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Reproductive strategies of two sympatric species of *Hyaella* Smith, 1874 (Amphipoda, Dogielinotidae) in laboratory conditions

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Abstract

This study was performed in order to test theoretical predictions that sympatric species might show different reproductive strategies to facilitate their coexistence. The duration of precopulatory mate-guarding behaviour, body size of ovigerous females, embryonic and postembryonic development time, fecundity, and viability of the sympatric amphipod species *Hyaella pleoacuta* and *H. castroi* were investigated under laboratory conditions ($19 \pm 1^\circ\text{C}$ and 12 h light/dark photoperiod). Specimens were collected with nets in autumn and spring 2004, and summer and winter 2005 in the Vale das Trutas, São José dos Ausentes County, southern Brazil, and transported to the laboratory. Both species of *Hyaella* showed similar reproductive strategies, especially in respect to the duration of the precopula, duration of the embryonic period, and fecundity. However, these species differed in body size of ovigerous females, duration of the postembryonic period, viability, and body size of juveniles that remained in the marsupium and those released by females. These differences in the reproductive traits of *H. pleoacuta* and *H. castroi* may facilitate their coexistence in nature.

Keywords: Embryonic and postembryonic periods, fecundity, *Hyaella*, precopulatory mate-guarding time, viability

Introduction

Freshwater amphipods of the genus *Hyaella* Smith, 1874 are widely distributed throughout North America, and occur also in Central and South America (González and Watling 2002). The life cycles of the species of *Hyaella* that occur in Brazil are not known. The majority of studies on *Hyaella* in Brazil have emphasized the systematic of the group (Pereira 1989, 2004; Bond-Buckup and Araujo 1998; González et al. 2006).

Amphipods are frequently used as bioindicators and biomonitors in a variety of aquatic ecosystems because they are found in marine, terrestrial, and freshwater environments (Borgmann et al. 1989; Buyle 1989; Nelson and Brunson 1995; Duan et al. 1997; Rinderhagen et al. 2000). Some features of the life cycle of these crustaceans, in particular

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their reproductive strategies, may be appropriate to interpret the results of ecotoxicological studies that utilize these organisms as bioindicators, especially in the species of *Hyaella* native to southern Brazil. In Brazil, as in the USA and Canada, ecotoxicological studies have used the North American species *Hyaella azteca* to evaluate the effect of different heavy metals on sediment quality of freshwater environments (Hogg et al. 1998). Assessment of the impacts caused by pollutants in a certain environment should use species endemic to the locale to be evaluated, because the effects may be under- or over-estimated when exotic species, for example *H. azteca*, are used.

Most of the species of *Hyaella* are found in a variety of permanent freshwater habitats, from large lakes to small ponds and marshes, clinging to vegetation, swimming in open water, or burrowing in bottom sediments (Kruschwitz 1978; Wellborn 1995; Grosso and Peralta 1999). However, some species are sympatric, such as *Hyaella pleoacuta* González, Bond-Buckup, and Araujo, 2006 and *H. castroi* González, Bond-Buckup, and Araujo, 2006; others live in caves, such as *H. caeca* Pereira, 1989; and one species, *H. rionegrina* Grosso and Peralta 1999, is found in wet soils (Grosso and Peralta 1999). The main aim of this study was to test theoretical predictions that sympatric species might show different reproductive strategies to facilitate their coexistence. The precopulatory mate-guarding time, the duration of embryonic and postembryonic development, the body size of ovigerous females, fecundity, and viability were investigated under laboratory conditions, to aid in understanding the reproductive strategies of *H. pleoacuta* González, Bond-Buckup, and Araujo, 2006 and *H. castroi* González, Bond-Buckup, and Araujo, 2006.

Materials and methods

Collection of amphipods

Specimens of the two species of *Hyaella* were collected with a net (autumn and spring 2004 and summer and winter 2005) in two ponds used for culturing trout, *Oncorhynchus mykiss* (Walbaum, 1792). The ponds are located in the Vale das Trutas, near the headwaters of the Antas River in São José dos Ausentes County, southern Brazil (28°47'00"S, 49°50'53"W). Both species are found together, living under the roots of aquatic macrophytes of the genera *Senecio* Linnaeus, *Hydrocotyle* Linnaeus, *Ludwigia* Linnaeus, and *Callitriche* Linnaeus, on the edges of the ponds. The local climate is medium mesotherm, with cold winters and mild summers, because of the altitude of 1100 m (Nimer 1989).

Laboratory procedures and data analysis

The amphipods were transported to the laboratory, where they were identified. They were maintained in the laboratory at 19°C and a 12 h light/dark photoperiod. During a 15-day acclimation period, the specimens of both species were kept in two aquariums, each with a volume of 15 litres of water. During this period, plants of *Salvinia* Séguier were added to the aquarium, to provide substrate and food. Every other day, fish food (TetraDiskus®—43% protein) was added *ad libitum* as a food supplement. The water used in laboratory cultures was collected together with the amphipods, from the trout-culture pond, without previous filtering. Some physical and chemistry water parameters are shown in Table I.

To determine the length of the precopulatory period, about 120 males and 100 females of *H. pleoacuta* and about 70 males and 50 females of *H. castroi* were kept together in a container in 1 litre of water. *Salvinia* was provided as food and substrate, and fish food was

Table I. Physical and chemical parameters of the water of the culture pond holding trout used in laboratory cultures.

Parameter	Value
O ₂ (mg l ⁻¹)	6.9
O ₂ (%)	78
pH	6.4
Electrical conductivity (μS cm ⁻¹)	29.3
Nitrogen (TKN) (mg l ⁻¹)	0.7
Nitrogen total (mg l ⁻¹)	1.0
Phosphorus total (mg l ⁻¹)	0.16
Potassium total (mg l ⁻¹)	1.5
Calcium total (mg l ⁻¹)	1.6
Magnesium total (mg l ⁻¹)	0.56
Copper total (mg l ⁻¹)	<0.02
Zinc total (mg l ⁻¹)	0.01
Iron total (mg l ⁻¹)	2.2
Manganese total (mg l ⁻¹)	<0.01
Sodium total (mg l ⁻¹)	2.4
Boron total (mg l ⁻¹)	0.01
Sulphate (mg l ⁻¹)	2.0
Hardness total (mg CaCO ₃ l ⁻¹)	3.7
N-NH ₄ ⁺ (mg l ⁻¹)	0.3
N-NO ₃ ⁻ + N-NO ₂ ⁻ (mg l ⁻¹)	0.3

Values are the means of two samples of water (April 2005 and April 2006).

added every other day. The containers were checked twice a day. Precopulatory pairs were transferred from the containers to an aquarium that had six connected compartments. The pairs were observed twice a day until they separated. Ovigerous females were separated from the males and observed daily to determine the embryonic and postembryonic development time. During the same period, another container with males and females was set up, to obtain more ovigerous females in order to characterize the embryonic and postembryonic periods. The embryonic development time was recorded as the period from oviposition until the release of juveniles into the marsupium; the postembryonic development time was recorded as the period from the release of juveniles into the marsupium until their liberation from the marsupium.

To assess the extent to which *Hyaletella* species mate assortatively by size, the correlation between male and female head length (HL) was examined for mating pairs in the laboratory. The mean size of paired males and females was compared by a *t* test ($\alpha=0.05$) (Zar 1996). The head length (tip of the rostrum to the posterior head margin in lateral view) was measured with a dissecting microscope and an ocular micrometer (0.01 mm). According to Edwards and Cowell (1992) and Pickard and Benke (1996) the head length has positive correlation with the total length of *Hyaletella azteca* (Saussure, 1858), and so this dimension can be used to represent the animal's size.

The contents of the marsupium of ovigerous females were characterized as one of three embryonic developmental stages, based on Hynes (1955) and Subida et al. (2005), and one postembryonic stage (juvenile remaining in the marsupium). These stages were distinguished easily by means of a stereoscopic microscope, and recognized as follows: stage I, with little or no cleavage, orange coloration, and yolk occupying the entire egg space; stage II, the beginning of the cellular cleavage is visible, but the body parts are not defined; stage III, the yolk is consumed, reduced to a tiny spot on the embryo, and the eyes

are easily visible; stage IV, with hatching juveniles present in the marsupium. These stages correspond to stages 1–2, 3, 4–5, and 6, respectively, as described by Sheader and Chia (1970) for *Gammarus obtusatus* Dahl, 1938. The term “egg” is used in this report synonymously with “embryo”, representing all developmental stages before hatching.

The fecundity (number of eggs) and viability (number of juveniles released by females) were determined under laboratory conditions (19°C and 12 h light/dark) for *H. pleoacuta* and *H. castroi*. About 250 males and 200 females of each species were kept together in an aquarium with a final volume of 15 litres of water. After they were fertilized, the females containing eggs at different stages of embryonic development were killed, and the number of eggs and/or juveniles was counted by means of a stereomicroscope. The mean number of eggs and the body size of ovigerous females were compared between *H. pleoacuta* and *H. castroi* using a *t* test ($\alpha=0.05$) (Zar 1996). Analyses of variance, complemented by a Bonferroni test, were used to compare the number of eggs produced in each embryonic developmental stage and the number of juveniles remaining in the marsupium in each species ($\alpha=0.05$) (Zar 1996).

We carried out regression analyses of egg numbers for each embryonic developmental stage, the number of juveniles remaining in the marsupium (*y*), and the head length (mm) of ovigerous females (*x*) ($y=ax-b$). Pearson’s correlation coefficient (*r*) was calculated for each relationship (Zar 1996). To determine whether the egg production at each embryonic developmental stage differed in size-specific fecundity, the embryonic stages were compared by covariance analysis, with the number of eggs and/or juveniles as the response variable, head length as the covariate, and the embryonic developmental stage as the grouping variable ($\alpha=0.05$) (Zar 1996).

After the juveniles hatched, some females were isolated in containers with a final water volume of 100 ml, in order to determine the mean number of released juveniles (viability). The mean number of released juveniles was compared with the mean number of juveniles remaining in the marsupium during the postembryonic development for each species, by means of a *t* test ($\alpha=0.05$) (Zar 1996). The mean number of released juveniles was compared between the species, by means of a *t* test ($\alpha=0.05$) (Zar 1996).

The head length was measured in juveniles born from ovigerous females (40 juveniles born from eight females), with a dissecting microscope and an ocular micrometer (0.01 mm). Student’s *t* test for mean comparisons ($\alpha=0.05$) was used to compare the mean size of juveniles inside the marsupium of females with those released by females (Zar 1996).

Results

Precopulatory mate-guarding time

The duration of the precopulatory mate-guarding phase is shown in Table II. The *t* test indicated no significant differences in the duration of this behaviour between *H. pleoacuta* and *H. castroi* ($t=0.585$; $P > 0.05$).

Table II. Duration (days) of the precopulatory mate-guarding phase in *Hyalella pleoacuta* and *H. castroi*.

	<i>Hyalella pleoacuta</i>	<i>Hyalella castroi</i>
Minimum–maximum	4.0–12.0	2.0–11.0
Mean $\pm$ SE	5.6 $\pm$ 0.36	5.4 $\pm$ 0.40
Number of mating pairs	31	20

Table III. Body size (head length in mm) of paired males and females of *Hyaella pleoacuta* and *H. castroi*.

	<i>Hyaella pleoacuta</i>		<i>Hyaella castroi</i>	
	Males	Females	Males	Females
Minimum–maximum	0.61–1.12	0.58–0.94	0.80–1.25	0.66–0.99
Mean ± SE	0.88 ± 0.009	0.72 ± 0.007	0.99 ± 0.009	0.80 ± 0.007
Number of individuals	100	100	92	92
<i>t</i> test	13.618		16.744	

Paired males of both species were significantly larger than the females (*H. pleoacuta*: $t=13.618$; *H. castroi*: $t=16.744$; $P < 0.05$) (Table III). A propensity to mate assortatively by size was observed in *H. pleoacuta* ($r=0.52$; $P < 0.05$), with large males generally paired with large females, and small males with small females. However, size-assortative mating in *H. castroi* was less pronounced ($r=0.23$; $P < 0.05$) than in *H. pleoacuta* (Figure 1).

Figure 1. Assortative mating, estimated as the relationship of male and female body size (head length in mm) for precopulatory mating pairs in *Hyaella pleoacuta* and *H. castroi*. HLm, head length of males; HLf, head length of females; n, number of individuals.

Table IV. Duration (days) of the embryonic and postembryonic periods in *Hyalella pleoacuta* and *H. castroi*.

	<i>Hyalella pleoacuta</i>			<i>Hyalella castroi</i>		
	EP	PP	TP	EP	PP	TP
Minimum–maximum	11–15	1–5	13–20	10–14	3–6	14–18
Mean $\pm$ SE	12.2 $\pm$ 0.12	3.2 $\pm$ 0.12	15.3 $\pm$ 0.20	12.0 $\pm$ 0.23	4.1 $\pm$ 0.13	16.1 $\pm$ 0.21
No. of ovigerous females	39	39	39	23	23	23

EP, embryonic period; PP, postembryonic period; TP, total embryonic period.

Duration of embryonic and postembryonic development

The duration of embryonic and postembryonic development is shown in Table IV. The length of the embryonic period was not significantly different between the two species ($t=0.629$; $P > 0.05$). However, the postembryonic period was significantly longer in *H. castroi* than in *H. pleoacuta* ($t=-5.741$; $P < 0.05$). The total embryonic period was significantly longer in *H. castroi* (16.1 ± 0.21) than in *H. pleoacuta* (15.3 ± 0.20) ($t=-2.966$; $P < 0.05$).

A total of 59 females of *H. pleoacuta* were observed after fertilization. Of these females, 66.1% carried the young until they completed embryonic development, 13.6% aborted, 5.1% ate the juveniles after they left the marsupium, and 15.3% died during the incubation period. In *H. castroi*, 35 females were observed after fertilization. Of these, 65.7% carried the young until they completed their development, 20% aborted, and 14.3% died during the incubation period.

During the time when juveniles were inside the female marsupium, no more-developed parental care was observed in either species, for example, feeding the juveniles, except for aeration by beating their pleopods and sometimes by flexing the uropods. The juveniles did not return to the marsupium after they were released.

Fecundity and viability

Eggs within a single brood developed almost synchronously, and all eggs hatched on the same day in both species (observations performed in the laboratory). The number of eggs (stage I + stage II + stage III) produced by ovigerous females of *H. pleoacuta* in the laboratory ranged from 10 to 59 ($n=147$), and the number of juveniles from 15 to 40 ($n=35$). The egg production of *H. castroi* ranged from 7 to 42 ($n=57$), and the number of juveniles from 16 to 36 ($n=29$). There was no significant difference between the mean number of eggs produced by *H. pleoacuta* (26.6 ± 0.75) and *H. castroi* (26.1 ± 1.10) ($t=0.477$; $P > 0.05$). There was no significant difference between the species in the mean number of juveniles that remained in the marsupium (*H. pleoacuta*: 25.1 ± 1.05 ; *H. castroi*: 24.9 ± 0.94 ; $t=-0.464$; $P < 0.05$). There was no significant difference between the mean number of eggs in each embryonic developmental stage and the mean number of juveniles remaining in the marsupium during the embryonic period in either species ($P > 0.05$) (Table V). Furthermore, females were not observed moulting during the embryonic and postembryonic period in either species of *Hyalella*.

Ovigerous females of *H. pleoacuta* had a smaller mean head length $\pm$ SE (0.75 ± 0.001 mm) than did females of *H. castroi* (0.86 ± 0.001 mm) ($t=-9.594$; $P < 0.05$). The females of *H. pleoacuta* carrying juveniles in the marsupium had a smaller mean size (0.75 ± 0.001 mm) than those of *H. castroi* (0.85 ± 0.001 mm) ($t=5.947$; $P < 0.05$).

Table V. Comparison of the mean number of eggs in each embryonic developmental stage and number of juveniles remaining in the marsupium of *Hyaella pleoacuta* and *H. castroi*.

Development stage	<i>Hyaella pleoacuta</i>			<i>Hyaella castroi</i>		
	Minimum–maximum	Mean $\pm$ SE	No. of ovigerous females	Minimum–maximum	Mean $\pm$ SE	No. of ovigerous females
Stage I	12–46	26.0 $\pm$ 1.12	54	16–45	26.3 $\pm$ 0.93	48
Stage II	16–36	25.5 $\pm$ 0.89	35	17–40	26.0 $\pm$ 1.03	34
Stage III	16–38	25.3 $\pm$ 1.03	32	15–45	25.4 $\pm$ 1.60	28
Stage IV	15–40	25.1 $\pm$ 1.05	35	16–36	24.9 $\pm$ 0.94	29

There was a positive linear correlation between the number of eggs in all embryonic developmental stages and size (head length) of females of *H. pleoacuta* and *H. castroi* ($P < 0.05$). There was also a correlation between the number of juveniles remaining in the marsupium and the head length of females in both *H. pleoacuta* (Figure 2) and *H. castroi* (Figure 3) ($P < 0.05$). Table VI shows the results of the analysis of covariance of the number of eggs or juveniles produced by *H. pleoacuta* and *H. castroi* at the different embryonic developmental stages. There was no significant difference between the number of eggs and juveniles in either *Hyaella* species ($P > 0.05$).

The mean number of juveniles that hatched in the laboratory ($\pm$ SE) was higher in *H. pleoacuta* (24.9 ± 1.13 juveniles; $n=64$) than in *H. castroi* (23.8 ± 1.46 juveniles; $n=40$) ($t=2.588$; $P < 0.05$). The mean number of hatching juveniles did not differ from the mean number of juveniles remaining in the marsupium during postembryonic development in either species of *Hyaella* ($P > 0.05$).

The mean head length ($\pm$ SE) of juveniles carried by females in the marsupium and juveniles liberated by females was 0.19 ± 0.001 mm in *H. pleoacuta* and 0.22 ± 0.002 mm in *H. castroi*. These results showed that juveniles of both species did not moult during the time that they remained inside the marsupium after hatching. The juveniles that remained in the marsupium and those released by females of *H. castroi* showed a greater mean head length than juveniles of *H. pleoacuta* ($t=9.644$; $P < 0.05$).

Figure 2. Regression analyses between body size of ovigerous females (head length—HL) and number of eggs in embryonic developmental stages and number of juveniles of *H. pleoacuta*. F, fecundity.

Figure 3. Regression analyses between body size of ovigerous females (head length—HL) and number of eggs in embryonic developmental stages and number of juveniles in *H. castroi*. F, fecundity.

Discussion

Precopulatory mate-guarding time

The reproductive behaviour of *H. pleoacuta* and *H. castroi*, like that of *H. azteca* Saussure, 1858 (Strong 1973; Wen 1992; Nelson and Brunson 1995), involves a precopulatory mate-guarding phase, during which the male holds and carries the female. The large second gnathopods of the male are used for manoeuvring the female into a carrying and copulatory position, while the small first gnathopods are used to carry the female during the precopulatory mate-guarding phase. A precopulatory pair remains attached, with the male dorsal to the female, until the female moults; the male continues to guard as the eggs pass

Table VI. Results of the analysis of covariance of size-specific fecundity among embryonic developmental stages of *Hyalella pleoacuta* and *H. castroi*.

	Slope (parallelism)		Intercept	
	F	P	F	P
<i>Hyalella pleoacuta</i>				
Stage I × Stage II*	0.84	0.36	5.43	0.02
Stage I × Stage III	2.16	0.14	3.83	0.05
Stage I × Stage IV	3.32	0.07	3.53	0.06
Stage II × Stage III	0.44	0.50	0.17	0.67
Stage II × Stage IV	0.85	0.35	0.19	0.66
Stage III × Stage IV	0.10	0.74	0.004	0.94
<i>Hyalella castroi</i>				
Stage I × Stage II	0.06	0.80	0.03	0.84
Stage I × Stage III	3.33	0.07	0.36	0.54
Stage I × Stage IV*	0.003	0.95	7.11	0.009
Stage II × Stage III	2.87	0.09	0.09	0.76
Stage II × Stage IV*	0.03	0.85	6.08	0.01
Stage III × Stage IV*	3.49	0.06	10.11	0.002

*Significant difference ($P < 0.05$).

into the marsupium, and the pair separates after fertilization occurs. Precopulatory mate-guarding ensures that the male is present during the short period when the female is receptive, and also secures the female against other males while her cuticle is soft after moulting (Strong 1973; Borowsky 1984; Dick et al. 1990). The duration of the precopulatory mate-guarding phase in the species of *Hyaella* analysed in the present study was similar to that observed by Othman and Pascoe (2001) for *H. azteca* and Strong (1973) for *H. azteca* from Hunter Hot Springs. However, Geisler (1944) reported that pairs of *H. azteca* remained attached for 7 days, and Strong (1973) reported that pairs of *H. azteca* from Siltcoos Lake and Suttle Lake remained together for 3.29 and 1.05 days, respectively (at 22°C). The duration of the precopulatory mate-guarding phase can vary with temperature and salinity (Hartnoll and Smith 1978), predation intensity (Strong 1973), the number of competitors, and the number of available females (Ward 1983). The duration of mate guarding also depends on the length of the period in which females can be mated after moulting, and the ability of the females to delay ovulation (Conlan 1991). In *Gammarus pulex* Linnaeus, 1758, females cannot be mated after 12 h following moulting (Birkhead and Clarkson 1980). On the other hand, in *Orchestia gammarellus* (Pallas, 1766), mating is still possible up to 72 h after the female moults (Campbell-Parmentier 1963).

Paired males of both *Hyaella* species were larger than paired females. This sexual dimorphism is considered essential for male performance during precopulatory mate-guarding behaviour (Sexton 1924, 1928; Vlasbom 1969), because the larger the male, the more easily it can carry the female (Adams and Greenwood 1983; Adams et al. 1985). Furthermore, large males have two advantages over small males: (1) they are better able to successfully “take over” females in competition with another male, and (2) they are better able to resist such take-over attempts themselves while paired (Ward 1983).

Both *Hyaella* species investigated in the present study showed size-assortative pairing, with larger males carrying larger females during the precopulatory mate-guarding phase. Probably, this size-assortative pairing is explained by loading constraints (Adams and Greenwood 1983; Greenwood and Adams 1984) and the male–male competition hypothesis (Ward 1983). According to the male–male competition hypothesis, larger males have two advantages over smaller males: they are better able to affect a takeover, and they are able to resist takeover attempts while paired (Ward 1983). Under the loading constraints hypothesis, males are constrained to select females much smaller than themselves because only those females can be easily carried during precopulatory mate guarding (Adams and Greenwood 1983). In laboratory experiments, Wen (1992) observed that precopulatory behaviour in *H. azteca* supports the male–male competition hypothesis of Ward (1983) and Elwood et al. (1987). Size-assortative pairing was also observed in *H. pleoacuta* and *H. castroi* in field conditions (Castiglioni and Bond-Buckup forthcoming a), and in *Gammarus troglophilus* Hubricht and Mackin, 1940 (Jenio 1980), *G. duebeni* Lilljeborg, 1851 (Ward 1985; Hatcher and Dunn 1997), *G. pulex* (Linnaeus, 1758) (Birkhead and Clarkson 1980; Elwood and Dick 1990; Ward and Porter 1993; Hume et al. 2002; Bollache and Cézelly 2004), *G. leopoliensis* Jazdzewski and Konopacka, 1989 (Zielinski 1998), and *Paracalliope fluviatilis* (Thomson, 1879) (Lefebvre et al. 2005).

Duration of embryonic and postembryonic development

Embryonic development, from ovulation to hatching, of *H. pleoacuta* and *H. castroi* requires about 12 days at 19°C. This is similar to periods reported for *H. azteca* by Strong (1972), and Edwards and Cowell (1992), in which the embryonic period lasted 10.9–13.4 days at

20°C and 11.1 days at 25°C, respectively. However, Geisler (1944) and Cooper (1965) observed that embryonic development in *H. azteca* was 21 days at 25°C and 9.3 days at 25°C, respectively. The duration of embryonic and postembryonic development in amphipods is strongly influenced by the temperature and photoperiod to which the females are submitted during this period (Strong 1972; De March 1978; Kruschwitz 1978; Borowsky 1980a; Shillaker and Moore 1987; Takeuchi and Hirano 1992; Maranhão and Marques 2003). Another factor that may cause differences in estimates of the duration of the embryonic period is the possibility of the existence of cryptic species. For instance, although *H. azteca* has been traditionally considered a single, widely distributed species, recent molecular genetic studies have demonstrated that it is a species complex (Thomas et al. 1997; Hogg et al. 1998; McPeck and Wellborn 1998; Witt and Hebert 2000).

The juveniles of both species of *Hyaletta* investigated in the present study remained in the marsupium after the embryonic period of 1.0–6.0 days. Geisler (1944) reported a similar period of 3 days in the marsupium for *H. azteca*. While the juveniles were in the marsupium, no parental care was observed in either species of *Hyaletta*. Juveniles of both species did not moult during their development inside the marsupium. This was confirmed by the similar head length observed in juveniles inside the marsupium and those liberated by the females. Juvenile permanence inside the marsupium occurs in other amphipod species, such as *Parallorchestes ochotensis* Brandt, 1851, in which the females display no parental care (Kobayashi et al. 2002), *Gammarus palustris* Bousfield, 1969 (Borowsky 1980b), and *G. pulex* (Welton et al. 1983). Extended parental care in amphipods can improve the survival and growth rates of offspring, which has been confirmed by experimental studies (Aoki 1997; Thiel 1999; Kobayashi et al. 2002).

Fecundity and viability

Females of *H. pleoacuta* and *H. castroi* could produce consecutive broods (iteroparity) under laboratory conditions, which were confirmed by the presence of developed (dark green) ovaries in females that were carrying eggs or juveniles in the marsupium. Taking into account the results for the duration of embryonic development, and the extrapolated values for the time of female maturation and the estimated female life-span (Castiglioni and Bond-Buckup forthcoming b), one should expect more than two generations per year in both species (multivoltine life cycle). Multivoltinism has been reported in other species of gammarideans, such as *Pseudorchestoidea brasiliensis* (Dana, 1853) (Cardoso and Veloso 2001), *Gammarus locusta* (Linnaeus, 1758) (Costa and Costa 1999), *Echinogammarus longisetosus* Pinkster, 1973 (Guerao 2003), and *G. chevreuxi* Sexton, 1913 (Subida et al. 2005).

Ovigerous females of *H. pleoacuta* produced more eggs than *H. castroi* in field conditions, even though the females of the former species are larger. The lower egg production observed in *H. castroi* may be compensated by the production of larger eggs and also by low brood mortality (Castiglioni and Bond-Buckup forthcoming c). However, these species produced fewer eggs under laboratory conditions, compared to females in the natural environment (Castiglioni and Bond-Buckup forthcoming c). Temperature and photoperiod may influence egg production, and therefore the females may produce fewer eggs under laboratory conditions (Kinne 1961; Steele and Steele 1969; Steele 1981). Laboratory experiments suggest that the quantity and quality of food is also important in determining brood size, as has been suggested in studies on other amphipods (Fish and Preece 1970; Van Dolah et al. 1975; Sheader 1983; France 1992; Muskó 1993).

No decrease in egg number during development was observed in either *H. pleoacuta* or *H. castroi*, as occurs under natural conditions in both species (Castiglioni and Bond-Buckup forthcoming c), as well as in other species of amphipods (Beare and Moore 1996; Dick et al. 1998, 2002; Cunha et al. 2000; Kevrekidis 2004, 2005). This may be related to the available space in the marsupium for incubating eggs, which is sufficient to maintain the number of eggs from the initial development stage to the moment of hatching. The absence of predators in the laboratory experiments may also account for the lack of any decrease in egg number.

There was a significant correlation between the number of eggs or juveniles and the size of ovigerous females in both species, as also observed in wild populations (Castiglioni and Bond-Buckup forthcoming c). Brood size in gammarideans is often reported as being proportional to body size of females (e.g. Beare and Moore 1996; Costa and Costa 1999; Persson 1999; Cunha et al. 2000; Othman and Pascoe 2001; Alcocer et al. 2002; Maranhão and Marques 2003; Kevrekidis 2004, 2005).

Conclusions

Both species of *Hyaella* showed similar reproductive strategies under laboratory conditions, especially in respect to the duration of the pre-copula and the embryonic period and fecundity. However, these species differed in the body size of ovigerous females, duration of the postembryonic period, viability, and body size of juveniles which remained in the marsupium and those liberated by females. These differences in the reproductive traits of *H. pleoacuta* and *H. castroi* might also facilitate their coexistence in nature. This is the first account of the reproductive strategy of endemic species of *Hyaella* in Brazil. Further studies on reproductive behaviour, feeding, and sensitivity to contaminants will increase our understanding of the reproductive strategies of these freshwater amphipods and their possible future use as bioindicators and biomonitors.

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