

## Variations on the larval incubation of *Anodontites trapesialis* (Unionoida, Mycetopodidae): Synergetic effect of the environmental factors and host availability

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### Abstract

The unionid *Anodontites trapesialis* (Lamarck, 1819) like most freshwater mussels is a parasite of fish. So it is trivial to assume that the availability of hosts is an important factor for the maintenance of unionoid populations. What seems obvious is not always so easy to demonstrate. This study proposes to investigate the effects of abiotic and biotic variables related to the incubation of larvae in *A. trapesialis*. For this, we estimate different biological indexes and try to capture the dimensionality of the fish, along with the temporal variation of environmental variables. From the application of a CCA, it was demonstrated that there was a synchronicity among the factors and variables proposed here, and we infer that the flood pulse acts as a synergistic factor in this process.

**Keywords:** reproduction, freshwater mussels, fishes, host relationship, wetlands.

### Variações na incubação larval de *Anodontites trapesialis* (Lamarck, 1819) (Bivalvia Unionoida, Mycetopodidae): efeito sinérgico de fatores ambientais e disponibilidade de hospedeiros

### Resumo

O bivalve *Anodontites trapesialis* (Lamarck, 1819), como a maioria dos mexilhões de água doce, é parasita de peixes. Então, seria trivial supor que a disponibilidade de hospedeiros é um fator importante para a manutenção das populações dos unionoideos. Porém, o que parece óbvio nem sempre é tão simples de ser demonstrado. Este estudo se propõe a investigar os efeitos de variáveis abióticas e bióticas relacionadas com a incubação de larvas de *A. trapesialis*. Para tanto, nós estimamos diferentes Índices Biológicos e tentamos capturar a dimensionalidade da comunidade de peixes juntamente com a variação temporal das variáveis ambientais. A partir da aplicação de uma CCA, demonstramos que há uma sincronicidade entre os fatores e as variáveis aqui propostas, e inferimos que o pulso de inundação atua como fator sinérgico desse processo.

**Palavras-chave:** reprodução, bivalves de água doce, peixes, relação parasito-hospedeiro, áreas úmidas.

## 1. Introduction

Unionoida mussels have a complex life cycle. After fertilization, the early stages of development occur in the inner demibranch (Bonetto and Ezcurra, 1962; 1965). The planktonic larvae, called glochidium or lasidium, complete their development cycle as temporary parasites of fish (Fryer, 1970, Mansur et al., 1987). Around 27 days after fixation, they complete metamorphosis and unfasten themselves from the fish and become a benthic organism (Bonetto and Ezcurra, 1962; Castellanos and Landoni, 1990).

Some limiting factors such as temperature (Bauer, 2000; Strayer, 2008; Oliveira et al. 2010), food availability and parasite-host interaction, influences directly in the reproductive dynamics of these mussels (Haag and Warren-Junior, 1997; Avelar and Mendonça, 1998; Beasley et al., 2000). To know which environmental factors are most important to each one of the reproduction attributes will depend on the combined effect provided by the environment in which these bivalves occur (Galbraith and Vaughn, 2009). Few studies have examined the environmental influences in the reproductive dynamics of the population and information is unknown related to the incubation period, elimination of larvae and parasite-host interaction for neotropical species.

*Anodontites trapesialis* (Lamarck, 1819) (Unionoida, Mycetopodidae) is widely geographically distributed and may represent the highest percentage of benthic biomass in rivers, lakes and flood plains (Colle and Callil, 2012). Although on the national list of endangered species, (Brasil, 2004), this species can be found in abundance in farm ponds and fish farming (Guardia-Felipi and Silva-Souza, 2008) and has been considered a species for cultivation and potential source of protein (Tello-Panduro et al., 2003). However being sedentary and excellent filter feeders it has been used as a sentinel organism (Callil, 2003) for studies of environmental monitoring and an effective biomarker of pesticides (Lopes et al., 1992) and heavy metals like mercury (Callil and Junk 1999, 2000), lead and cadmium (Tomazelli et al., 2003).

In order to understand the processes related to the larval development and completion of hatching process, we intend is to answer: i) If biometrics Indexes vary between incubated and non-incubated individuals? ii) Is there a relation between frequency of the individuals incubated with richness and abundance of fish? iii) Is the Incubation is influenced by limnological variables or the presence of hosts? These questions were elaborated on the assumption that environmental factors are subject to changes resulting from the periodic flood pulse, inherent in the Pantanal, and these influencing reproduction and host availability. The assessment and thread of this premise is the object of this study.

## 2. Material and Methods

### 2.1. Sampling design

Samples of mussels were collected in an artificial ponds, which acts as shallow lake (16° 32' 29" S and

56° 26' 16" W) under Indirect Influences of the floodplain of the Cuiabá River near to RPPN SESC Pantanal. Every month during November 2006 to October 2007, the mussels were collected touching the substrate by hand and feet. Over every sampling event depth and transparency were measured with a Secchi disk and temperature (°C), pH, dissolved oxygen (OD mg.L<sup>-1</sup>) using a multi probe.

For all collected specimens (n = 180; 15 per month), height (H, mm), total length (Lt, mm), demibranch length (Ltd, mm) were obtained with a digital caliper. The measures related to the mass as total weight (Wt, g), weight of soft parts (Wb, g), shell weight (Ws, g) (Callil and Mansur, 2007) and weight of demibranch (Wd, g) were obtained with an analytical balance. All individuals had their demibranches examined under the microscope to detect the presence of eggs and larvae. These material can be found at the Invertebrate Aquatic Collection of UFMT – CIAMT under the following numbers: CIAMT 593, CIAMT, CIAMT 595, CIAMT 596, CIAMT 597, CIAMT 598, CIAMT 599, CIAMT 600, CIAMT 601, CIAMT 602, CIAMT 603 and CIAMT 604.

Data on the composition of local fish fauna were collected together with the capture of the bivalves. For this, we used two cast nets (of 3 m and 2 cm between nodes) and a trawl net (20 × 4 m with 1 cm between nodes). The cast nets were launched 100 times in each sampling and the trawl net twice. All specimens were identified using the literature (Britski et al., 2007; Buckup et al., 2007). After data collection, in order to verify the presence of larvae (*lasidia*) of *A. trapesialis*, we examined by the naked eye and stereoscopic microscope, the body surface, fins and gills of all fishes collected. The richness, relative and total abundance for samples of fish were determined.

### 2.2. Analytical design

With the biometric variables, the possible differences among incubated and non-incubated individuals were investigated by ANOVA (significance level of 5%). From length and weight variables, three biological indexes were estimated: Condition Index (CI), Condition Somatic Index (SCI) and Incubation Index (ICI). For this, the allometric method was used for selection, the results of which are not subject to distortions due to the variation in length of individuals, and which allows comparison of samples formed by individuals of different sizes (Braga, 1986; Lima-Junior et al., 2002). These biological indexes were calculated for each individual from the expression:  $CI = 1000(Wt/Lt^b)$ ,  $SCI = 1000(Wb/Lt^b)$  and  $ICI = 1000(Wd/Ld^b)$ . To calculate the coefficient <sup>b</sup>, total sample was used for adjusting the expression of weight x length ( $W = aL^b$ ), using the least squares method applied to the data converted to their natural logarithms (Lima-Junior et al., 2002).

The differences between the indexes (CI, CSI and ICI) of the incubated and non-incubated specimens were tested by analysis of variance (ANOVA). A simple linear regression was used among the incubated *A. trapesialis* and richness and abundance of fishes captured during the sampled period.

The influence of environmental characteristics on the abundance and richness of fish, biological indices and frequency of incubated and non-incubated was observed using a canonical correspondence analysis (CCA). We first ran a correspondence analysis using SYSTAT 13, at function smart correspondence analyses with canonical standardization, that uses a multiway frequency (environmental variables, the index and the incubated and non-incubated frequency) table format with the first column indicating row labels (months) on the response matrix to obtain an estimate of total inertia or variation explained in the matrix.

### 3. Results

#### 3.1. Limnological characteristics of Baía do Meio Lake

During the sampled period the water though warm, as well as oxygenated (8.50 to 10.10 mg.L<sup>-1</sup>), remained neutral (6.37 to 7.46). The temperature oscillated from 23 to 32.30 °C, and the largest variation was detected for the depth that was 95 to 168 cm and for transparency ranging from 23 to 110 cm.

#### 3.2. Biometry x incubation

Were sampled 180 individuals that varied from 58 to 210 mm in length and 9 to 330 g in weight. The monthly average value of body weight was 52.24 to 125.93 g and the Incubation Index from 1.364 (March) to 3.159 (August). The linear regression was significant for the relationships between the Lt x Ld ( $p < 0.001$ ;  $r^2 = 0.605$ ) and the Ld x Ldi ( $p < 0.001$ ;  $r^2 = 0.671$ ) but the Ld x Wd ( $p < 0.001$ ;  $r^2 = 0.320$ ) and Ldi x Wd ( $p < 0.001$ ;  $r^2 = 0.302$ ), but although it was statistically significant, the correlation coefficient was low.

The larval incubation of *A. trapesialis* always occurred on the central part of inner demibranch, characterised by intumescences of the gill filaments of a whiteish colour. During the sampled period, larvae were observed in the inner demibranch in 71% of the mussels ( $n = 127$  incubated) of which the monthly variation of the relative frequency of incubated ranged from 13 to 93% (Figure 1). The total length classes (Lt) varied from 60 and 185 mm for all specimens and from 115 to 185 mm for the incubated ones. The total weight classes (Wt) varied from 20 to 340 g for all specimens, and from 80 to 340 g for the incubated ones. It has been noted that the smaller specimens found in the classes of Lt of 55 to 110 mm and Wt 10 to 70 g were not incubating larvae. When comparing the non-incubated and incubated individuals, the biometric variables were different for Lt ( $p = 0.046$ ;  $F_{1,178} = 4.055$ ), Wt ( $p = 0.05$ ;  $F_{1,178} = 3.617$ ), Wd ( $p < 0.001$ ;  $F_{1,178} = 49.661$ ), and Ld ( $p = 0.019$ ;  $F_{1,178} = 5.560$ ). The Wb ( $p = 0.13$ ;  $F_{1,178} = 2.243$ ) and h ( $p = 0.27$ ;  $F_{1,178} = 1.209$ ) were not different.

#### 3.3. Biological indexes x incubation

The equations of the length x weight relationship adjusted to obtain the b coefficient for each index are the following:  $Wt = 0.0001 Lt^{2.551}$  for Condition Index (CI),  $Wb = 0.0001 Lt^{2.7848}$  for Somatic Condition Index (SCI)

and  $Wd = 0.002 Ld^{1.6257}$  for Incubation Index (ICI). The ranges of variation were CI: 0.071 to 0.282; SCI: 0.052 to 0.186 and ICI: 0.616 to 4.815.

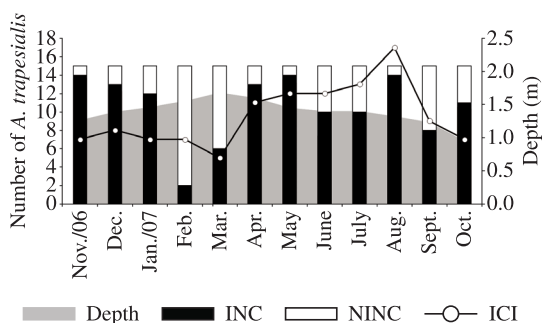
The CI ( $df = 178$ ,  $F = 3.182$ ,  $P = 0.076$ ) and SCI ( $df = 178$ ,  $F = 1.270$ ,  $P = 0.261$ ) did not show differences among incubated and non-incubated, but when considering the Incubation Index, as expected (Figure 2), significant differences (ICI:  $df = 178$ ,  $F = 51.804$ ,  $P < 0.001$ ) were shown.

#### 3.4. Fish fauna

1733 individuals were sampled in the Baía do Meio distributed among 61 species, five orders and 14 families. Among the five orders found, the one that showed greater wealth of species was the Characiformes (32 species), followed by the Siluriformes (19 species), the Perciformes (7 species), the Gymnotiformes (2 species), and one species of the Cyprinodontiformes. The most abundant family was the Characidae (Characinae) with 358 individuals, followed by the Curimatidae with 329. The least abundant families were the Characidae (Cheirodontinae), the Characidae (Iguanodectinae), the Hypopomidae, the Sternopygidae, and the Poeciliidae, with only one individual.

#### 3.5. Environmental factors x fish fauna x incubation

The canonical correspondence analysis explained 93.32% of the total variation data represented by the temporal gradient. The first axis accounted for 84.73% of the variation explained mainly by abundance and transparency, while the second axis represented 8.58% variation. All axes from CCA (sum of all eigenvalues - Total Inertia = 0.219,  $c^2 = 1198.614$ ,  $df = 121$ ,  $P = 0.0001$ ) were significant in this analysis. Figure 3 shows that incubation is often associated with dry season months (July, August and September), and that richness and abundance of fish are associated with October, November and December, months of flooding. On the other hand, the frequency of non-incubated individuals is related to the months of the rainy season (January, February and March) along with depth and transparency.



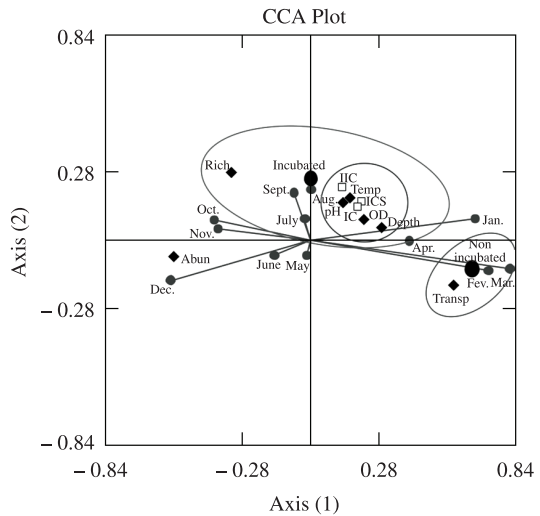
**Figure 1.** Temporal variation of depth (cm) incubated (I) and non-incubated (NI) and Incubation Index (ICI) over the sampled period in the Baía do Meio lake in the Pantanal of Poconé, Mato Grosso state.

**4. Discussion**

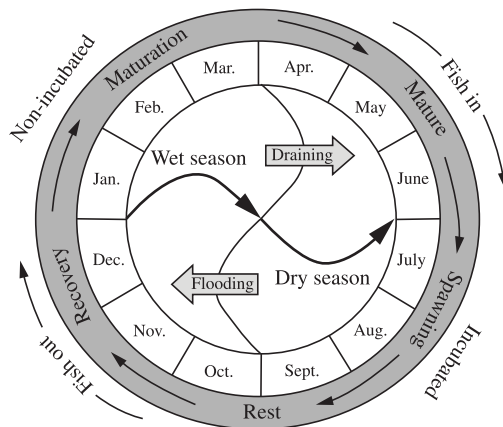
The Baía do Meio Lake is an artificial habitat that has been undergoing the water regime conditions of the Pantanal over the past ten years. Artificial environments contribute to the degradation of the Unionoida, because they restrict their distribution and can retain isolated populations. However, the environment is housed in an area subject to periodic flood pulse, characteristic of the Pantanal. Maintaining the connection with the floodplain adjacent to the rivers makes possible the dispersal of the bivalves' larvae.

During the whole sampled period, incubated individuals were found with more frequency during November, May, and August; agreeing with Callil and Mansur (2007) who observed *A. trapesimalis* incubated in April and May in the Pantanal. Bonetto and Ezcurra (1962) also found *A. trapesimalis forbesianus* (Lea, 1860) in Argentin, incubated between August and December. In the gametogenesis and larval morphology study of *Popenaias popeii* (Lea, 1857) the species shows a short period of incubation and a long period of release of gametes, that makes it considered to be an asynchronous reproducer (Smith et al., 2003). The synchrony of the reproductive cycle maybe a specific characteristic of each population, or it may vary in populations that occur in different geographical areas (Eversole, 1989). The Unionidae *Anodonta* Lamarck, 1799 have multiple reproductive peaks in a single period (Heard, 1975) and *Glebulina rotundata* (Lamarck, 1819) also has a cycle of up to three reproductive periods (Parker et al., 1984). As to the reproductive aspects of the *A. trapesimalis* (Lamarck, 1819) (Unionoida) studied on the Pantanal region of the state of Mato Grosso by Callil and Mansur (2007), it is possible to say that the gametogenesis is continuous, however, with trends to the gonadal maturation stage (Silva-Souza et al., 2011). Oovogenesis has its maximum maturation period in April and May, whereas spermatogenesis from April to July. Gametogenesis is continuous with maturation peaks and elimination of gametes during the dry season. In this study, a continuity of specimens with larvae and/or eggs was observed in the demibranch over the sampling period. In the months of November of 2006, and May and August of 2007, there was a higher relative frequency of incubated specimens, also demonstrated by CCA. When this period is compared with Callil and Mansur's (2007) dates, conformity is noted related to the period in which there is the presence of embryos at the gills (April and May). Furthermore, the period with higher frequency of incubated species found in this work occurs at the onset of the dry season, when water leaves the flood plains and returns to the main channels (May) and in the dry season (August to November), also noted by Callil and Mansur (2007). In this, a relation can be traced between this period of higher maturation and elimination of gametes with the exit of fish from the lakes and flooding of adjacent areas, which are characteristic of the studied region. Thus, it is possible to associate the relation of dependence of the bivalves with the fish fauna.

From the environmental variables considered in this study, depth and transparency seem to have a negative relationship with the reproduction of the bivalves. The variation of the water depth refers to the fluctuation of the water level of the studied site, and this characteristic associated with the load of suspended solids seems to influence the reproductive period of freshwater bivalves (Beasley et al., 2000), mainly in tropical regions where the water temperature is constant. The hydroperiod, marked for



**Figure 2.** Canonical correspondence analysis (CCA) biplot of the first two axes showing the relationship between Frequency of Incubation (INC, NINC, •); Index (IC, ICS, IIC, □); environmental variables (Rich, Abund, pH, OD, Temp, Depth, Transp, ♦) and temporal variations - months (arrows).



**Figure 3.** The floodpulse-driven wet-and-dry cycle in the Pantanal giving the synergistic effect of abiotic and biotic factors demonstrated by the synchronicity of reproduction in freshwater mussels with basic patterns of fish migration. Adapted from Wantzen et al. (2008).

a seasonal flood pulse in the Pantanal (Junk et al., 1989; Junk and Cunha 2005), makes it possible for the lakes to receive a higher amount of organic matter consequently increasing the amount of food available for those bivalves. The favourable conditions with great availability of food promote an increment of energy reflected by the Condition Index. This energy increase will later be transferred from the digestive glandules to the gonads, thus the amount and quality of available food, as an intrinsic factor, is also capable of controlling reproduction (Grotta and Lunetta, 1980).

Regarding *A. trapesialis*, larval incubation has been noted on the central part of the inner demibranch for all the analysed specimens, and the characteristics of the incubated demibranch were intumescence and whiteish in colour, as noted in other species for this genus (Bonetto and Ezcurra, 1962; Maldonado et al., 1990). It has been noted that the demibranches of *P. popeii* destined for the marsupial function differ from the outer demibranch by presenting a modified septum (Smith et al., 2003), a condition also described for *A. trapesialis* by Callil (2003). Yeager and Neves (1986) researched the *Quadrula cylindrical* (Say, 1817), and noticed that 65% of the incubated females kept larvae only on the outer demibranch, and 35% used all four. According to Ituarte (1984), the behaviour of incubating eggs and larvae on specific spots of the demibranch may be related to the protection and the development of the offspring as a reaction to the biotic and abiotic characteristics of the freshwater environments.

As to the biometry of *A. trapesialis*, it has been noted that there is significant relation between the length of the demibranch and the total length of the shell demonstrating that the longer the length is, the longer the spot used for incubating the eggs and/or larvae will be. For *Sphaerium striatinum* (Sphaeriidae), a diminute bivalve that incubates the eggs that exhibit development without the larval planktonic stage, do not show a significant relation between the length of the bivalve and the length of the larval development region they have nevertheless, at the moment of the maturation peak, it could be predicted that the size of the spot used for the maturation increases due to the total length (Beekey and Hornbach, 2004). This inference can be confirmed by McIvor and Aldridge's (2007) study on reproductive biology of *Pseudanodonta complanata* (Rossmössl, 1835) (Unionidae), in which it was noted that the number of incubated glochids increases due to the total length of the bivalve (Figure 3).

The larvae of most unionoids are parasitic on fishes, so it is reasonable to suspect that the distribution and abundance of host fish might be an important factor in limiting the unionoid population (Strayer, 2008). *A. trapesialis* fits into this scenario characterised as a temporary parasite (Bonetto and Ezcurra, 1962) and most often forms a cyst on the tissue, which does not cause severe lesion to the hosts (Silva-Souza and Eiras, 2002). This relation was described by Arey (1932) and Murphy (1942) as a nutrition relation between the larvae and the degeneration of the host tissue, which is the source of nutrients during metamorphosis. There is a wide gap of information regarding the host-

parasite relationship in unionoids: almost nothing is known about the infection of fish and the parasite-host relation in the Neotropical region (Silva-Souza and Eiras, 2002; Callil and Mansur, 2007) and little is available with this approach in the world (Hoggarth, 1992; Strayer, 2008).

Biological Indexes are much used in studies on oysters widely grown for commercial purposes, due to the fact that they reflect the period of sexual maturation as well as the nutritional conditions (Absher and Christo, 1993), and they are also applied to biomonitoring (Callil, 2003; Rebelo et al., 2005). The Condition Index reflects changes in the animal's physiology, especially during larvae incubation, and has also been broadly used to estimate mean weight from a known length and to calculate the index of physical condition of populations (Albuquerque et al., 2009). It would be obvious that the CI and SCI were directly associated with the gonadal maturation period (Aswani and Hamilton, 2004) and therefore would be expected to be closely related to larval hatching and/or nutritional value of the bivalve, however the results of this study did not show differences for the CI and SCI among incubated and not incubated mussels. Abujanra et al. (2009) considering that difficulties such as the strong correlation between the input parameters (a and b of the length-weight relationship), the existence of allometry in the growth of many species (Braga, 1986), and the limitations in comparing different populations are some controversial points regarding the use of condition factors. However, the Biological indexes may be affected by a wide range of factors, such as temperature, the gametogenic cycle of the species as well as the availability of food, for these last two are the most influential ones (Orban et al., 2002). Stressing conditions or environmental situations that require greater energy expenditure (release of gametes, for instance) may be responsible for the low condition indexes observed in certain periods of the year.

The factors that exert greater influence on the bivalves' condition are the availability of food, temperature, and salinity (Hickman and Illingworth, 1980; Smaal and Van Stralen, 1990). During this investigation it has been noted that limnological variables are related to the Indexes (CI, SCI and ICI) (Figure 2). These factors may be considered an important exogenous factor for the control of the reproduction of the Unionoida bivalves (Jones et al., 1986; Avelar and Mendonça, 1998; Beasley et al., 2000). It is known that temperature influences gametogenesis, incubation, and of larval development of larvae in temperate regions (Araujo and Ramos, 2000). Still, the regulation of gametogenesis and larval incubation is not restricted to a single factor. Haag and Warren-Junior (2003) analysed the behaviour of larval release of seven species of freshwater bivalve family Unionidae, and the results showed that the physical interaction with fish is necessary for the stimulation of large releases of larvae. Although we did not identify the long-awaited statistical significance between the composition of icitiofauna with factors that reflect incubation, this study demonstrated that there is, rather,

a relation between wealth and abundance of fish with the frequency of incubated bivalves.

Summarising, the weight (Wd) and the length of the demibranch (Ldi) have shown significant differences between the incubated and non-incubated specimens. There is a relationship among environmental variables and the Biological Indexes (CI, SCI and ICI). The fish fauna composition seems to be synchronised with the number of incubated *A. trapesialis*. These statements lead us to suggest that there is a synergism between endogenous and exogenous factors in relation to the reproduction of freshwater bivalves. The knowledge on the parasite-host relation as well as on the unionoides reproductive aspects is a way of contributing to the preservation of the species. The fish dependence on completing the metamorphosis up to the juvenile stage increases its susceptibility to any environmental disturbance, since the decline in the abundance of fish, the introduction of exotic fish or changes to the groups of fish may provoke drastic consequences to the ability of the bivalves for having success in their reproduction (Kneeland and Rhymer, 2007).

Among the 115 species (underestimated) freshwater mussels recorded in Brazil, 26 (11 Hyriidae and 15 Mycetopodidae) are in the red list of threatened species (Brasil, 2008), in the EUA and in Canada 300 species and subspecies of Unionoida, about 72% are threatened (Vaughn and Hakenkamp, 2001). This group is considered to be endangered due to the frailty of the riparian sites, which are subjected to anthropic actions. Research on gametogenesis, incubation period, and the relations established between these bivalves with their hosts are scarce in Brazil, mostly when it comes to the Pantanal region, which has a characteristic flood pulse. Among various factors, knowledge on the reproductive cycle is essential for decision making about the sustainable management of this group.

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