



## Incubation cycle of eggs and larvae of *Anodontites trapesialis* (Lamarck, 1819) (Bivalvia, Mycetopodidae) in fish farming

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**Abstract.** The aim of this study was to determine the period in which eggs and larvae *lasidia* occur in the marsupium of *Anodontites trapesialis*, in different conditions of fish farming. We studied 240 specimens collected from two properties located in the municipality of Londrina, Paraná, Brazil. We determined the period of egg incubation and presence of larvae for each cultivation tank. We analyzed distinct characteristics regarding dimensions, fish species and management: in one property/tank, fishes are raised for the sport fishing practice of “fish-and-pay” (Point A - Toca do Jacaré) while in the other, they are raised/reared exclusively for tilapia fry (Point B - Paraíso da Tilápia). We measured conductivity and water temperature during the collection of bivalves and evaluated these values with monthly rainfall levels in order to determine the relation of these parameters to the development of *lasidia*. We concluded that, under these conditions of fish farming, *Anodontites trapesialis* does not show the seasonal pattern of egg incubation reported for the majority of freshwater bivalves in natural environments. The cultivation conditions and management of the tanks appear to constitute preponderant factors for the differences found between the two points with respect to reproduction in this bivalve.

**Key words:** reproduction, *lasidium*, demibranchs, aquaculture, management

**Resumo. Ciclo de incubação dos ovos e larvas de *Anodontites trapesialis* (Lamarck, 1819) (Bivalvia, Mycetopodidae) em tanques de piscicultura.** Teve-se como objetivo determinar o período em que ovos e as larvas *lasidia* ocorrem no marsúpio de *Anodontites trapesialis* e o efeito de fatores ambientais, em diferentes condições de piscicultura. Foram estudados 240 espécimes de duas propriedades localizadas no município de Londrina, Paraná, Brasil. O período de incubação dos ovos e a presença de larvas de foram determinados para dois tipos de tanques de cultivo com características distintas com relação às dimensões, espécies de peixes e manejo, onde um é utilizado para pesca esportiva de “pesque-pague” (Ponto A - Toca do Jacaré) e a outra serve exclusivamente para a criação de tilápia (Ponto B - Paraíso da Tilápia). A condutividade e a temperatura da água foram medidas no momento de captura dos bivalves e, juntamente com os níveis mensais de pluviosidade, foram avaliadas para determinar a relação desses parâmetros com o desenvolvimento das *lasidia*. Conclui-se que, em tanques de piscicultura, *Anodontites trapesialis* não mostra o padrão sazonal de incubação dos ovos reportado para a maioria dos bivalves de água doce em ambientes naturais. As condições de criação e o manejo dos tanques parecem constituir fatores preponderantes para as diferenças encontradas entre os dois pontos, com respeito à reprodução desse bivalve.

**Palavras-chave:** reprodução, *lasidium*, demibrânquias, aquicultura, manejo

### Introduction

The genus *Anodontites* Bruguière, 1792 is one of the twelve genera that make up the family Mycetopodidae and it is native to South America. In Brazil, it corresponds to the genus with the greatest number of species (15), among which *A. trapesialis* stands out (Simone 2006).

In Peru, *A. trapesialis* has been indicated as an alternative source for aquaculture, because it is considered of good nutritional value, with protein content close to that of fish (Tello-Panduro *et al.* 2003; Tello-Panduro *et al.* 2004). However, its larva, called *lasidium*, is an obligatory parasite of fishes (Veiteinheimer-Mendes & Mansur 1978; Silva-Souza *et al.* 2011). The effects of parasitism by bivalve larvae include reduced growth in fish,

increased sensitivity to pollutants, secondary infections by opportunistic pathogens, and even the host's death, depending on the intensity of infestation (Silva-Souza & Eiras 2002; Felipi & Silva-Souza 2008).

Over the last years, in the municipality of Londrina and in its region, it has been demonstrated that *A. trapesialis* occurs in large numbers on fish farms. Their presence in culture tanks, especially of tilapia, has caused damage to fish and consequent losses for producers. However, nothing is known about its development under these conditions, which makes it difficult to decide on appropriate actions to control these bivalves in fish farming.

The aim of this study was to determine the period in which eggs and larvae of *Anodontites trapesialis* occur in two tanks for fish farming with distinct characteristics and how this relates to environmental conditions.

### Materials and Methods

The collection sites are located in two properties in Distrito Administrativo Espírito Santo, eastern region of Londrina: Points A and B.

Point A (23°24'46.8"S and 051°08'33.5"W) consisted of a tank of 1,400 m<sup>2</sup> where fish species were cultivated. The species included: *Oreochromis niloticus* (Linnaeus, 1758), known popularly as "tilapia", *Piaractus mesopotamicus* (Holmberg, 1887), known as "pacu", and a hybrid of a male pacu (*Piaractus mesopotamicus*) and a female "tambaqui" (*Colossoma macropomum*, Cuvier, 1816), called "tambacu". The fish tank was used for sport and leisure fishing at the fish-and-pay "Pesque-Pague Toca do Jacaré". It was surrounded by eucalyptus trees and it contained a large quantity of eucalyptus leaves in its substrate.

Point B (23°25'16.7"S and 051°09'13.6"W) consisted of a tank of approximately 300 m<sup>2</sup>, free of arboreal vegetation along its edges, and it was used for rearing only *Oreochromis niloticus*. The tank was located on the fish farm "Paraíso da Tilápia."

We collected specimens of *Anodontites trapesialis* at each point every month for a year (May 2006 to – April 2007), with an interval of four weeks between each collection. Ten animals were collected monthly at each location, totaling 240 individuals.

At the time of each collection, we determined the means of water conductivity ( $\mu\text{s}/\text{cm}$ ) and temperature ( $^{\circ}\text{C}$ ) with the help of the instruments Tecnopon MB-11 and MB-10, respectively. Rainfall data were obtained from IAPAR (Instituto Agronômico do Paraná, Londrina) and corresponded to total precipitation in the 30 days preceding each collection.

We found the specimens by tapping at the bottom of the tanks with our feet. We then collected manually and transported them in plastic containers with water from the tank itself, to Laboratory of Ecology of Aquatic Organisms Parasites (LEPOA), of the Department of Animal and Vegetal Biology, State University of Londrina, Londrina, PR. In the laboratory, we washed the animals in running water, identified and weighed them, obtaining the total weight (Wt), body weight (Wb) corresponding to the soft parts, and the shell weight (Ws), expressed in grams (Callil & Mansur 2007). These weights were used to calculate the condition index (IC), which corresponded to the weight of the soft parts (body) in relation to total weight (Wb/Wt), and the shell index (IS), which is the weight of the shell in relation to total weight (Ws/Wt), both expressed as a percentage.

A digital pachymeter was used to measure total length (Lt) – corresponding to a line that bisects the shell into dorsal and ventral regions –, and height of the shells (h) – that corresponds to a line that divides the umbo perpendicular to the line of the length and divides the shell into anterior and posterior regions, in millimeters (Mansur *et al.* 1987).

In order to determine the presence of eggs and larvae, we analyzed the demibranchs under a stereomicroscope. To confirm the occurrence of larvae, we examined temporary preparations under a light microscope.

The mean and standard deviation were determined for each biometric variable. The non-parametric statistics test the Mann-Whitney U test was applied to compare the values for total weight (Wt), shell weight (Ws), body weight (Wb), and length (Lt) and height of shell (h) between the two locations. Parametric tests require data conditions, such as independence and normality, which should be fulfilled for their safe usage. This was not the case on some data in the present study and so the non-parametric test was employed.

The condition index (IC %) and shell index (IS %) of the bivalves collected in the two locations were analyzed using Fisher's exact test for a 2x2 contingency table.

Student's t-test was employed to compare the values for water temperature and conductivity recorded in each month, between the collection points.

A multiple regression analysis was performed to test the relation of water temperature and conductivity and rainfall with occurrence of individuals with eggs and with larvae, separately, in each tank.

In all comparisons made, data were considered significantly different when a two-tailed probability of  $p < 0.05$  was obtained.

## Results

The collections started in May, at the end of fall, when the water temperature was 19.6°C in both tanks. In the following month, the beginning of winter, the lowest temperatures were recorded, 17.6°C at Point A and 13.8°C at Point B (Table I). In the subsequent collections, the water gradually showed higher temperatures reaching 29.6°C at Point A in February and 28.6°C at Point B in January. However, on average, the water temperature did not differ between locations ( $p = 0.43$ ).

Conductivity varied more at Point A, where minimal values of 91.2  $\mu\text{s/cm}$  were recorded in April 2007, and maximum values of 270.2  $\mu\text{s/cm}$  in

September 2006, and 226.4  $\mu\text{s/cm}$  in March 2007. At Point B the minimal value obtained was 40.1  $\mu\text{s/cm}$ , also in April 2006, and a maximum value of 241.1  $\mu\text{s/cm}$  in December 2006 (Table I). However, when comparing the mean values, no differences were found between the two locations ( $p = 0.12$ ).

The specimens of *Anodontites trapesialis* collected at the two points showed, on average, similar length, height and weight (Tables II and III). The condition index (IC %) and shell index (IS %) were also similar (Table III).

During the study period, specimens with eggs in the gills were found in all months at Point A, except in October 2006, in which 20% of specimens contained larvae and the rest presented an empty marsupial. We only found individuals containing larvae recently hatched in the gills in September and November of 2006 and in February and March of 2007 (Fig. 1).

**Table I.** Water temperature (°C) and conductivity ( $\mu\text{s/cm}$ ) recorded in each collection, from May 2006 to April 2007, at points A (Toca do Jacaré) and B (Paraíso da Tilápia), Londrina, PR, Brazil and resulting p values using Student's t-test.

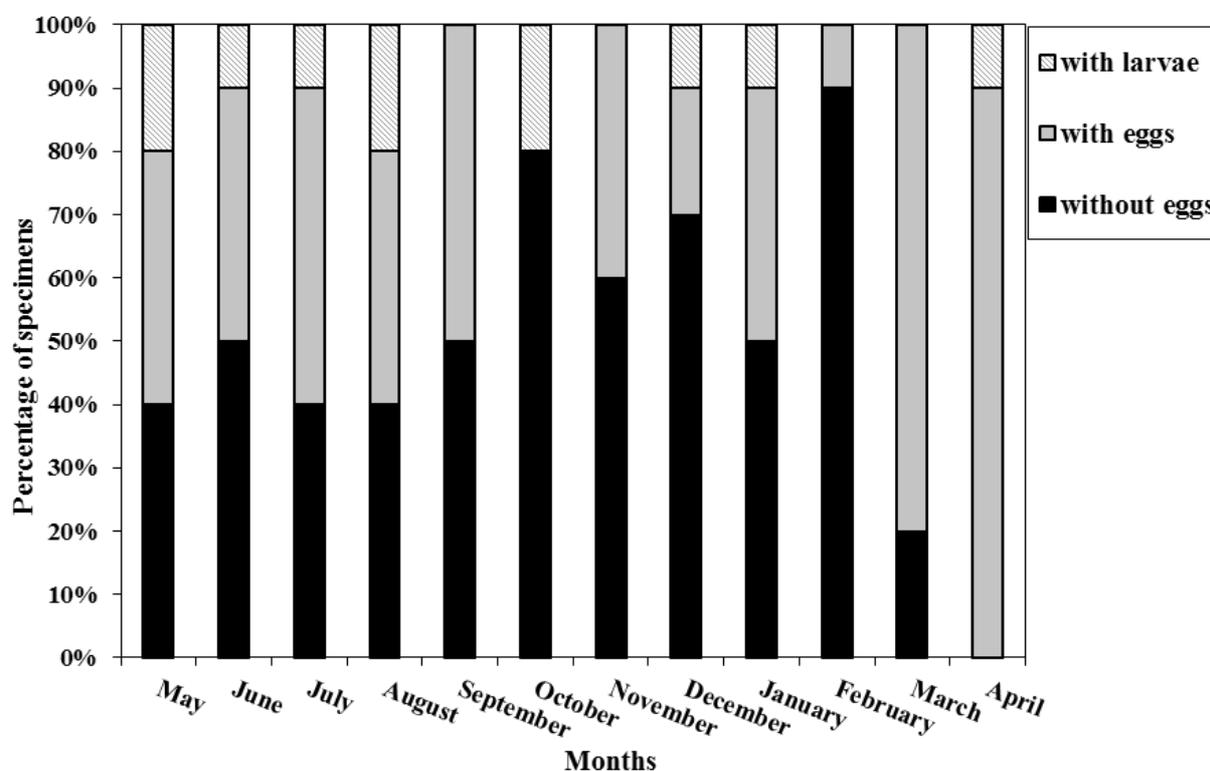
Months of study	Water temperature (°C)		Conductivity ( $\mu\text{s/cm}$ )	
	Point A	Point B	Point A	Point B
May/06	19.6	19.6	92.2	108.4
June/06	17.6	13.8	98.5	93.8
July/06	19.2	17.6	112.5	99.7
August/06	23.7	20.3	109.8	104.6
September/06	22.4	20.1	270.2	120.4
October/06	28.9	27.0	197.1	116.8
November/06	28.5	25.0	198.2	151.7
December/06	28.0	28.2	174.1	241.1
January/07	25.5	28.6	116.1	133.8
February/07	29.6	27.7	171.2	91.4
March/07	27.5	26.2	226.4	128.5
April/07	26.8	25.2	91.2	40.1
<b>Mean±SD</b>	24.8±4.19	23.3±4.82	179.8±109.36	119.2±47.29
<b>t-test</b>	p = 0.43		p = 0.12	

**Table II.** Means ± Standard Deviations (SD) and minimum and maximum values of total length (Lt) and height (h) of specimens of *A. trapesialis* collected at Points A (Toca do Jacaré) and B (Paraíso da Tilápia), Londrina, PR, Brazil, from May 2006 to April 2007, and results of the Mann-Whitney "U" test (p).

Sites	Lt (mm) ± SD (min.- max.)	h (mm) ± SD (min.- max.)
Point A	118.277 ± 6.63 (62.59-146.31)	57.17 ± 4.05 (29.97-78.74)
Point B	119.73 ± 13.94 (75.92-154.38)	55.67 ± 8.25 (32.86-89.65)
<b>P</b>	0.21	0.89

**Table III.** Means and standard deviations (SD) and minimum and maximum values of total weight (Wt), body weight (Wb) and shell weight (Ws), condition index (IC %) and shell index (IS %) of specimens of *Anodontites trapesialis* captured at Points A and B, from May 2006 to April 2007, and results of the Mann-Whitney U test and Fisher's exact test (p).

Site	Wt (g) ± SD	Wb (g) ± SD	Ws (g) ± SD	IC % ± SD	IS % ± SD
	(min.-max.)	(min.-max.)	(min.-max.)	(min.-max.)	(min.-max.)
<b>Point A</b>	157.69±64.62 (24.69-322.71)	110.43±42.80 (19.76-265.11)	47.25±23.97 (4.93-111.17)	70.91±4.45 (47.57-84.38)	29.08±4.45 (15.62-52.43)
<b>Point B</b>	156.48±80.54 (34.59-322.45)	116.47±58.73 (27.01-230.67)	40.00±22.51 (7.58-91.78)	75.21±2.92 (67.77-84.42)	24.79±2.71 (15.58-32.23)
<b>U test</b>	p = 0.82	p = 0.53	p = 0.08	-	-
<b>Fisher's test</b>	-	-	-	0.63	0.63



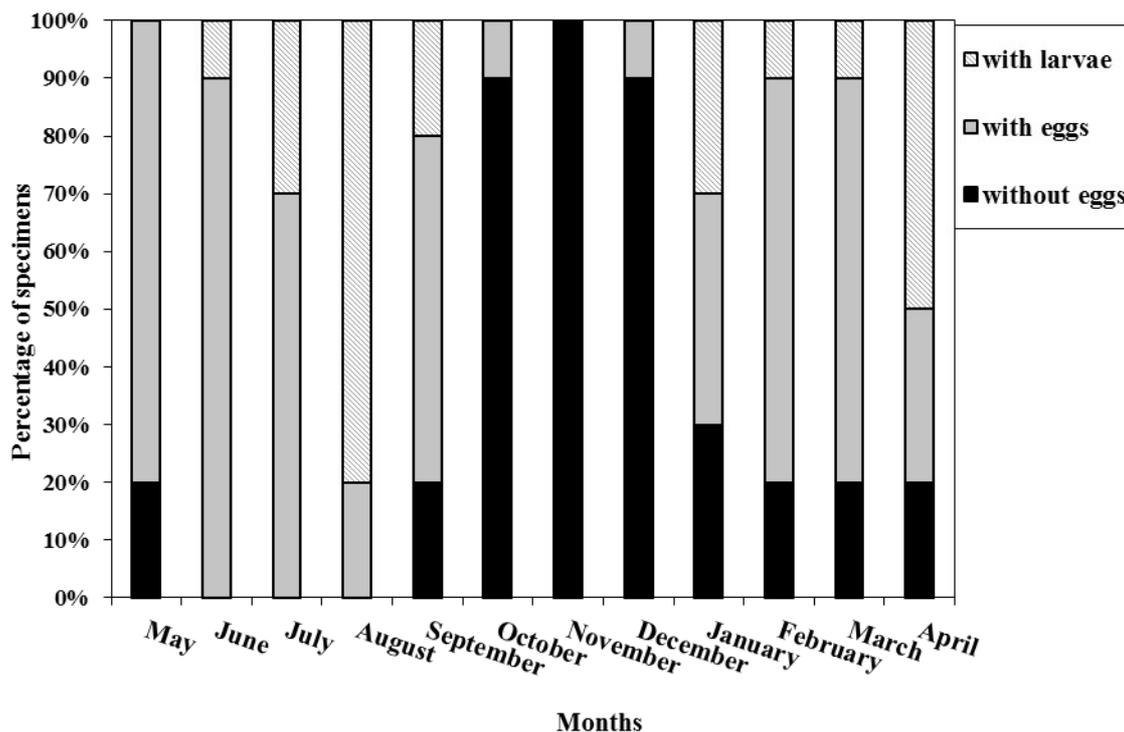
**Figure 1.** Variation in percentage of specimens of *Anodontites trapesialis* without eggs, with eggs and with larvae in the gills, at Point A (Toca do Jacaré), Londrina, PR, Brazil, from May 2006 to April 2007.

At Point B, two periods appear to correspond to incubation of the eggs and release of larvae: from May to September and from January on. In October, November and December, 90 to 100% of specimens collected were found with the inner demibranchs empty (Fig. 2).

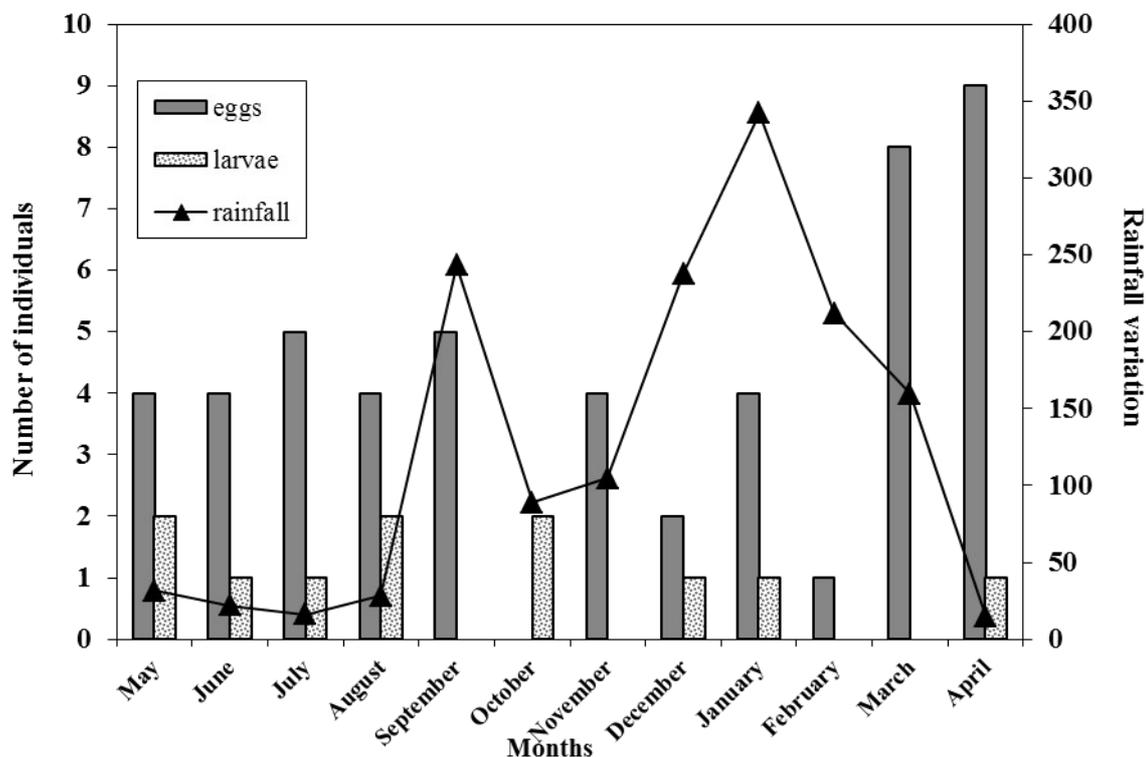
The incubation cycle in the specimens of *Anodontites trapesialis* was not correlated with the variations in water temperature, either at site A

( $r^2=0.37$ ,  $p=0.12$ ) or site B ( $r^2=0.18$ ,  $p=0.41$ ). The same held true for conductivity, at both site A ( $r^2=0.45$ ,  $p=0.07$ ) and site B ( $r^2=0.42$ ,  $p=0.09$ ).

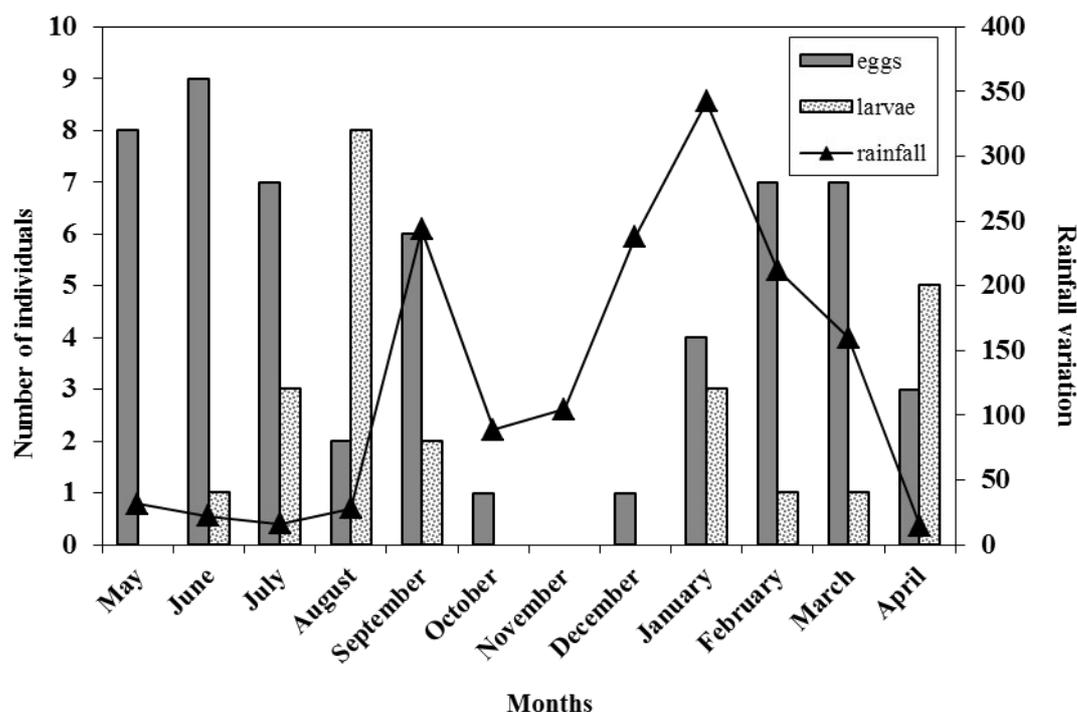
In periods with low rainfall and in rainy periods, we collected individuals with eggs and with larvae in the marsupium, in both tanks (Figs. 3 and 4). Under cultivation conditions, rainfall did not influence larval development in either location ( $r^2=0.35$ ,  $p=0.15$ ;  $r^2=0.09$ ,  $p=0.67$ , respectively).



**Figure 2.** Variation in percentage of specimens of *Anodontites trapesialis* without eggs, with eggs and with larvae in the gills, at Point B (Paraíso da Tilápia), Londrina, PR, Brazil, from May 2006 to April 2007.



**Figure 3.** Variation in rainfall (mm) and number of individuals of *Anodontites trapesialis* with eggs and with larvae in the gills, at Point A (Toca do Jacaré), Londrina, PR, Brazil, from May 2006 to April 2007.



**Figure 4.** Variation in rainfall (mm) and number of individuals of *Anodontites trapesimalis* with eggs and with larvae in gills, at Point B (Paraiso da Tilápia), Londrina, PR, Brazil, from May 2006 to April 2007.

## Discussion

Seasonal variations in temperature have been indicated as being important in the initiation of reproduction in unionoid bivalves. According to Nagabhushanam & Lohgaonker (1978), Jones *et al.* (1986) and Avelar & Mendonça (1998), gonadal development begins with the increase in temperature, and the release of gametes coincides with a decrease in temperature. On the other hand, Peredo & Parada (1986) observed spawning with an increase in water temperature and the interruption of gamete emission at low temperatures, during fall and winter.

Maldonado *et al.* (1990) reported the occurrence of *Anodontites (A.) soleniformes* (d'Orbigny, 1835) in Bolivia, with incubation of eggs from January to July. Bonetto & Ezcurra (1962) noted the presence of larvae in the gills of *A. trapesimalis* in a defined period of August to January, in natural Argentinian environments. In Brazil, larvae of this species were recorded parasitizing specimens of *Tilapia rendalli* (Boulenger, 1897) in the lake, from August to October (Silva-Souza & Eiras, 2002).

In regions where water temperature is more constant, some factors that appear to influence the reproductive period of freshwater bivalves are: water level, flow and load of suspended solids (Beasley *et al.*, 2000).

In the Pantanal (Mato Grosso, Brazil), gametogenesis and release of gametes in *Anodontites trapesimalis* are conditioned to the system

of periodic inundations (Callil & Mansur 2007). Gametogenesis of this bivalve species appeared to be continuous, with peaks of maturation and elimination of gametes between April, May and June and presence of embryos in the marsupium in individuals collected in April and May.

Therefore, it is evident that although freshwater bivalves can reproduce all year long, they show a defined period of egg incubation, which varies according to the species and region where they occur.

Under culture conditions, the water volume in the tanks can be controlled by managing the rainy and dry periods, so that the water level does not change with rainfall. The water temperature is expected to be an important factor for the reproduction of bivalves in tanks. However, differing from that reported in the literature, specimens of *Anodontites trapesimalis* with eggs and with larvae in the marsupium were found in most months. At Point B, however, the bivalves appeared to show two periods of defined egg incubation – from May to September and from January to April. On the other hand, at Point A, there were individuals in incubation during all months, but also several of which had empty marsupia. It is possible that these differences are consequence of management and environmental conditions of the tanks.

According to Byrne (1998), gametogenesis can be continuous and the reproductive yield can be high in bivalve specimens of the family Hyriidae, which inhabit eutrophized locations. However, in

oligotrophic environments, gametogenesis is generally seasonal and reproductive disturbance appears to be more common. According to Simone (1994), species of Mycetopodidae commonly show a certain resistance to pollution, but specimens found in polluted rivers generally have deformations in shells or various organs.

Point A comprises a large tank, where various fish species are cultivated at high concentrations of individuals of different sizes, and which receive large quantities of food offered by fishers. In addition, the substrate has large amounts of leaves – that fall from the nearby trees. Besides lime – used to correct pH – chemical products are frequently added to the water to control parasites of fish and are responsible for the peaks of conductivity recorded in September and March. In this tank, most specimens collected showed deformed shells, with dark spots and worn points in the periostracum, which, according to Simone (1994), suggests low water quality.

On the other hand, point B is small and only tilapia fry are reared in this tank, with number of individuals, management and food controlled. At this location, the surface and color of shells of *A. trapesialis* specimens appeared more uniform and without deformities.

Thus, under the cultivation conditions evaluated, *Anodontites trapesialis* did not show the same seasonal pattern of incubation of eggs reported for the majority of freshwater bivalves in natural environments (Callil & Mansur 2007). Fish farming conditions and the management of tanks constitute preponderant factors for differences in the reproduction of this bivalve in fish ponds.

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