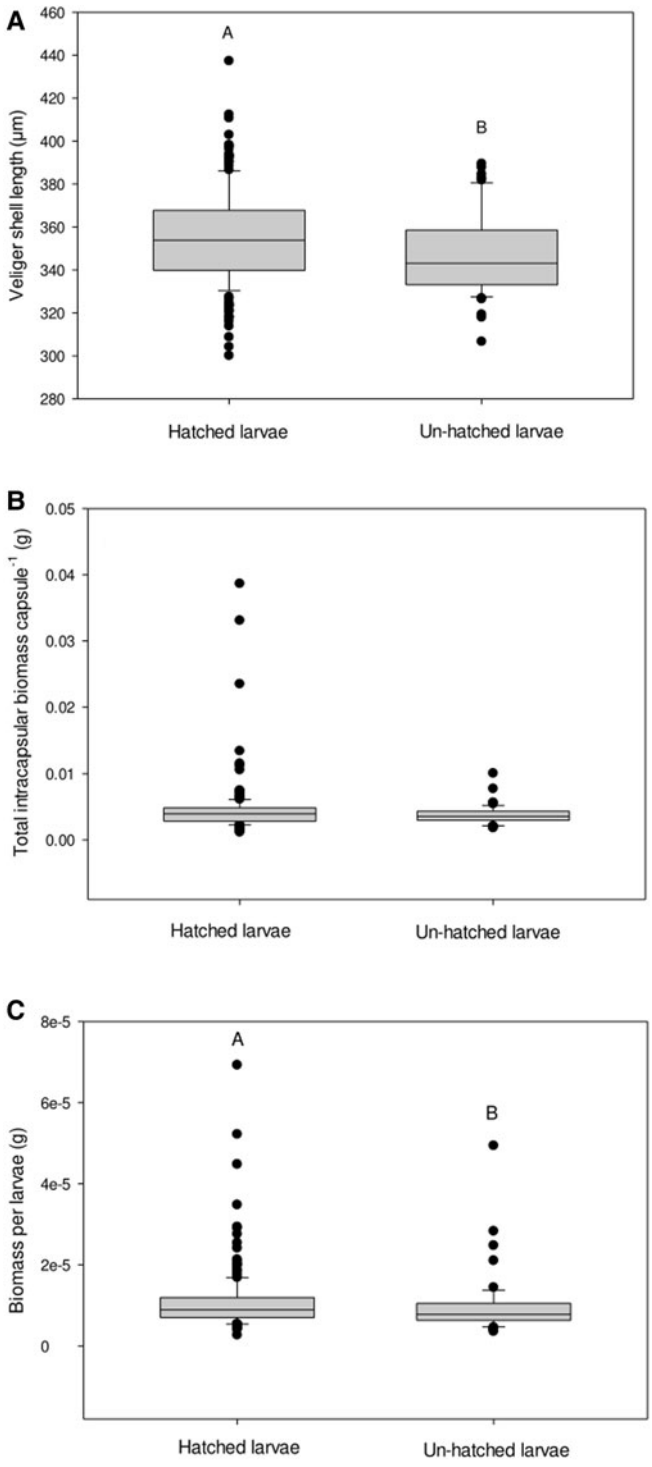

Figure 2. *Crepipatella peruviana* (A) Mean shell length $P = 0.007$, Total $n = 245$, (B) larval biomass $P = 0.222$, Total $n = 245$ and (C) average individual dry weight of larvae $P = 0.047$, Total $n = 245$ of hatched and unhatched *C. peruviana* veligers. Different letters above the bars indicate significant differences between means.

Capsular biomass vs *hatching time*

No significant relationship was found between the hatching time (order of capsule hatching) and the total biomass of the larval capsule at the time of hatching (Linear regression: $y = 8e^{-6}\,x + 0.00397$, $R^2 = 0.002$; $F_{(1,171)} = 0.035$, $P = 0.85$) (Figure 5B).

Female dry weight vs *days until hatching*

The hatching order of capsules from the same egg mass showed a significant relationship between time until hatching and dry weight of the female (non-linear regression: $0.0055x^2 - 0.009x + 0.9747$, $R^2 = 0.2115$; $F_{(1,175)} = 23.06$, $P < 0.001$) (Figure 5C), with capsules from larger females requiring more time to hatch.

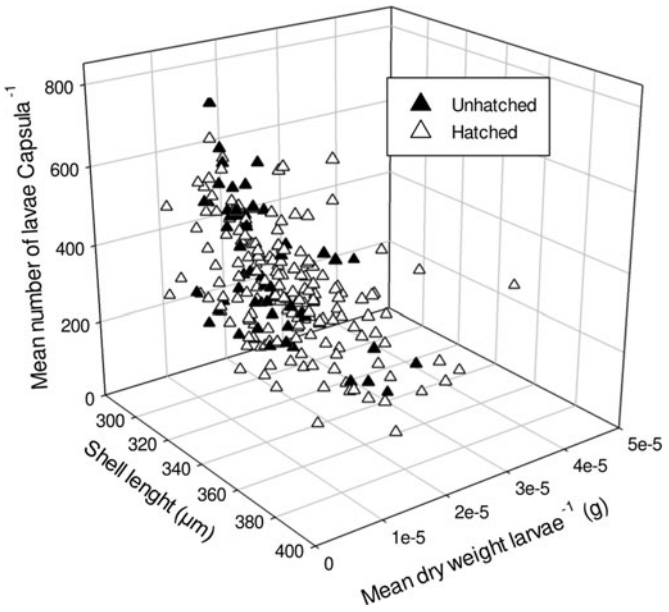

Figure 3. *Crepipatella peruviana.* Relationship between mean dry weight of each larva, number of larvae per capsule, and the size of *C. peruviana* veligers from hatched and unhatched capsules.

The order in which capsules from the same egg mass hatched depended on the size of the larvae at the hatching and the number of encapsulated larvae (number of larvae/capsule, Figure 6, Table 1). The first hatched larvae corresponded to larger larvae from capsules with fewer siblings.

Discussion

In our experiments with the marine gastropod *C. peruviana*, the absence of the mothers did not appear to have a direct effect on the timing of larval hatching from the egg capsules. Nevertheless, the possibility that the incubating female may have some involvement, especially in the synchronization of hatching, should not be excluded. In general, the capsular hatching process responds to a set of intracapsular conditions and is strongly associated with the physiological and/or morphological characteristics of the embryos (Andrade-Villagrán *et al.*, 2018b). However, in some encapsulating marine invertebrates, the mother plays an active role in hatching (Clare, 1997; Oyarzun and Strathmann, 2011; Warkentin, 2011; Branscomb *et al.*, 2014; Lesoway *et al.*, 2014), and in some species this female role may be driven by environmental changes, such as temperature and food availability, or biological changes, such as the presence of predators (Oyarzun and Strathmann, 2011; Branscomb *et al.*, 2014).

In several species of incubating invertebrates, including a number of other calyptraeid species (e.g. *Crepidula navicella*, Lesoway *et al.*, 2014, *Calyptrea lichen*; McDonald *et al.*, 2014), female presence is associated with larval hatching through active rupture of the enveloping walls (Clare, 1997; Saigusa, 2000; Oyarzun and Strathmann, 2011; Oyarzun and Brante, 2015). In some cases, parents can manipulate the timing of hatching (Warkentin, 2011). In the case of the estuarine crustacean *Sesarme haematocheir*, hatching of zoeae larvae is more synchronous when stimulated by the mother than when the larvae hatch after their removal from the maternal pleopods (Saigusa, 2000). In contrast, in some species of the gastropod family Calyptreidae, the

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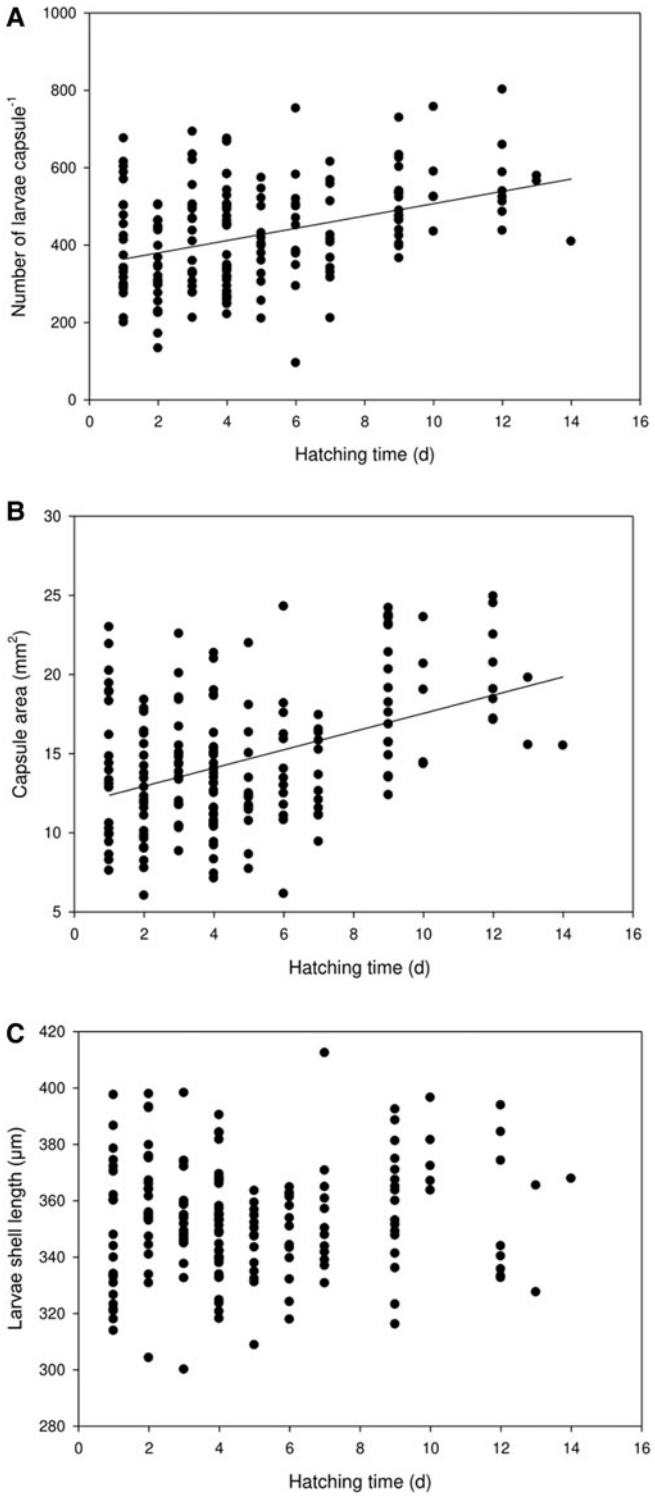

Figure 4. *Crepipatella peruviana.* (A) Positive relationship between the hatching time (compared to the first hatched capsule from the same egg mass clutch) and the number of larvae contained in each capsule. $P < 0.001$, Total $n = 175$. (B) positive relationship between the hatching time and the area of each capsule (mm^2, surface area of the lateral side of a capsule) from the same egg mass $P < 0.001$, Total $n = 175$. (C) Relationship between the hatching time for capsules from the same egg mass and the size of the hatched veligers (µm SL) from those capsules $P = 0.224$, Total $n = 174$. Each dot corresponds to one capsule.

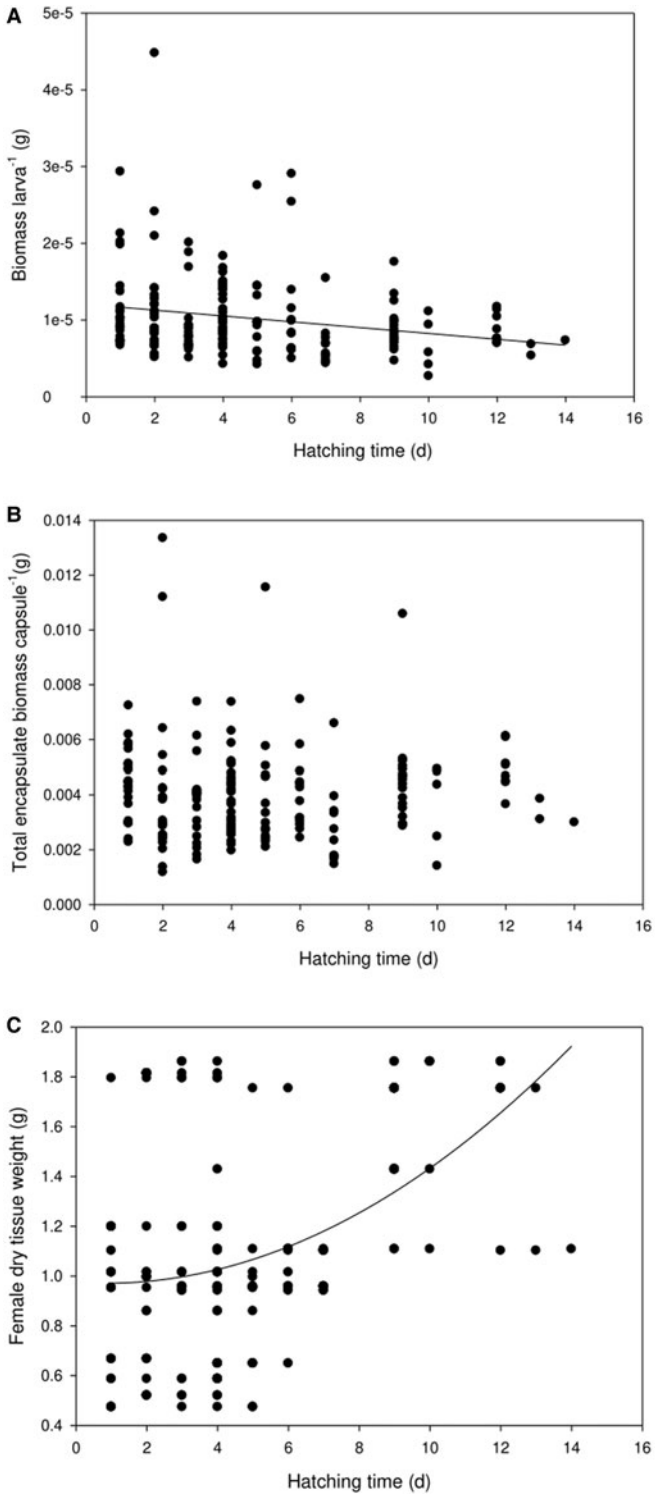

Figure 5. *Crepipatella peruviana.* (A) Relationship between when hatching occurred from an egg capsule and the mean biomass of each hatched larva, $P = 0.03$, Total $n = 172$. (B) relationship between capsule hatching time and total biomass of hatched larvae from each capsule, $P = 0.85$, Total $n = 172$. (C) relationship between hatching capsule time from the same egg mass and dry tissue weight of the producing *C. peruviana* female, $P < 0.001$, Total $n = 176$. Each symbol corresponds to one capsule.

mandatory involvement of the mother in hatching has been discarded (*C. dilatata*, Andrade-Villagrán *et al.*, 2018b), as in our results with *C. peruviana*.

The presence of hatching capsules in an egg mass did not induce the hatching of the sister capsules in *C. peruviana*, as has also been documented for the sibling species *C. dilatata* (Andrade-Villagrán *et al.*, 2018b). The hatching process clearly has an internal capsular origin in this species, such that hatching depends on the state of the embryos encapsulated in a given capsule and not on the state of the hatching process in the sister capsules within the same egg mass. This non-incidence of hatching sister capsules is related to the hatching method described for *C. peruviana*, where the process is associated with osmotic

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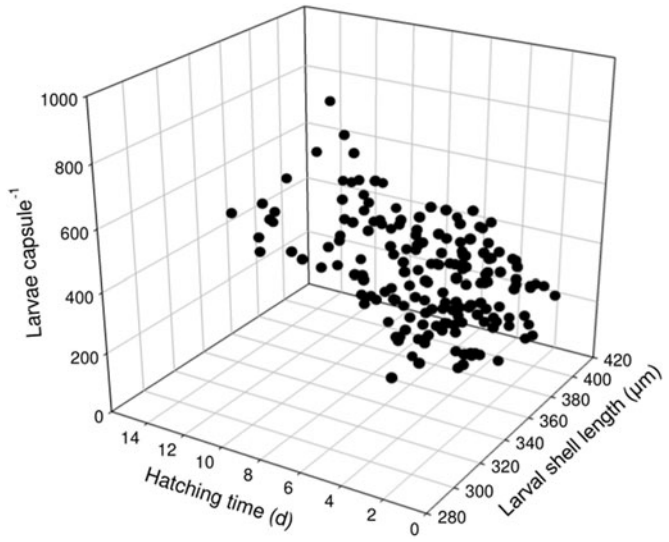

Figure 6. *Crepipatella peruviana.* Relationship between the average size of hatched *C. peruviana* larvae (µm SL), the hatching time, and the number of larvae in each capsule.

changes inside the capsules and the entry of water, which facilitates the rupture of the capsular closure and the subsequent hatching of the larvae (Andrade-Villagrán *et al.*, 2018b). It should be noted that the capsule wall in *C. peruviana* thins with larval development, which may promote larval hatching (Ojeda and Chaparro, 2004).

Hatching vs non-hatching

Our results indicate that capsular opening in the hatching process is closely related to the size of the encapsulated veligers; in the present study, size at hatching averaged about 354 µm. Previously reported hatching sizes for this species (329.5 mm SL Chaparro *et al.*, 2005; 337–340 µm SL, Chaparro *et al.*, 2002) show hatching sizes relatively similar to those obtained in the present investigation.

A second factor associated with larval hatching in this species was the mean biomass per larva. Larvae from hatched capsules had a higher average weight than larvae from sister capsules that had not yet hatched. However, the total weight of larvae in the capsule was not a determinant of the timing of hatching, as this may be due to capsules with many siblings that have not yet reached hatching size. This scenario probably results in a delay in hatching time because additional time is needed for both size and weight to reach the levels associated with the hatching process.

In summary, the timing of hatching of *C. peruviana* capsules is related to a combination of larger larval size, higher individual larval weight, and a lower number of larvae inside the capsules, which is consistent with what Andrade-Villagrán *et al.* (2018b) reported for the same process in *C. dilatata*.

Hatching order in capsules from the same egg mass

The present study shows that the number of siblings per capsule can affect the hatching order in *C. peruviana*. In this species, we observed a range of up to 14 days between the first hatching and the last hatching from capsules hatching within the same egg mass. This range should be even wider in nature, considering that in our experiments we controlled at most 60% of the hatched capsules, since the remaining capsules of the same egg mass that had not hatched were used to identify the differences with the hatched ones. The range of time between the first hatching and the last hatching for capsules from the same egg mass could be related to the time it takes the female to deposit the entire egg mass. Unfortunately, there is no information available for *C. peruviana* on how long it takes females to complete the oviposition process. However, the information available for the related species *C. dilatata* indicates that the oviposition process would take approximately only 9% (approx. 0.7 d) of the time it takes for all of the capsules from the same egg mass to hatch (Andrade-Villagrán *et al.*, 2018b). In the case of *C. navicella*, a tropical calyptreid gastropod, estimates of the time required to deposit capsules from an egg mass ranged from 2.6 to 24 h, while hatching of all capsules from an egg mass took from a few hours to a few days (Lesoway *et al.*, 2014). This information could suggest that the difference between the first and last hatch is not related to the time elapsed from the first to the last capsule deposited by the *C. peruviana* females.

Our results indicate that when capsules hatch from the same egg mass of *C. peruviana* is related to the number of larvae inside the capsules. The first capsules to hatch in an egg mass tended to enclose fewer larvae, which in turn is related to the smallest capsules from an egg mass. The timing of hatching varied considerably between capsules in the same egg mass, suggesting that this process varies according to the stage of embryonic development within each capsule.

As in our results with *C. peruviana* (which does not produce nurse egg), in the sister species *C. dilatata* (with nurse egg), it was also found that the unhatched capsules from the same egg mass were those with smaller offspring and more numerous (Andrade-Villagrán *et al.*, 2018b). The diameter of the eggs deposited in the capsules of *C. peruviana* shows a very conspicuous and narrow modal distribution (Gallardo, 1977). Considering this distribution as a proxy of similar egg sizes, the faster development to hatching size in the less densely populated egg capsules does not seem to be related to the endogenous energetic supply to the embryos and may be related instead to the availability of dissolved organic material (DOM) in the intracapsular fluid that bathes the embryos (calyptraeids, Ojeda and Chaparro, 2004; Brante *et al.*, 2009; Leroy *et al.*, 2012). Embryos are able to use the inner capsule layers and the DOM in the intracapsular fluids as an extraembryonic energy source, and so with fewer embryos per capsule, there will

Table 1. Multiple regression and the importance of the variables that explain the moment of hatching and the order in which the capsules hatched from the same egg mass of *C. peruviana*

	Coefficient	Std. Error	*t*	*P*	VIF
Constant	−6.478	3.822	−1.695	0.092	
Number of larvae	0.00856	0.00184	4.648	<0.001	1.208
Larva shell length	0.0237	0.0105	2.245	0.026	1.027
Larval biomass	− 61,323	−1.331	−1.331	0.185	1.233

VIF, Variance inflation factor.

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be less competition for those resources. The ability of embryos to use DOM has been recognized in several species of encapsulating invertebrates (Taylor, 1973; Stöckmann-Bosbach and Althoff, 1989; Brante *et al*., 2009; Büchner-Miranda *et al*., 2018) including *C. peruviana* (Ojeda and Chaparro, 2004).

In later hatching capsules with a high number of siblings, their slower growth could also be related to unfavorable conditions associated with a higher density of embryos, which affects, for example, oxygen availability; oxygen deprivation has been shown to slow the development of encapsulated embryos in *Crepidula coquimbensis* and *C. fornicat*a (Brante *et al*., 2009). In some gastropod egg capsules the availability of intracapsular oxygen is known to vary according to the developmental stage of the embryos (Segura *et al*., 2014). Advanced prehatching stages (e.g. the gastropod *Acanthina monodon*, Chaparro *et al*., 2020) may suffer from hypoxic conditions within the capsules at prehatching stages, which may explain slower embryonic development rates. Previous records for *C. dilatata* have also shown that periods of hypoxia increase the time until hatching (Segura *et al*., 2014).

The larval shell length at hatching in the first hatched capsules from a given egg mass is very similar to that of capsules hatched later from the same egg mass, reinforcing the idea that larval size is a good indicator of the ability of larvae at hatching to function in the open environment (Warkentin, 2011). The length of the larval shell is a critical factor in the hatching process. Consequently, the availability of the elements necessary for shell growth may act as a limiting factor in this process. For instance, in the intracapsular fluid of the species *Crepidula fornicata*, it has been demonstrated that as development progresses, the ions 'packaged' by the mother (e.g. Cl^{-}, Na^{+}, Mg^{2+}, Ca^{2+}) are not sufficiently abundant (Maeda-Martínez, 2008) to meet the needs of the embryo as it develops its shell. In more advanced larval stages, an additional influx of ions into the intracapsular fluid would occur, but only after the capsule wall has become more permeable due to the dissolution of part of the capsule wall (Maeda-Martínez, 2008). Given the close relationship between the availability of these ions and shell growth, it is likely that their availability during the early stages of shell development is a limiting factor for embryonic growth while encapsulated, particularly for densely-packed capsules, where embryos must share these ions with many more siblings, slowing larval development. Hatching in *C. peruviana* is also associated with an osmotic mechanism (Andrade-Villagrán *et al*., 2018a). Intracapsular osmolality is lower when veligers are of prehatching size, implying that water entry into the capsule is associated with a larval size that has developed capacities that favor water entry into the capsule and whose internal pressure forces the opening of the apical ´zipper´ of the capsule, with the consequent exit of the larvae (Andrade-Villagrán *et al*., 2018a).

In *C. peruviana*, siblings from the same capsule tend to be quite similar in size at hatching, which seems to be explained by the same maternal origin and the absence of nurse eggs as extraembryonic food (and thus the absence of competition for this type of food) inside the capsule. In *C. peruviana*, the diameter of the eggs deposited inside the capsules exhibits a narrow range, with a markedly pronounced mode and limited hatch size variation (Gallardo, 1977). This is in contrast to its sister species, *C. dilatata*, which displays a broad range of egg sizes and individual sizes at hatching (900–1300 µm SL, Gallardo, 1977). This disparity appears to be related to the availability and unequal distribution of nurse eggs among siblings, which are needed to feed the embryos during the encapsulated phase.

Hatching of capsules from the same egg mass in *C. peruviana* occurred at different times in our study, and the hatching order appears to be related to the size and number of encapsulated veligers within an egg capsule. Thus, in some egg masses, the large time lag between hatching of the first and last egg capsule in an egg mass suggests that females apparently have limited control over the total number of eggs contained in each capsule. However, this apparent disadvantage may increase offspring survival in changing environments (Moran and Emlet, 2001; Marshall *et al*., 2008). In adverse situations, the delayed encapsulated embryos may find more favorable conditions at hatching, allowing them to cope with temporary adverse situations and thus avoid total loss of the egg mass. Future research should address this question.

Data. The data that support the findings of this study are available on request from the corresponding author ORC.

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