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## ***Branchiura sowerbyi* reared in two sediments for ecotoxicological bioassays**

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### **Abstract**

The present work compared the survival, growth, and reproduction of *Branchiura sowerbyi* reared in natural sediment and in artificial tropical sediment (composed of sand, clay, and coconut fibers). The artificial tropical sediment, in the tested composition, appeared not to be suitable for conducting bioassays with this species, since the results showed a high mortality of the juveniles and low reproduction of adults as compared to those observed for the natural sediment.

*Key Words:* peat extract, coconut fiber, oligochaete, artificial sediment.

### **INTRODUCTION**

For conducting bioassays, rearing a test species in a laboratory is recommended above field-collection on animals (ASTM, 2005). Aquatic oligochaetes require sediments that are able to guarantee an adequate level of survival, growth and reproduction of the test-species. The international protocols for sediment toxicity testing with oligochaetes have recommended the use of natural sediments from places free of contamination, or formulated (artificial) sediments constructed using pre-defined proportions of sand, silt/clay and organic material (Environment Canada, 1995; ASTM, 2005; OECD, 2008).

Some authors, such as Kembel *et al.* (1999), Arrate *et al.* (2004), and Sloomweg *et al.* (2010), successfully conducted bioassays with limnic oligochaetes using artificial sediments. For constructing the sediment, they used sand, clay and peat; ingredients all easily obtained in temperate climates. However, for culturing and testing with tropical species, it may be necessary to adapt that composition, because peat may be logistically and financially out of reach in tropical countries. As an alternative, Garcia (2004) replaced peat by coconut fiber powder (*Coco nucifera* L.) in some toxicity tests using *Eisenia foetida* (Savigny), a terrestrial oligochaete. The two compositions (peat and coconut fiber powder) yielded

similar results with respect to growth, reproduction, and survival, leading to the successful adoption of this substitute substrate by other authors (Buch *et al.*, 2011; Nunes & Espíndola, 2012). Yet there are still no records of aquatic bioassay studies in tropical regions that have used coconut fiber powder to formulate sediments.

With the goal of increasing the use of artificial sediments for toxicity bioassays in tropical regions, we sought to compare rates of survival, growth, and reproduction of the oligochaete *Branchiura sowerbyi* Beddard, kept in natural sediment (NS) and in artificial tropical sediment (ATS), using coconut fiber powder as a substitute for peat. This species was chosen because it is a tropical species that can be cultured along a range of sediment types (Aston & Milner, 1981; Casellato *et al.*, 1992; Marchese & Brinkhurst, 1996; Ducrot *et al.*, 2007; Nascimento & Alves, 2008; Lobo & Alves, 2011), and has been used as test species in ecotoxicological bioassays (Lobo & Espíndola, 2014).

### **MATERIAL AND METHODS**

The NS was collected in Córrego Perdizes (Brotas, São Paulo, Brazil: 19°47'04.6X; 75°43'013.73Y; 23K). After collection, the sediment was sieved (mesh sieve 0.5mm) in order to remove undesired organisms, and kept at 4°C for up

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to 30 days. Córrego Perdizes is classified as oligo-mesotrophic (Sanchez, 2012), and its sediment has been used as a control sediment in other (non-published) studies, as well as a substrate to rear *B. sowerbyi*, at the Núcleo de Ecotoxicologia e Ecologia Aplicada (NEEA) of the Universidade de São Paulo (São Carlos, Brazil).

The ATS was prepared in accordance with the instructions of the protocol EPS 1/RM/30 of Environment Canada (1995), using sand (Brazilian Standard Sand, NBR 7214/82), white clay (kaolin U.S.P. 26, Synth<sup>®</sup>) and coconut fiber powder (Golden Mix T-80; Amafibra<sup>®</sup>), instead of peat extract as organic matter (see Report EPS 1/RM/30 of Environment Canada, 1995 for more details). The ATS was stored at 4°C until test set-up (storage time did not exceed 7 days). In Table 1, the compositions of the two sediments are displayed. The NS are classified as medium to coarse sand and the ATS are muddy sands. We aimed to maintain the characteristics of the artificial sediment recommended by Environment Canada (1995), formulating a sediment with an organic matter content between 2 and 10%. The percentage of phosphorus was also different between the sediments since coconut fiber has a considerable phosphorus content (Abad *et al.*, 2002).

The bioassays followed the protocol developed by Lobo & Alves (2011). In the bioassays, we used *B. sowerbyi* originating from the cultures kept at NEEA. Two bioassays were run to compare the sediments: one with adult organisms (10 weeks old), for 28 days, and another with juveniles (one week old) for 14 days. In the first test, survival, reproduction, and growth rates were assessed as endpoints, while in the second only survival was considered. Sixty healthy organisms (with normal movement and the adults with the presence of egg's in the ovisac) were selected for each bioassay (30 individuals per sediment treatment). Six replicates, containing five individuals per 250mL beaker each, were prepared for the respective sediments. Each beaker was filled with 100mL sediment, 100mL reconstituted water, and 0.21g of Tetramin<sup>®</sup> as food supplement (food was provided only at the beginning of the experiments). The beakers were kept at 25 ± 1°C, under moderate aeration, with photoperiod of 12L:12D, for 28 days. The water level was adjusted weekly in order to compensate for the loss of water due to evaporation. At the end of the 28 days, the sediments from the beakers were washed in a 0.25mm mesh and analyzed on a light box to verify the

number of surviving adults, cocoons, eggs and juveniles. To determine the biomass, adults were kept for 4h in Petri dishes containing only reconstituted water, to empty their intestinal content (feces were removed by pipette to avoid coprophagy), and then weighed on an analytic scale with a precision of 0.1mg. The average daily growth rate ( $G_w\%$ ) was calculated according to Reynoldson (1987):  $G_w\% = (\ln W_2 - \ln W_1) \times 100t^{-1}$ , where  $W_1$  = initial biomass (mg);  $W_2$  = final biomass (mg); and  $t$  = time in days.

The pH (Micronal B374, São Paulo, Brazil), electrical conductivity (Thermo Orion M145, Beverly, USA), concentration of dissolved oxygen (Yellow Springs YSI-55, Ohio, USA) and total ammonia concentrations (spectrophotometry method, APHA 1995) were measured every other day. All the equipment used were calibrated according to the manufacturer's instructions. Statistical analyses were undertaken with the free software R (R Core Team, 2013), using the Student *t* test ( $p = 0.05$ ) to compare the two treatments.

## RESULTS

Physical and chemical parameters of the water were very similar between the treatments (pH = 7.79 ± 0.26 and 7.79 ± 0.10; OD = 6.94 ± 0.27 and 6.64 ± 0.31 mg L<sup>-1</sup>; ammonia = 0.75 ± 0.10 and 0.84 ± 0.09 mg L<sup>-1</sup>, for NS and ATS respectively) with the exception of conductivity (NS: 254.19 ± 34.07 and ATS: 404.14 ± 34.70 μS cm<sup>-1</sup>,  $p < 0.05$ ). All parameters remained within the tolerance limits of *B. sowerbyi* (Chapman *et al.*, 1982) and in accordance with the standards for chronic bioassays established for oligochaetes (ASTM, 2005).

At the end of the 28-day test period, 100% and 70% survival rates were recorded for NS and ATS, respectively. The organisms kept in NS presented increases in their biomasses, while those kept in ATS showed biomass reduction, resulting in a significant difference between the final weights, demonstrated by the  $G_w\%$  (Table 2). Only the average number of eggs per cocoon and the initial weights did not differ significantly between organisms exposed to NS and ATS.

In the second experiment, the survival rate after 14 days of juveniles kept in NS was 90.74%, contrasting with only 3.7% of those kept in ATS.

**Table 1** – Characteristics of Natural Sediment (NS) and Artificial Tropical Sediment (ATS) used in bioassays with *Branchiura sowerbyi* (all values refer to dry weight).

Composition	NS <sup>a</sup>	ATS
Grain size		
Coarse sand (0,5 – 0,25mm)	46.28%	0%
Medium sand (0,25 – 0,105mm)	48.43%	75%
Fine sand (0,105 – 0,053mm)	4.51%	0%
Silte and clay (< 0,053mm)	0.78%	21%
Organic Matter (LOI <sup>b</sup> )	0.75%	4%
Total Nitrogen	0.09%	0.09%
Total Phosphorus	0.204 μg g <sup>-1</sup>	0.657 μg g <sup>-1</sup>

<sup>a</sup> - Values after sieving (0.5mm of mesh). <sup>b</sup> - Lost of Ignition

**Table 2** – Biological parameters observed in bioassays with adult *Branchiura sowerbyi*, kept in Natural Sediment (NS) and Artificial Tropical Sediment (ATS).

Biological Parameters	NS	ATS	Statistics
Survival (%)	100 ± 0.00	70 ± 11.55	*
Average growth rate (%)	0.35 ± 0.24	-0.44 ± 0.24	**
Initial average weight (mg)	23.79 ± 0.56	25.56 ± 1.97	
Final average weight (mg)	26.34 ± 1.41	21.43 ± 1.68	*
Average number of cocoons	19.00 ± 6.24	2 ± 0.001	*
Average number of eggs per cocoon	1.74 ± 0.09	1.33 ± 0.29	
Average of cocoons per adult per day	0.14 ± 0.04	0.02 ± 0.001	*

\* -  $p < 0.05$ ; \*\* -  $p < 0.001$ .

## DISCUSSION

There are no records on the use of artificial sediments for the rearing of *B. sowerbyi*, however, although they have frequently been used in ecotoxicological tests with other benthic organisms (Kembel *et al.*, 1999; Goedkoop & Peterson, 2003; Sloomweg *et al.*, 2010).

For the organisms kept in NS, the Gw% found for *B. sowerbyi* was very similar to that found for this species in other studies (values close to 0.5%; Marchese & Brinkhurst, 1996; Lobo & Alves, 2011). Concerning individual fertility, Ducrot *et al.* (2007) and Lobo & Alves (2011) recorded postures of 0.16 and 0.11 cocoons day<sup>-1</sup>, respectively, with an average of 2.2 ± 0.4 and 1.73 ± 0.57 eggs cocoon<sup>-1</sup>; values close to those found in the present study. These results, added to a survival rate of 100%, demonstrate that the NS used can be considered suitable for rearing *B. sowerbyi*.

Concerning the results obtained for ATS, all endpoints analyzed were worse than observed for NS. Organism survival after 28 days was 70%, which was lower than 90%, i.e. the limit recommended by ASTM (2005) for *T. tubifex* tests.

Following Arrate *et al.* (2004), increased ammonia concentration originating from organic material decomposition and food supplements can cause negative side effects, including the death of adults. However, in the present work, the concentration of total ammonia was below 1mg L<sup>-1</sup>, which is not considered toxic for many benthic invertebrates (Monda *et al.*, 1995).

Despite the analyses of several parameters we conducted, it was not possible to determine the cause of the high mortality of the organisms in the artificial tropical sediment (ATS). In accordance with Garcia (2004), the coconut fiber powder used to formulate artificial soils does not cause negative side effects in terrestrial oligochaetes, but from our results it thus appears not to be a viable option for freshwater oligochaetes.

Due the higher concentration of organic matter in the ATS (4.00%, against 0.75% in NS), we expected to find better fitness and reproduction for organisms in this treatment. One of our hypothesis for this opposite result is that the coconut fibers probably decompose less quickly than the peat extract, demanding a longer period of hydration and decomposition by bacteria. Garcia (2004) left the coconut fiber powder decompose for 30 days before use in the soils, a week more than in the present study, where the time of decomposition was 21 days (in accordance with Environment Canada, 1995). This longer period could provide greater food availability for the oligochaetes, once it will allow the establishment of a higher density of bacteria (primary food source for oligochaetes, Brinkhurst & Chua, 1969; Wavre & Brinkhurst, 1971) and consequently a higher degree of mineralization of the organic matter, with the breaking of the coconut fiber into simpler compounds that could be assimilated by the gut of the oligochaetes (subject for future studies). Rodriguez *et al.* (2001) observed a high content of vegetal fibers from peat components in fecal pellets of *T. tubifex* maintained

in artificial sediment, suggesting that this fiber was neither digested nor assimilated by the oligochaetes.

Another hypothesis is that the coconut fiber may release some toxic substance during its decomposition. According to Rosa *et al.* (2001), the coconut fiber presents a high concentration of tannin, a toxic substance that could adversely affect aquatic organisms (Hedmark & Scholz, 2008). Even with the wash process to obtain the fibers, reported by the manufacturer, the toxic substances could remain and reach lethal levels to the oligochaetes and/or to the bacteria, food source for *B. sowerbyi*. This hypothesis should be tested in future works.

From our results, we may conclude that the ATS, in the composition previously successfully used for terrestrial oligochaete tests, is not a viable option for tests with the aquatic oligochaete *B. sowerbyi*, given that it caused a high mortality rate in adults and juveniles when compared with the NS tested. However, new studies are necessary to assess why ATS affected the species during the bioassays and what alternative ATS could be used for aquatic organisms.

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