COMPARISONS OF FECUNDITY, EGG SIZE, AND EGG MASS VOLUME OF THE FRESHWATER PRAWNS *MACROBRACHIUM POTIUNA* AND *MACROBRACHIUM OLFERSI* (DECAPODA, PALAEMONIDAE)

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A B S T R A C T

Some reproductive features of *Macrobrachium potiuna* and *M. olfersi* females were compared, correlating brood pouch volume with the number and volume of eggs during the incubation period. These data contribute to the understanding of the diversity of reproductive strategies observed in the family Palaemonidae. *Macrobrachium olfersi* and *M. potiuna* were collected in Ratones basin, Santa Catarina Island, Brazil, and the total lengths of the ovigerous females were determined in the laboratory. The eggs were measured, and the individual fecundity was determined. Volume of the eggs, egg mass volume, and brood pouch volume were estimated. Females of *M. potiuna* had a mean body length of 34.9 mm, mean brood pouch volume of 71.3 mm^3, mean fecundity of 35 eggs, and egg mass volume of 70.3 mm^3. *Macrobrachium olfersi* females had a mean body length of 46.7 mm, mean brood pouch volume of 127.9 mm^3, mean fecundity of 1917.2 eggs, and egg mass volume of 96.6 mm^3. Differences of fecundity and egg volume in *M. potiuna* and *M. olfersi* indicate the adoption of different reproduction strategies. However, proportional egg loss and increase of egg volume during the embryogenesis reflect particularities of the reproductive process of the females in the Palaemonidae.

Caridean females carry yolky eggs in an external brood pouch formed by the growth of the abdominal pleura and the bristles of the pleopods (Charniaux-Cotton and Payen, 1992). The strategy of carrying eggs until hatching contributes to a greater survival of the embryos, optimizing the reproductive success of the species (Shakuntala, 1977; Nazari *et al.*, 2000).

Different reproductive strategies can be observed in palaemonid species, such as carrying a great number of smaller eggs, as previously reported for *Macrobrachium olfersi* (see Corey and Reid, 1991); *Macrobrachium amazonicum* (see Odinetz-Collart and Rabelo, 1996); and *Macrobrachium acanthurus* and *Macrobrachium carcinus* (see Müller *et al.*, 1999b). Other species, for instance *Macrobrachium borelli* (see Pereira and Garcia, 1995); *Macrobrachium potiuna* (see Müller and Carpes, 1991); *Macrobrachium nipponense* (see Mashiko, 1990); and *Macrobrachium nattereri* (see Magalhães and Walker, 1988), have few eggs of larger volume.

Total body length (Valenti *et al.*, 1989; Müller and Carpes, 1991) and length and volume of the abdomen (Corey and Reid,

1991) are the dominant factors in the amount of eggs transported by palaemonid females. Other biological variables are also important in the determination of fecundity, such as the length of the pleopods and the mortality rate of the eggs (Annala, 1991).

The freshwater prawns *M. potiuna* (Müller, 1880), a Brazilian endemic species, and *M. olfersi* (Wiegmann, 1836), which has a large geographic range, are frequently found living alongside each other in inland waters in southern Brazil (Bond-Buckup and Buckup, 1989; Müller *et al.*, 1999b).

Macrobrachium olfersi belongs to a group of *Macrobrachium* species that depends on estuarine water to successfully complete the larval development, whereas *M. potiuna* completes its whole life cycle in freshwater environments. As with other palaemonids, such as *Palaemonetes argentinus* (see Müller and Araujo, 1994) and *P. pugio* (see Wirth *et al.*, 2001), these species have ecological importance in the freshwater food chain and can be used as biomarkers of aquatic contamination.

This paper compares the reproductive features of *M. potiuna* and *M. olfersi* females,

Table 1. Values of length, brood pouch volume, and fecundity in females of *M. potiuna* and *M. olfersi* in Groups I and II.

	M. potiuna	*M. olfersi*
Female length* (mm)		
Range (n = 45)	27.3–48.6	33.2–64.0
Mean (n = 45)	34.9 (± 4.54)	46.8 (± 7.20)
Group I (n = 22)	34.6 (± 4.64)	47.6 (± 8.38)
Group II (n = 23)	35.2 (± 4.52)	46.8 (± 6.14)
Brood pouch volume* (mm³)		
Range (n = 45)	39.3–128.8	33.8–240.8
Mean (n = 45)	71.3 (± 19.0)	127.9 (± 44.9)
Group I (n = 22)	69.9 (± 19.5)	131.2 (± 49.3)
Group II (n = 23)	72.5 (± 19.0)	127.0 (± 40.8)
Fecundity* (eggs)		
Range (n = 45)	19–65	1029–6320
Mean (n = 45)	35.0 (± 15.2)	1917.2 (± 1104.1)
Group I (n = 22)	40.2 (± 12.67)**	2209.7 (± 1337.2)**
Group II (n = 23)	30.3 (± 16.02)**	1651.4 (± 778.2)**
Apparent egg loss	9.9 (± 4.9)	558.3 (± 289)

* Data shown in mean (± standard deviation).
** Indicates significant statistical variation between Groups I and II.

correlating brood pouch volume with the number and volume of eggs in two groups of ovigerous females defined according to the incubation period. These data contribute to the understanding of the diversity of reproductive strategies observed in the family Palaemonidae.

MATERIALS AND METHODS

Ovigerous females of *Macrobrachium potiuna* and *Macrobrachium olfersi* were collected in the Ratones basin (27°28′S, 48°30′W), Santa Catarina Island, Brazil, in freshwater streams. *Macrobrachium potiuna* preferentially inhabits small, clear-watered stony streams surrounded by arboreous vegetation. *Macrobrachium olfersi* was captured in wider streams with sand and gravel bottoms, large amounts of aquatic vegetation, and no tree or shrub cover at the margins.

In the laboratory, the females were cooled at 4°C for the determination of the total body length, defined as the distance between the distal extremities of the rostrum and the telson. Subsequently, the individuals were fixed in formaldehyde solution and stored in 70% alcohol. The egg mass was removed from the brood pouch for the determination of the individual fecundity by manual count and characterization of the developmental stages.

Eggs were classified in two developmental stages, based on the presence or absence of eye pigmentation. This allowed the categorization of the ovigerous females into two groups: Group I was represented by females bearing eggs without eye pigmentation; and Group II corresponded to females carrying eggs with eye pigment visible.

Subsequently, the long and short axes of eggs were measured using an optical microscope with an eyepiece scale (20× for *M. potiuna* and 40× for *M. olfersi*). The number of eggs to be measured from each female was determined by the equation $N = 1.96 \cdot s/l$, where s = standard deviation of the initial sample (n = 5) and l = 10% of the average of the initial sample (Müller *et al.*, 1999b). The egg volume was calculated from the formula $EV = (\pi l h^2/6)$, where l = long axis and h = short axis (Odinetz-Collart and Rabelo, 1996). The egg mass volume was obtained by $EMV = $ (mean fecundity) × (mean egg volume).

Brood pouch volume was calculated from $BPV = 1/6 LWD$, where L = length (measured between the anterior and posterior brood pouch extremities), W = maximum width of the abdomen, and D = maximum depth of the brood pouch.

Difference in mean length of females, brood pouch volume, fecundity, and egg volume between Groups I and II was tested using ANOVA (and Tukey's test). Linear regression and the correlation coefficient was calculated to assess the relationship between brood pouch volume and egg mass volume in each species.

RESULTS

In this study, females of *M. potiuna* had a mean length of 34.9 mm and a mean brood pouch volume of 69.9 mm³ in Group I and 72.5 mm³ in Group II. In *M. olfersi*, females had a mean length of 46.8 mm, and the mean brood pouch volume was 131.2 mm³ and 127.0 mm³ for Groups I and II, respectively (Table 1).

In both species, the eggs in the brood pouch were loosely attached to one another by chorion filaments and surrounded by mucus. They were continuously exposed to the external medium during all of the incubation period. However, the eggs of *M. potiuna* formed a less compact egg mass than *M. olfersi* and were rarely observed beyond the distal limit of the abdominal pleura (Fig. 1a). The mean fecundity of *M. potiuna* was 40.2 eggs for Group I and 30.3 eggs for Group II, with an apparent loss of 9.9 eggs (24.6% of the total number of eggs) during the incubation period (Table 1).

The eggs of *M. olfersi* were tightly attached to one another, and a large portion of them were attached to the external ovigerous setae of the pleopods (Fig. 1b). The mean fecundity was 2209.7 eggs in Group I and 1651.4 eggs in Group II, corresponding to an apparent loss of 558.3 eggs (25.2% of the total number of eggs) during the incubation period (Table 1).

The eggs of both species underwent a significant increase of egg volume from Group I to
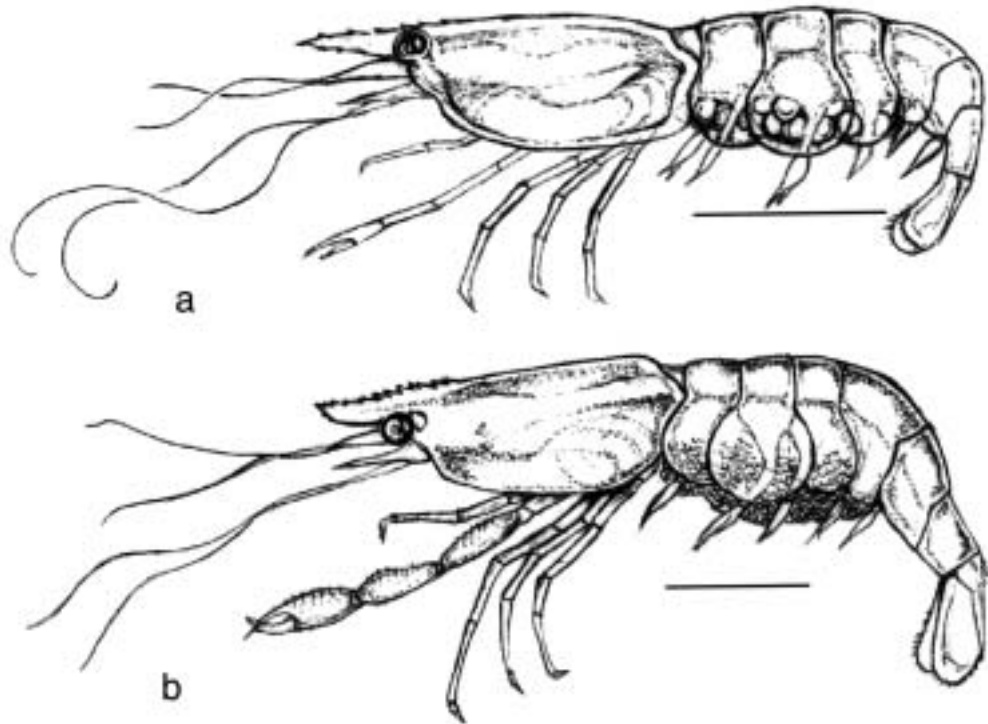
Fig. 1.   Ovigerous females of (a) *M. potiuna* and (b) *M. olfersi* in lateral view. Scale bar: 1 cm.

Group II, because of the growth of the egg axes. In *M. potiuna*, the short and long axes increased 2.9% and 21.2%, respectively. The increase in *M. olfersi* was 13.1% in the short axis and 23.4% in the long axis.

In *M. potiuna*, the mean volume of the eggs from Groups I and II was 1.789 and 2.290 mm^3, respectively, which corresponds to an apparent increase in the egg volume of 28%. The eggs of *M. olfersi* had a mean volume of 0.035 mm^3 in Group I and 0.056 mm^3 in Group II, indicating an apparent increase of 60% during the incubation period (Table 2).

The mean egg mass volume from Group I was 71.92 mm^3 for *M. potiuna* and 77.34 mm^3 for *M. olfersi*. In *M. potiuna*, the egg mass volume decreased 3.6%, reaching a mean value of 69.37 mm^3 in Group II. However, in *M. olfersi*, an increase of 19.6% in the egg mass volume could be observed from Group I to Group II, which had a mean value of 92.48 mm^3 (Fig. 2).

A positive significant correlation between the brood pouch volume and the egg mass volume in both Groups I and II in *M. potiuna* ($F = 14.6$, $P < 0.001$, $r = 0.77$; $F = 31.0$, $P < 0.0005$, $r = 0.78$) was observed (Fig. 3a). According to

Table 2.   Egg measurements of *M. potiuna* and *M. olfersi* in Groups I and II.

	Group I*			Group II*		
	Axis (mm)			Axis (mm)		
Species	Short	Long	Volume of the egg (mm^3)	Short	Long	Volume of the egg (mm^3)
M. potiuna	1.37	1.79	1.789	1.41	2.17	2.29
($n = 110$)	($\pm$ 0.08)	($\pm$ 0.13)	($\pm$ 0.27)**	($\pm$ 0.09)	($\pm$ 0.17)	($\pm$ 0.38)**
M. olfersi	0.38	0.47	0.035	0.43	0.58	0.056
($n = 110$)	($\pm$ 0.023)	($\pm$ 0.038)	($\pm$ 0.004)**	($\pm$ 0.036)	($\pm$ 0.077)	($\pm$ 0.009)**

* Data shown in mean ($\pm$ standard deviation).
** Indicates significant statistical variation between stages, ANOVA ($P \leq 0.05$).
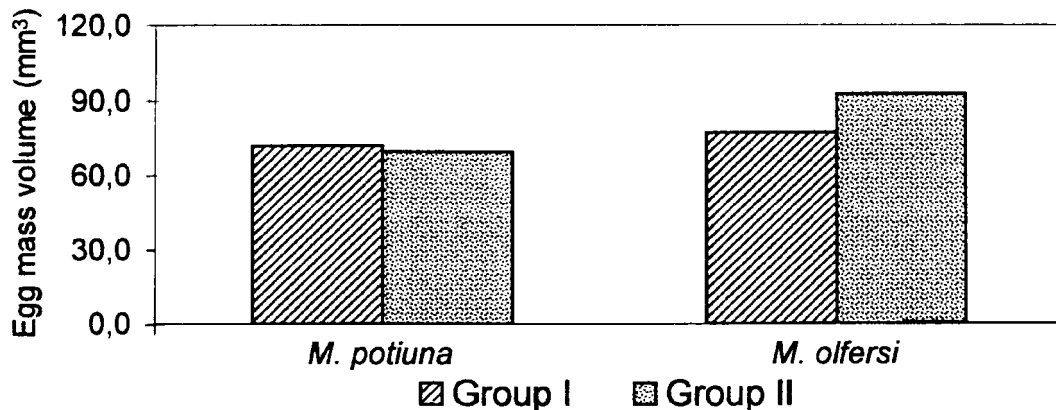
Fig. 2. Egg mass volume in females of Groups I and II in *M. potiuna* and *M. olfersi*.

the critical values (Zar, 1974), the Pearson's coefficient obtained in the regression analysis of both groups showed a strong correlation between the brood pouch volume and the mass of eggs.

In the Groups I and II of the *M. olfersi* females (Fig. 3b), there was also a positive correlation between the volume of the brood pouch and the volume of the egg mass ($F = 4.6$, $P < 0.04$, $r = 0.51$ for Group I; $F = 0.57$, $P < 0.02$, $r = 0.17$ for Group II). According to the critical values (Zar, 1974), the Pearson's coefficients obtained indicate a good dependency in this relationship for Group I , whereas in Group II the variables analysed had a lower level of dependency.

DISCUSSION

In this study, there was no representative differences of body size in Groups I and II of both species, suggesting that the apparent egg loss is not directly related to the size of the females. Instead, this loss seems to be a result of pleopod beating, swimming, and removal of the nonviable eggs (Levi *et al*., 1999).

Reduction of fecundity during the incubation period seems to be a common feature in the reproduction of crustaceans and was also observed in other species, such as the amphipod *Crangonyx pseudogracilis* (see Dick *et al*., 1998), the anomuran crab *Psidia longicornis* (see Sampedro *et al*., 1997), and the lobster *Homarus americanus* (see Talbot and Helluy, 1995). This apparent egg loss may result in a better accommodation of the eggs in the brood pouch, allowing a greater circulation of water

through the egg mass, thus increasing the rate of oxygen around embryos.

According to Dick *et al*. (1998), the embryo's demand for oxygen increases during the later stages of development, because of the greater number of cells present and the process of cell differentiation. As a result, the female increases the frequency of pleopod beating as hatching approaches, maximizing the chances of premature ejection of eggs from the brood pouch.

In *M. olfersi*, the strategy of carrying a large number of small eggs allows a greater egg loss without affecting the reproductive success of the species. *Macrobrachium potiuna* has a reduced egg loss, because this species spawns a smaller number of voluminous eggs, and a larger loss could compromise its reproductive investment. However, the proportional egg loss is equivalent in *M. olfersi* and *M. potiuna*. In palaemonids, the apparent egg loss has a wide range of values, as reported in *Macrobrachium acanthurus* and *Palaemon pandaliformis* (23%) (Anger and Moreira, 1998) and *Exopalaemon modestus* (7%) (Oh *et al*., 2002).

The marked increase in the volume of the egg observed during the process of embryonic development, as observed by Mossolin and Bueno (2002), is associated, among other parameters, with the absorption of water to obtain cellular mobility, structural organization, and the growth of the embryo (Green, 1965; Kobayashi and Matsuura, 1995).

In *M. potiuna*, the small reduction in the *EMV* from Group I to Group II is a consequence of the decrease of fecundity. However, because of the increase of egg volume, this reduction is minimized. Although the two species had
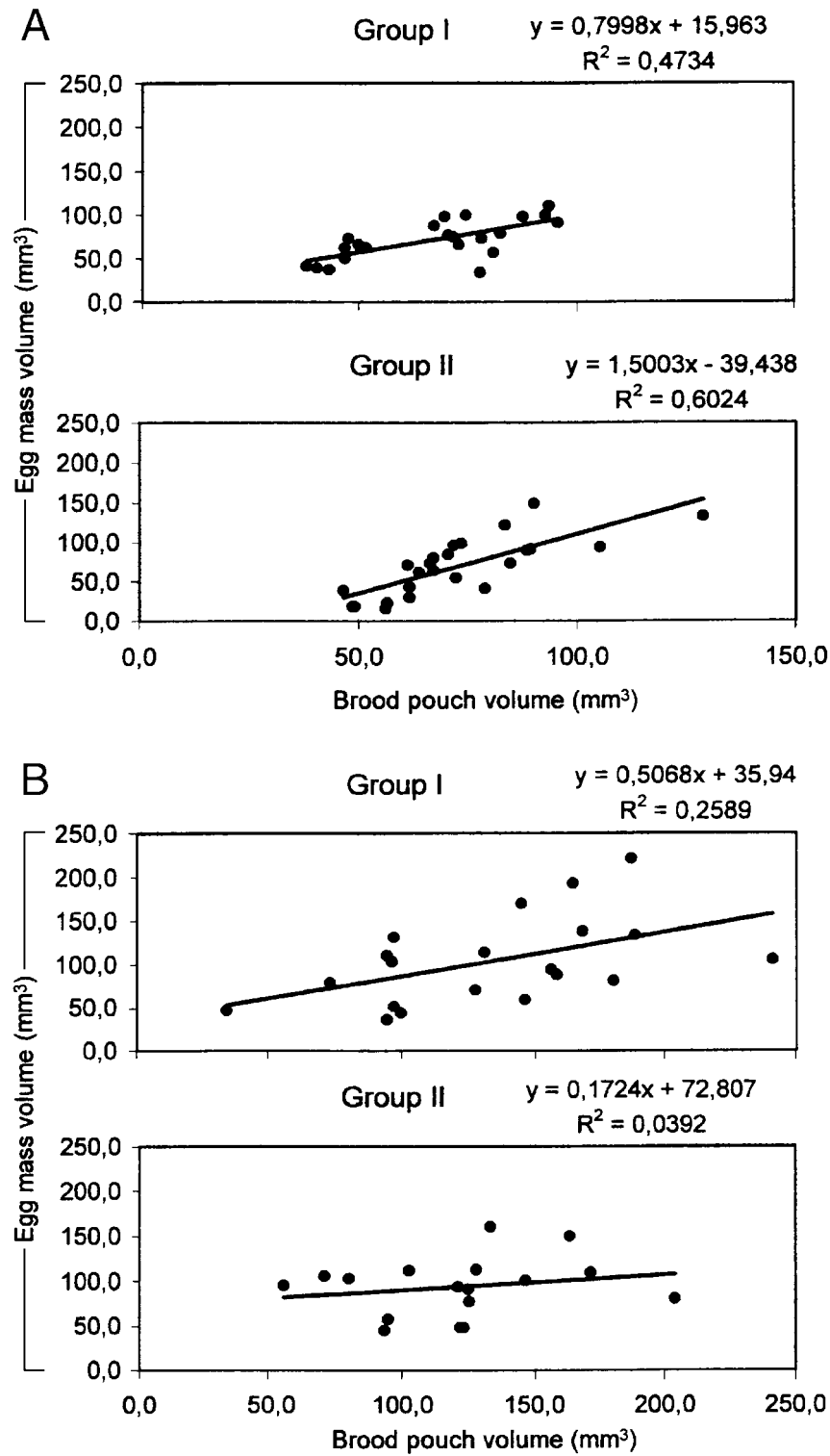
Fig. 3. Relationship between brood pouch volume and egg mass volume of Groups I and II in (A) *M. potiuna* and (B) *M. olfersi.*

a similar proportional egg loss, the larger increase of egg volume observed in *M. olfersi* results in a substantial growth in the *EMV* in Group II.

The greater increase in the long axis of the egg reflects the growth of the embryonic structures in the cephalic-caudal axis (Anderson, 1982). At the end of the incubation period, the growth of egg volume is an important feature of the embryogenesis, allowing a greater motility of the embryos and the hatching of the larvae (Müller *et al.*, 1999a; Nazari *et al.*, 2000).

In *M. potiuna*, the positive correlation between brood pouch volume and egg mass volume transported by Groups I and II shows a strong relationship between these two variables, suggesting that in this species, the egg production is proportional to the space available to accommodate the eggs.

In *M. olfersi*, the females from Group II present a slightly smaller brood pouch volume, although they are carrying a larger egg mass volume when compared to Group I. However, the smaller available space in the brood pouch of females of Group II does not entirely affect their capacity to carry eggs, because there is a large portion of the egg mass attached to the ovigerous setae of the pleopods, outside the space available in the brood pouch. This explains the positive correlation established between brood pouch volume and egg mass volume in *M. olfersi*, which is less significant than the ones observed in both groups in *M. potiuna*.

The comparison of the reproductive features of *M. potiuna*, found exclusively in freshwater streams, and *M. olfersi*, captured in sites connected to estuarine waters, are consistent with the observations of Jalihal *et al.* (1993), who stated that species from interior continental waters have an increase in egg size and a reduction of fecundity.

Therefore, the different reproductive features observed in the two species studied sustain the hypothesis that the genus *Macrobrachium* is still in the process of adapting to the freshwater environments, as stated by Mashiko (1990), Jalihal *et al.* (1993), and Odinetz-Collart and Rabelo (1996).

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