

Reproductive aspects of *Hyalessa carstica* (Amphipoda: Hyalessidae) in a natural environment in southeastern Brazil

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ABSTRACT: The reproductive aspects of populations are important determinants of their ability to maintain themselves throughout time. In the case of amphipods from the genus *Hyalessa* Smith, 1874, the reproductive period is easily determined by the presence of ovigerous females and pre-copula pairs in the population. This study evaluated the fecundity, pairing patterns and reproduction period of *Hyalessa carstica* Bastos-Pereira and Bueno, 2012 in a karst environment in the West of Minas Gerais, Brazil. Monthly, from April, 2010 to April, 2011, amphipods were sampled using a hand net with 15 cm diameter and 250 µm mesh size. In the laboratory, animals were sexed and measured, and the ovigerous state of females was evaluated. Couples in pre-copula and ovigerous females were found throughout the year, with the exception of the dry months (September–November). During drought, the stream's superficial water dried out, and only a few refuges for the population remained. Although couples were found in all months with a water flow, a higher number of unpaired males and females was found after the dry season. In all months, ovigerous females were more abundant than non-ovigerous, and the relationship between head length and the number of eggs in the marsupium was positive. Most eggs were in the first developmental stage, although all other stages were found before the dry season. Considering the frequency of pre-copula pairs, as well as ovigerous females, the reproduction in this *H. carstica* population seems to be continuous throughout the year, without a distinct peak.

Key words: Pre-copula, pairing, ovigerous females, life history.

INTRODUCTION

Two reproductive strategies are relevant for gammarid amphipods: the male's active search for a mate and the pre-copula behavior. During pre-copula, days before the females' molt, the males grab the females using their gnathopods, and the couple remains swimming together until the copula itself occurs (Strong, 1973). This particular behavior happens due to the fact that females only copulate when they are "soft", i.e., soon after they molted and the new exoskeleton is not sclerotized yet (Strong, 1973; Conlan, 1991).

Environmental conditions, such as food availability, are extremely important for the

reproductive success of a population. In adverse conditions, an increase in the egg and juvenile sizes trades-off with a decrease in the number of eggs in some crustaceans (Clutton-Brock, 1991). The female size is also an important factor in fecundity, since larger females produce more eggs (Steele and Steele, 1991).

Hyalessa Smith, 1874 is a genus of freshwater amphipods found only in the American continent. *Hyalessa carstica* Bastos-Pereira and Bueno, 2012 is a newly described species, and it is present in the Minas Gerais state of Brazil, a region with peculiar characteristics due to an intense environmental degradation caused by mining (Bastos-Pereira and

Bueno, 2012). Here we studied the reproductive ecology of *H. carstica* in a natural environment, addressing its fecundity, pairing patterns and reproductive period throughout the year.

MATERIAL AND METHODS

This study was conducted in southeastern Brazil, in the West of the Minas Gerais state, in an area located between Arcos and Pains municipalities (20°20'01.5"S 45°36'40.7"W). Along with Doresópolis, the geology and speleology of these two municipalities were well studied due to interest in their unique rock formations (Menegasse *et al.*, 2002). The subtropical region where the municipalities are located has wet summers, dry winters, and an annual mean precipitation of 1,344 mm (Menegasse *et al.*, 2002). Since the dry season often leads to the complete disappearance of some streams in the region, the landscape is subject to changes in the hydric dynamics of the water bodies.

The stream where the samplings were taken belongs to the São Francisco river watershed. This area is considered an important karst aquifer due to its potential of hydric reloading (Menegasse *et al.*, 2002). During the dry season the stream water flow is interrupted at a little dam set in its course (personal observation). The drought period lasts from May to September, and the regularization of the flow usually occurs in October (Menegasse *et al.*, 2002).

Monthly, from April, 2010 to April, 2011, *Hyalella carstica* were sampled for 20 minutes using a hand net with 15 cm of diameter and 250 µm mesh size, in a backwater area of the stream where the flow was slower. Oviparous females and couples in pre-copula were individualized in microtubes, so in case they were separated, the respective pairs would not get lost.

After sampling the crustaceans, and in the same place, environmental parameters (conductivity, dissolved oxygen (DO), temperature and pH) were measured using a digital portable meter placed below the water surface.

The ratio of paired and unpaired males and females was evaluated, and the developmental stage of the eggs was recorded. To categorize the eggs, the five stages described by Dick *et al.* (1998) for

Amphipoda were used: Stage 1) dark, homogeneous eggs; Stage 2) recently formed embryos are visible, but without eyes; Stage 3) embryos are more developed, with visible eyes; Stage 4) embryos fill the egg and have an orange color; Stage 5) fully developed and hatched juveniles that have not abandoned the marsupium yet.

Data analysis

The individual's head length (HL) was measured with a stereomicroscope containing a millimetric lens (0.01 mm). We used the Pearson correlation to determine if there was an individual size preference among pairs, comparing the HL of each individual with their respective pair. The relationship between the females HL and the number of eggs was determined using a simple linear regression. Statistical analyses were performed in Bioestat 5.0 and SigmaStat 3.1, using a 5% level of significance.

RESULTS

Pairing

In this *Hyalella carstica* population, couples in pre-copula were found throughout all the year, except in April, 2010. In the months before the dry season (September–November) the number of couples was high and gradually decreased until the stream had disappeared completely. After the superficial course of the stream returned, the abundance of couples increased again every month. Considering the total number of adults in each month, the highest proportion of adults in pre-copula, compared to the unpaired adults, was observed in August, 2010, and February and April, 2011.

The relationship between the HL of paired males and females was not significant ($r = 0.26$; $p = 0.10$), what suggests that pairing in *H. carstica* is not related to the size of individuals.

The paired males/unpaired males (PM/M) ratio was low in all months, and always lower than 1, which indicates that in any month the number of males in pre-copula was lower than the number of unpaired males (Fig. 1A). However, the months with highest proportion of paired males were August, 2010, just before drought, and February 2011, after the return of the rain for the region. Regarding females, the paired females/unpaired

females (PF/F) ratio was also low. Only in February, 2011 there was a higher number of paired females when compared to unpaired ones (Fig. 1B).

Fecundity

Ovigerous females were found in all months, except in March, 2011 and during the dry season (September–November). A total of 207 ovigerous females were found, with eggs in different developmental stages and with juveniles. Ovigerous females were found in higher proportion when compared to non-ovigerous females after the dry season, with the exception of March (Tab. 1). The only month where the ratio was lower than 1 was May, 2010. This means that, during most of the study, most *H. carstica* females in this population

were ovigerous.

Regarding the fecundity, a mean of 12.6 ± 7.2 eggs per female was found. The months right after the dry season had a higher mean of eggs per female (Tab.1), with a significant difference among the sampling months ($p < 0.001$). A high number of females carrying eggs in the first stage of development was observed (Fig. 2). Eggs in different stages were found throughout the year.

The HL of females was directly related to the number of eggs they were carrying ($R^2 = 0.28$; $p < 0.001$), indicating that females with larger HL will carry more eggs in the marsupium (Fig. 3).

Environmental data

The mean water temperature over the months was

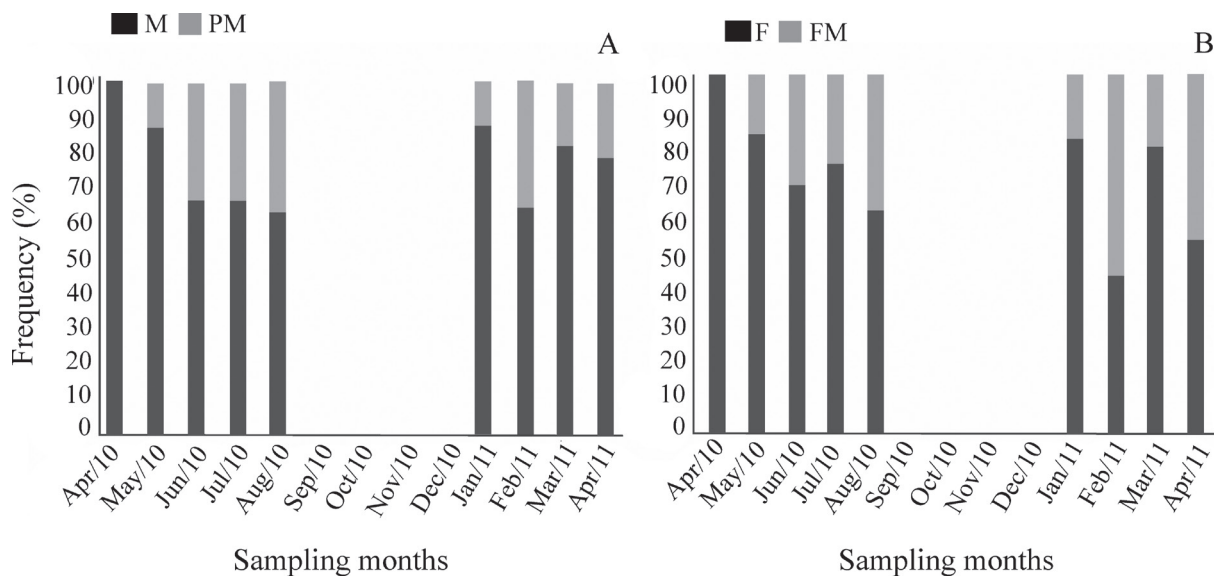


Figure 1. Proportion between paired and unpaired males (A) and females (B) in a population of *Hyalella carstica* in a stream in Arcos, Minas Gerais, Brazil, from April/2010 to April/2011. PM = paired males; M = unpaired males; PF = paired females; F = unpaired females.

Table 1. Ratio between ovigerous females in relation to the total of females, mean number of eggs per female and couples in pre-copula found in a population of *Hyalella carstica* in a stream in Arcos, Minas Gerais, Brazil, from April/2010 to April/2011. OF = ovigerous females; F = females; SD = standard deviation; CP = couples in pre-copula.

Collection months	Ratio OF/F	Eggs/♀ (mean±SD)	Number of CP
Apr/10	1.38	11.2 ± 3.7	-
May/10	0.47	8.7 ± 3.1	18
Jun/10	1.1	8.8 ± 4.9	-
Jul/10	1.2	6.4 ± 2.7	-
Aug/10	1.0	4.3 ± 2.1	-
Sep/10	-	-	-
Oct/10	-	-	-
Nov/10	-	-	-
Dec /10	-	-	-
Jan/11	2.0	14.1 ± 5.8	2
Feb/11	3.5	20.1 ± 7.8	5
Mar/11	-	-	6
Apr/11	1.4	15.4 ± 5.6	11

22.68°C ± 3.17, and it was higher after the drought. The mean dissolved oxygen was 6.63 mg.L⁻¹ ± 1.80, and the mean conductivity was 48.67 μS.cm⁻² ± 11.75. The pH varied slightly around neutrality, averaging 7.43 ± 0.29 (Tab. 2). During the dry season, it was not possible to measure these variables at the study site, since the sampling site had no water.

DISCUSSION

The proportion of couples in the population of *Hyaletta carstica* was slightly low, when compared to unpaired adults, even though couples were observed in all months. The same was observed for other species of *Hyaletta*: *Hyaletta castroi* Gonzalez, Bond-Buckup and Araujo, 2006 had 12.8% of males and 10.7% of females in pre-copula, and *Hyaletta pleoacuta* Gonzalez, Bond-Buckup and Araujo, 2006 had 16.5% of males and 14.4% of females

(Castiglioni and Bond-Buckup, 2008b).

The low occurrence of couples in this *H. carstica* population can be related to the fact that the individuals stay in pre-copula for a short time, approximately 3 days (personal observation), since this behavior can make individuals more vulnerable to predation (Strong, 1973; Wellborn, 1995). Furthermore, when in couples, individuals that inhabit locations where predators usually prey on larger organisms have a higher mortality rate. Besides, the paired individuals swim slower than unpaired ones (Cothran, 2004). Moreover, the sampling procedures may have led to the separation of individuals.

An important factor that must be considered is the drought period to which the *H. carstica* population was exposed. During the months when the water ceased to flow, and the organisms survived in ponds used as refuges, the conditions

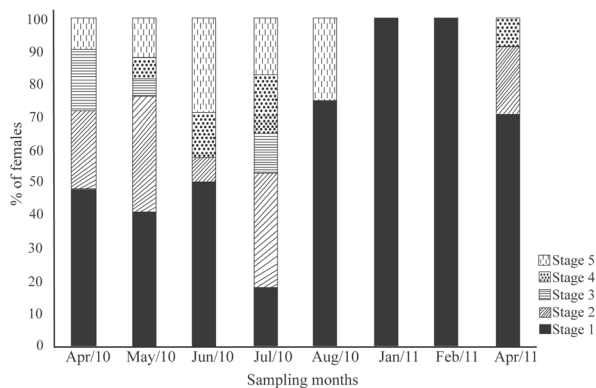


Figure 2. Percentage of females carrying eggs of each stage in a population of *Hyaletta carstica* in a stream in Arcos, Minas Gerais, Brazil, from April/2010 to April/2011.

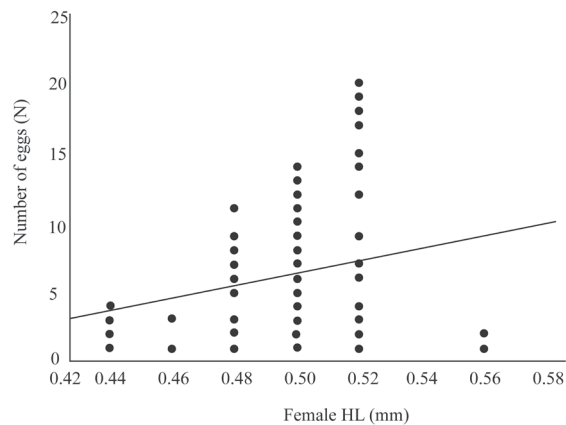


Figure 3. Linear regression between the females head length (HL) and the number of eggs contained in their marsupium for a population of *Hyaletta carstica* in a stream in Arcos, Minas Gerais, Brazil, from April/2010 to April/2011.

Table 2. Physico-chemical parameters of the stream in which *Hyaletta carstica* in a stream in Arcos, Minas Gerais, Brazil, from April/2010 to April/2011. Temp. = Temperature (°C); DO = dissolved oxygen (mg.L⁻¹); Cond. = Electrical conductivity (μS.cm⁻²); * dry period, unavailable data.

Collection months	Temp.	pH	DO	Cond.
Apr/10	24.1	7.3	6.6	56
May/10	19.8	7.5	5.9	63
Jun/10	19.1	7.5	5.5	64
Jul/10	15.8	7.4	4.7	75
Aug/10	20.1	7.8	8.2	56
Sep/10	*	*	*	*
Oct/10	*	*	*	*
Nov/10	*	*	*	*
Dec/10	23.6	6.69	6.01	48
Jan/11	25.9	7.4	5.37	45.1
Feb/11	25	7.4	8	43.7
Mar/11	24	7.4	10.8	41.1
Apr/11	21.6	7.68	6.38	38.7

for reproduction might not be optimal (Lake, 2003). Ovigerous females were observed upstream in the river, in permanently wet places. Once the population was reestablished, the amphipods resumed reproducing regularly at the sampling site. Temperature is the most important factor controlling the reproductive stage (March, 1977), and in this study, after dry period temperature (Tab. 2) was ideal for the reproduction of *H. carstica*.

Another factor that must be taken into consideration is the relative body size difference between genders. Some studies with different species of amphipods showed that larger males prefer to mate with larger females (Dick and Elwood, 1993; Wellborn, 1995; Castiglioni and Bond-Buckup, 2008b; Franceschi *et al.*, 2010; Hume *et al.*, 2005), but in other cases this did not occur (Wellborn, 1995; Wellborn and Bartholf, 2005). The lack of relationship can be due to the fact that there was no significant variation between the size of paired males, or to the fact that females of all sizes were found in pairs (Wellborn, 1995). The females of the *H. azteca* species complex seem to control when the pre-copula begins, as well as how long this behavior lasts (Wellborn and Bartholf, 2005), since females are only receptive to males a few days before molting (Cothran, 2004).

The reproduction of *H. carstica* population was continuous throughout most of the year. Many authors, studying different species of amphipods, also observed ovigerous females along the entire sampling period (Strong, 1972; Casset *et al.*, 2001; Guerao, 2003; Kevrekidis, 2005; Castiglioni and Bond-Buckup, 2008a). The proportion of females with eggs in marsupium can vary in a significant way, even though they were found in almost all months, presenting abundance peaks when the conditions were more favorable (Cooper, 1965). Ovigerous females of *H. carstica* were observed during the dry months in the upstream part of the river that does not dry, indicating that reproduction continues even during the drought. The same was observed in two other studies on different populations of *H. azteca* (Edwards and Cowell, 1992; Pickard and Benke, 1996).

The prevalence of ovigerous females, in relation to those without eggs, was observed in other

studies on freshwater amphipods (Kevrekidis, 2005; Kestrup and Ricciardi, 2010). In addition, this does not happen only in the freshwater, as shown by Maranhão *et al.* (2001) in a population of *Echinogammarus marinus* (Leach, 1815). For this marine amphipod, the percentage of ovigerous females was higher than that of non-ovigerous during the whole year.

Females of freshwater amphipods usually have larger eggs than females of marine amphipods (Steele and Steele, 1991). However, with respect to the number of eggs, *Hyalella* has a low number when compared to other gammarids, and their juveniles are larger when they hatch (Strong, 1972; Wellborn *et al.*, 2005). In the present study, the dry season was an important factor for the egg production, since in the months before drought the mean number of eggs was lower. However, after that period, females produced a higher number of eggs, maybe to recover the population size after the re-colonization of their habitat.

In this study, the developmental stage of the eggs of each female was also recorded and, as observed in *H. azteca* by Strong (1973), the reproduction was not synchronized. This means that most stages are found throughout the year, even though all eggs within a single female are at the same developmental stage. This happens not only in *Hyalella*, but also in other amphipods, including marine species (Maranhão *et al.*, 2001). The high number of *H. carstica* females with eggs in the first stage may be related to the fact that some eggs may be discarded, since some of them can be unfeasible and/or not fertilized yet (Cothran *et al.*, 2010). Besides, this stage lasts the longest. Females with eggs in the first stage were also the majority in a population of the amphipod *Crangonyx pseudogracilis* Bousfield, 1958: 65% of females had eggs in the first three stages. Usually, the number of eggs per female decreases from the first to the last stage (Dick *et al.*, 1998), due to embryo mortality.

As in many other crustaceans and in *H. carstica*, female body size is a good indicator of fecundity. Many authors already observed a positive correlation between HL and the number of eggs in the marsupium, thus larger females carry more eggs (Cooper, 1965; Strong, 1972; Steele and

Steele, 1991; Wellborn, 1995; Maranhão *et al.*, 2001; Guerao, 2003; Wellborn and Bartholf, 2005; Castiglioni and Bond-Buckup, 2007).

The reproduction of *H. carstica* was continuous along the year: couples in pre-copula and ovigerous females were found every month. Many authors stated that this characteristic is more common in tropical populations, where the food availability and the environmental factors are steadier and favor a longer reproduction period. Another aspect that corroborates the continuous recruitment hypothesis is the fact that, in a given moment, all egg stages were found in the population, but there was no synchronic hatching of juveniles. In this way, there are always new individuals entering the population. The positive relationship observed between the ovigerous females' HL and the number of eggs in the marsupium indicates that larger females contribute with a greater progeny to the population. Understanding the reproductive aspects of a population is important, since they allow us to infer about the capacity of such population to maintain itself through time in a given environment, thus helping the discussion of conservational policies.

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