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Reproductive cycle of *Branchiura sowerbyi* (Oligochaeta: Naididae: Tubificinae) cultivated under laboratory conditions

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ABSTRACT. The biology of *Branchiura sowerbyi* Beddard, 1892 has been the focus of many studies in temperate regions, where the species is exotic, according to literature data. Due to its high productivity and easy cultivation, *B. sowerbyi* is of great interest as a food source for fish farming. The present study reports information on the reproductive biology and growth of *B. sowerbyi* under laboratory conditions at 25 ± 1°C. Weekly observations during 52 weeks indicated that the time between cocoon laying and young hatching was 14 to 16 days, the specific daily growth rate was 0.91 ± 0.04% (mean ± SD) and the time to reach sexual maturity was 40.83 ± 6.88 days. As reported by other authors, the hatching rate observed was low (33.08%), but the survival rate of young was high, approximately 96%. Laboratory observations showed that *B. sowerbyi* has two annual reproductive cycles, the first (between the 5th and 24th week) being more pronounced than the second (between the 31st and 51st week) concerning the number of cocoons.

KEY WORDS. Cocoons; fish food; growth rate; oligochaete; tubificid.

Branchiura sowerbyi Beddard, 1892 is commonly found in organically enriched freshwater environments in tropical and temperate regions. In the latter, *B. sowerbyi* is used as a thermal pollution indicator (Aston 1968). The biology of the species has been the focus of many studies. Aston & Milner (1982) and Aston *et al.* (1982) studied the ideal conditions to breed it in activated sewage, and found better growth and reproductive rate in a mixture of activated sewage and fine sand (66% of activated sewage) at 25°C. Casellato (1984) studied the species' life cycle in a water-lily tank at the Botanical Garden of Padua and reported that *B. sowerbyi* presents the highest reproduction activity in the spring. Casellato *et al.* (1987) described the species' histological characteristics during gametogenesis. Bonacina *et al.* (1994) studied the population ecology of *B. sowerbyi* in mass cultivation and suggested a mathematical model to estimate its demographic parameters in natural environments. Ducrot *et al.* (2007) conducted a long study showing important aspects of the species' biology, aiming to use it in ecotoxicological tests (for other studies on this species, see Naqvi 1973, Aston 1984, Drewes & Zoran 1989, Casellato *et al.* 1992, Marchese & Brinkhurst 1996).

Most of the above studies were carried out in temperate regions, where *B. sowerbyi* is exotic, according to Aston (1968). In Brazil, studies in lentic environments and reservoirs show that *B. sowerbyi* is one of the most abundant species among the benthic organisms (Alves & Strixino 2000, Pamplin *et al.* 2005, Dorfeld *et al.* 2006). According to Raburu *et al.* (2002), in a study in Lake Naivasha (Kenya), this tubificid presented high annual productivity (7.43 g of dry matter per m^2) compared to the oligochaete *Limnodrilus hoffmeisteri* Claparede, 1862 (0.65 g of dry matter per m^2). These studies show the importance of the species within the benthic community of tropical aquatic environments.

Due to its larger size (20 to 185 mm long) and high productivity compared to other tubificid species (Yan & Wang 1999, Raburu *et al.* 2002), *B. sowerbyi* raises great commercial interest as live fish food. Many studies show that aquatic oligochaete represent a significant part in the diet of some fish species (Riera *et al.* 1991, Gophen *et al.* 1998, Rahman *et al.* 2006). Lietz (1987) advocated the potential of these animals as live fish food, since they are easily cultivated in large scales and are resistant to variations in temperature and dissolved oxygen levels.

Another interest is the use of *B. sowerbyi* in ecotoxicological studies (Keilty *et al.* 1988, Marchese & Brinkhurst 1996, Ducrot *et al.* 2007), since specimens are easy to handle (Marchese & Brinkhurst 1996) and are present in aquatic environments where *Tubifex tubifex* (Müller, 1774), commonly used in ecotoxicology, is either relatively rare or is not present (Ducrot *et al.* 2007). According to Casellato *et al.* (1992), in order to use a species in ecotoxicological studies with the aim to evaluate the level of sediment contamination of rivers and lakes by pollutants, it is first necessary to understand its biological attributes.

The present study reports on characteristics of the reproductive biology and growth of *B. sowerbyi* under laboratory conditions, and provides other information that could be incorporated in ecotoxicological studies on the species. The information provided here complements a previous study (Nascimento & Alves 2008) on the egg-laying and egg hatching rates of *B. sowerbyi* individuals maintained in clay substrate.

MATERIAL AND METHODS

Specimens of *B. sowerbyi* used in the experiment were obtained from a culture kept at the Benthonic Invertebrate Laboratory at the Universidade Federal de Juiz de Fora (Juiz de Fora, Minas Gerais, Brazil) under ambient temperature and photoperiod. The organisms are originally from Diogo Lake (Luis Antônio, São Paulo, Brazil). The collection method was described in Nascimento & Alves (2008).

The experiment was conducted in 250-mL beakers containing 100 mL of sand, 100 mL of water (dechlorinated and aerated) and 0.1 g of fish food (AlconBASIC®, Tab. I) as a source of organic matter. The sand used in the experiment, collected from the Peixe River (21°54'37"S and 43°33'24"W), located in southwestern Minas Gerais state (Brazil), had been previously analyzed under a stereoscopic microscope (40x magnification) to remove invertebrates. The granulometric sand fraction used was 0.25 to 1.00 mm. Six beakers were used for cocoon production. Each beaker contained five mature specimens (six weeks of age) and was kept in incubators at 25 ± 0.1°C, in the dark. Every other day for 20 days the substrate of each beaker was washed in a 0.25-mm sifter and analyzed under a stereoscopic microscope to collect and count the cocoons. The transparence of the cocoon allowed us to easily count the number of eggs inside.

Using a 3-mL Pasteur pipette, the cocoons were collected and put in 100-mL beakers containing 25 mL of substrate and 25 mL of dechlorinated and aerated water. The recipients containing the cocoons were kept in incubators at 25 ± 0.1°C, in the dark, and analyzed under a stereoscopic microscope every two days for observation and counting of eclosions.

Thirty young individuals (two days old), which presented normal movement and no body deformations, were selected for observation of growth and sexual maturation that was determined by the laying of the first cocoon. Six 250-mL beakers, each containing five individuals, were analyzed weekly during 364 days to record the weight of individuals and number of eggs and cocoons. To do this, the substrate was washed in 0.250-mm sifters and analyzed under a stereoscopic microscope. To avoid stress on the organisms, they were removed with a Stanley knife and transferred to a Petri dish containing dechlorinated water. After the collection of cocoons, our subjects were transferred to a new substrate with 0.1 g of fish food.

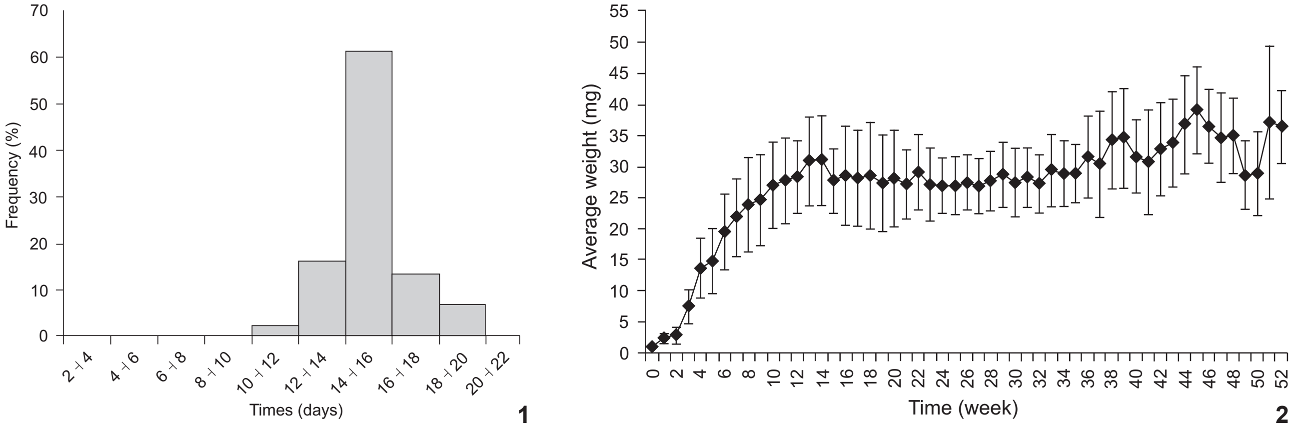
For each beaker, the time between cocoon laying and hatching, average rate of daily growth, time of sexual maturation, number of cocoons per adult per day and number of eggs per cocoon were recorded. Data are presented as mean ± standard deviation. The average rate of daily growth (G_w%) was calculated according to Reynoldson (1987):
$G_w\% = (LnW_2 - LnW_1) \times 100t^{-1}$; where: W1 = initial weight (mg); W2 = final weight (mg); and t = time in days.

Table I. Composition of the fish food Alcon Basic® used as organic matter source for the maintenance of *B. sowerbyi* in laboratory. Figures provided by the manufacturer (value per kilogram of the product).

Food composition	Quantity
Vitamin A	30,000 UI
Vitamin D3	5,000 UI
Vitamin E	83.3 mg
Vitamin K3	8.3 mg
Vitamin B1	6.7 mg
Vitamin B2	25.0 mg
Vitamin B6	6.7 mg
Vitamin B12	33.3 mg
Niacin	116.7 mg
Calcium Pantothenate	50.0 mg
Biotin	0.3 mg
Folic Acid	2.5 mg
Hill	520.0 mg
Iron	83.3 mg
Cupper	8.3 mg
Zinc	83.3 mg
Manganese	66.7 mg
Selenium	0.2 mg
Iodine	1.7 mg
Methionine	833.3 mg
Stabilized Vitamin C	250.0 mg

RESULTS

A rate of eclosion of 33.08% was determined from 67 cocoons, containing 1.99 ± 0.21 eggs·cocoon^{-1} (mean ± SD). There were no deaths among the adults during the 21-days period of cocoon collection. The time between cocoon laying and hatching varied from 10 to 20 days (Fig. 1). Most of the hatchings (90.91%) occurred between 12 and 18 days after the cocoon had been laid. Positive growth was observed until the organisms completed 14 weeks (Fig. 2), after which their weights stabilized. After the 36th week the weights of the subjects varied greatly among one another.



Figures 1-2. (1) Period between cocoon laying and eclosion and (2) average weight of *B. sowerbyi* cultivated in medium sand (0.250-1.000 mm) at 25 ± 1°C.

The average time to reach sexual maturity (laying the first cocoon) was 40.83 ± 6.88 days, varying between 35 and 49 days (after the 7th week, all organisms had produced cocoons), with an average weight of 17.56 ± 4.57 mg in the first week of laying. In this period the individual average growth rate was 0.41 ± 0.09 mg·day^{-1} and average daily growth (G_w%) of 6.98 ± 0.91%. At the end of the 364-days period the average daily growth was 0.91 ± 0.04%.

Cocoon laying started on the 5th and lasted until the 24th week, before resuming on the 31st week (Fig. 3), although with a smaller number of cocoons per adult and inconstant throughout the weeks that followed. Accordingly, there were two distinct periods of reproduction, the first between the 5th and the 24th week and the second between the 31st and 51st week. The number of eggs per cocoon varied between 1 and 6, with an average of 1.73 ± 0.57, and the average of cocoons per adult per day was 0.12 ± 0.13. The survival rate was 100% at the end of the first reproductive cycle (25th week), which slowly decreased to 63% at the end of the observations.

DISCUSSION

Branchiura sowerbyi specimens cultivated under laboratory conditions have low hatching rates, as reported by Marchese & Brinkhurst (1996), Nascimento & Alves (2008) and our results (34.4; 44.3 and 33.08% respectively). According to Wisniewsky (1979), the low hatching rate of tubificidae may be related to the attack of organisms that can harm embryo development. However, further studies are necessary to verify whether this low hatching rate is intrinsic to the species or whether it is related to the vulnerability of cocoons to microorganisms and laboratory handling.

The period between laying the cocoon and eclosion of the young in our experiment (90.01% in the period 12-18 days) was similar to the interval found by Bonacina *et al.* (1994) and Nascimento & Alves (2008) with the same temperature. This period does not seem to be correlated with the kind of sediment, once Nascimento & Alves (2008) used clay, but it is correlated with the temperature as showed by Bonacina *et al.* (1994).

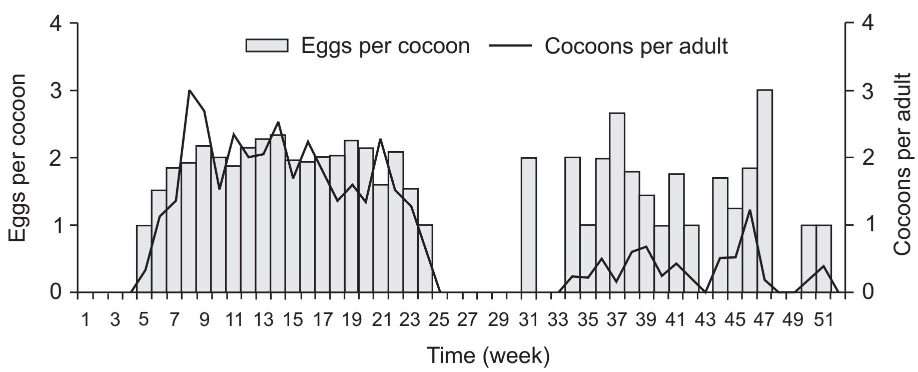


Figure 3. Number of cocoons per adult and average of eggs per weekly cocoons of *B. sowerbyi* cultivated in medium sand (0.250-1.000 mm) at 25 ± 1°C.

The growth rate of the *B. sowerbyi* individuals was high in the first weeks of life. Marchese & Brinkhurst (1996) reported a growth rate of 0.58 mg·day^{-1} in the first 35 days, when they reach sexual maturity, a higher value than that observed in the present study. According to Marchese & Brinkhurst (1996) and Ducrot *et al.* (2007), from the beginning of cocoon production, negative growth can be observed due to the use of energy resources in the production of eggs. Nevertheless, in the present study the organisms continued growing for 11 weeks after reaching maturity, after which their weights stabilized. According to Tuomi *et al.* (1983), continued feeding during the reproductive period may avoid weight loss in individuals, as occurred in this study.

The average weight of the oligochaetes after the first cocoon laying was similar to that observed by Marchese & Brinkhurst (1996) at 25°C, approximately 20 mg, but it was lower than the average found by Ducrot *et al.* (2007), 84.1 ± 6.5 mg, for the same species at 24°C. The average number of eggs per cocoon was lower than that observed by Aston *et al.* (1982) and Marchese & Brinkhurst (1996), 2.82 ± 0.87 and 1.94 ± 0.13, respectively, and greater than the 1.21 ± 0.08 reported by Nascimento & Alves (2008) for organisms kept in clay sediment at 25°C.

Aston (1968) studied the effect of temperature on the life cycle of *B. sowerbyi* and observed that the cocoons were laid during the hottest months of the year, concluding that seasonal temperature variations influenced their reproduction. Subsequently, Aston & Milner (1982) and Sobhana & Nair (1984) confirmed the effects of temperature on the reproduction of the species. When cultivated under controlled conditions, *B. sowerbyi* presented two cycles of annual laying, which lasted approximately 105 days, as well as an interval of approximately 52 days (Ducrot *et al.* 2007), similar to our findings.

Ducrot *et al.* (2007) found that the first reproductive cycle is synchronized, that is, all organisms are in the reproductive stage at the same time, while in the second cycle only a few organisms reproduce. According to Pianka & Parker (1975), animals of the same age differ in life expectancy and future reproduction. Individuals with a reproductive effort that is above average generate a larger number of descendants in the short term, but this increases their death rate and lowers their reproductive value for the next cycle. To those authors, variations of this kind in a population can result in an inexplicable individual variation in reproductive effort in each age group.

Ducrot *et al.* (2007) emphasized that, due to the lack of synchronization of reproductive cycles among individuals, the second reproductive phase is not a good parameter (endpoint) for use in tests involving toxic substances. By contrast, other reproductive aspects, such as number of cocoons per adult per day, time of sexual maturity and interval between egg laying are good parameters for use in sublethal toxicological tests.

With information on the life cycle of *B. sowerbyi* provided in the present paper, we can establish a work chronogram for the culture of *B. sowerbyi* in the laboratory. The great number of organisms (in all life stages) obtained in the culture during the entire year will allow the use of this species in ecotoxicological tests, as suggest by Organization for Economic Co-operation and Development (OECD 2008), as well as in fish feeding, since it is well known that oligochaete is one of the main food sources for benthophagous fishes (Riera *et al.* 1991, Kakareko 2001, Rahaman *et al.* 2006).

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