Assessment of benthic macroinvertebrates at Nile tilapia production using artificial substrate samplers

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(With 4 figures)

Abstract

Biomonitoring is a cheap and effective tool for evaluation of water quality, and infer on the balance of aquatic ecosystems. The benthic macroinvertebrates are bioindicators sensitive to environmental changes, and can assist in detecting and preventing impacts such as organic enrichment and imbalance in the food chain. We compared the structure of benthic communities on artificial substrate samplers located in places near and far from net cages for production of Nile tilapia (*Oreochromis niloticus*). Samplers were manufactured with nylon net, using substrates such as crushed stone, gravel, loofah and cattail leaves. Samples were collected after 30 days of colonization, rinsed and then the specimens were identified and quantified. The following metrics were calculated: richness of Operational Taxonomic Units, Margalef richness, abundance of individuals, Shannon index and evenness index. The macrobenthic community structure was strongly modified according to the proximity of the net cages. Metrics showed significant differences ($p < 0.05$) between near and distant sites, for both periods (dry and rainy seasons). The position of the samplers significantly affected the structure of macroinvertebrate community, as near sites showed higher values for the community metrics, such as richness and diversity. Near sites presented a larger number of individuals, observed both in the dry and rainy seasons, with a predominance of Chironomidae (Diptera) in the dry season and Tubificidae (Oligochaeta) in the rainy season.

Keywords: biomonitoring, aquaculture, *Oreochromis niloticus*, Brazil, artificial substrate sampler.

Avaliação da comunidade de macroinvertebrados bentônicos em produção de tilápia-do-Nilo com uso de coletores com substrato artificial

Resumo

O biomonitoramento é uma ferramenta com custo relativamente baixo e eficaz para avaliação da qualidade da água, além de inferir sobre o equilíbrio dos ecossistemas aquáticos. Os macroinvertebrados bentônicos são bioindicadores sensíveis às mudanças ambientais, e podem auxiliar na detecção e prevenção de impactos como o enriquecimento orgânico e o desequilíbrio da cadeia alimentar. Como objetivo, foi comparada a estrutura das comunidades bentônicas em coletores com substrato artificial situados em locais próximos e distantes de tanques rede para produção de tilápia do Nilo (*Oreochromis niloticus)*. Os coletores foram fabricados com rede de náilon, utilizando os substratos brita, cascalho, bucha vegetal e folhas de taboa. Foram realizadas coletas após 30 dias de colonização. As amostras foram lavadas e os espécimes identificados e quantificados. As métricas calculadas foram: riqueza de Unidades Taxonômicas Operacionais, riqueza de Margalef, abundância de indivíduos, índice de Shannon e índice de Pielou. A estrutura da comunidade macrobentônica foi fortemente modificada de acordo com a proximidade dos tanques-rede. As métricas apresentaram diferenças significativas ($p < 0,05$) entre os locais próximos e distantes, em ambos os períodos (estações seca e chuvosa). A posição dos amostradores afetou significativamente a estrutura da comunidade de macroinvertebrados, na medida em que locais próximos dos tanques rede apresentaram valores mais elevados para as métricas de comunidade, como riqueza e diversidade.Amostras próximas aos tanques rede apresentaram um maior número de indivíduos em ambas as estações do ano, com predomínio de Chironomidae (Diptera) na estação seca e Tubificidae (Oligochaeta) na estação chuvosa.

Palavras-chave: biomonitoramento, aquicultura, *Oreochromis niloticus*, Brasil, substrato artificial.

Braz. J. Biol., 2016, vol. 76, no. 3, pp. 735-742

735

1. Introduction

Aquaculture is the farming activity that had the highest rate of growth in recent years when compared to other categories of livestock such as pig, cow, and chicken. Besides that, it stands as one of the best alternatives to meet the growing demand for aquatic food (FAO, 2010). As the demand for fish increases, the risk of deterioration of water quality resulting from intensive production also rises. When proper management is lacking, the deterioration of water quality resulting from intensive farming can compromise the stability of the ecosystem causing disturbances to the physical and chemical environments, and changes in the structure and dynamics of biological communities, manifested by marked reduction in aquatic biodiversity. On the other hand, sometimes they can also result in increased biological richness.

Biomonitoring is widely used to evaluate water quality of rivers, lakes and ponds. However, the use of this technology to assess water quality in aquaculture is still incipient in Brazil. Benthic macroinvertebrates stand out as an important component of the substrates of rivers and lakes, playing a key role in the dynamics of nutrients, transformation of matter, and flow of energy (Silveira and Queiroz, 2006), and according to Matsumura-Tundisi (1999), aquatic invertebrates are those that best respond to changing environmental conditions.

Asaduzzaman et al. (2009) studied the effect of the addition of tilapia and substrates for periphyton development on ecology, production and economic performance in the rates of C/N, in a freshwater prawn (*Macrobrachium rosenbergii*) farm. The authors found that the addition of tilapia and periphyton substrates benefited shrimp production through: reduction of toxic inorganic nitrogen in the water, increasing the availability of plankton, periphyton, benthic macroinvertebrates and microorganisms, reducing the demand for additional food by tilapia, which improved survival and production, and brought economic benefits.

For the effluents produced by aquaculture to meet legal standards, especially the CONAMA Resolution 430/11 (Brasil, 2011), it is essential to adopt and implement Best Management Practices (BPMs). Nevertheless, to comply with more environmental friendly practices, it is essential that the management of water quality can be conducted in a simple, inexpensive, quick way that offers easy access to producers, with the use of low cost equipment (Queiroz et al., 2007).

The collection of benthic organisms can be made with the use of sediment samplers such as the Ekman grab, or samplers that simulate the natural environment of bottom sediments, such as artificial substrate samplers. Artificial substrate samplers have the advantages of standardizing sampling, reducing variability and processing time, and cutting operating costs, because they are easy to make and handle, and are made with cheap materials (Gibbons et al., 1993). On the other hand, the disadvantages are: it excludes certain organisms that cannot colonize it; when placed in public sites, it is subjected to vandalism; and two trips are needed: one for installation and another for removal, which can endear a draft long-term monitoring.

Artificial substrates are mostly used in marine ecology studies (Guerra-Castro and Cruz-Motta, 2014), but freshwater studies are also found in rivers (Volkmer-Ribeiro et al., 1984), coastal lagoons (Henriques-de-Oliveira et al., 2007), inland lagoons (Strixino and Trivinho-Strixino, 2006), small dam reservoirs in the savanna ecosystem in the State of Paraíba (Souza et al., 2008) and dams (Kipper et al., 2011). However, in aquaculture there are few studies that employ biomonitoring with the use of benthic organisms.

The objective of this study was to know the composition of the assembly of benthic macroinvertebrates in sites directly and indirectly influenced by farming cages of Nile tilapia (*Oreochromis niloticus*), in Rio do Corvo, located at Rosana Reservoir, Paraná State, Brazil.

2. Material and Methods

The Rosana Reservoir (Figure 1), located on the Paranapanema River basin, was closed in November 1986 and has a flooded area of 220 km². The axis of the dam is located between the municipalities of Diamante do Norte (PR) and
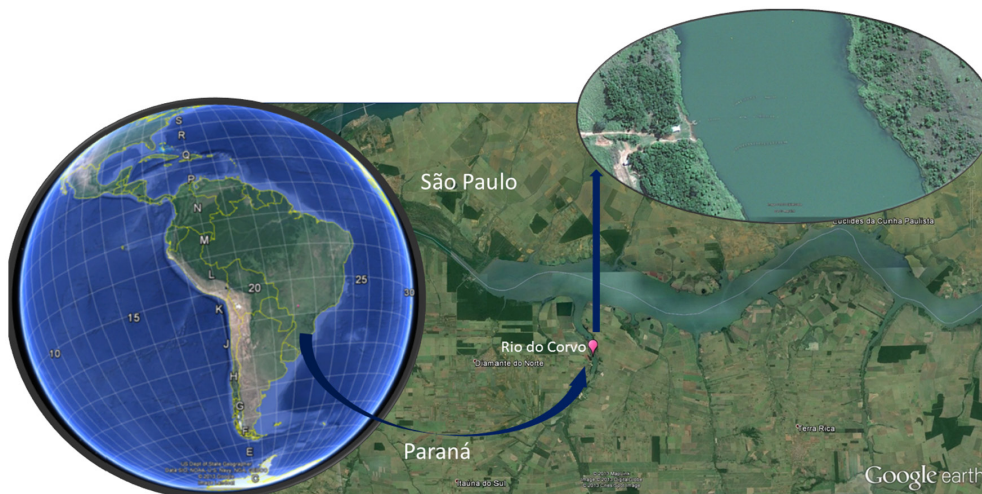

Figure 1. Study area: Rosana reservoir (Paranapanema River watershed) in Paraná State, Brazil, with sampling station in the Corvo River. Source: Google Maps (2015).

736

Braz. J. Biol., 2016, vol. 76, no. 3, pp. 735-742

Primavera (SP), 20 km from its mouth, the Paraná River. Two field campaigns at May (dry season) and November (rainy season) in 2010 were conducted at the experimental station of the Maringá State University (UEM), located in the Corvo River, where Nile tilapias are grown in cages. The geographic coordinates are: 22°39'28.03"S and 52°46'51.06"O (tilapia production); and 22°39'24".4S and 52°46'50".1W (junction between Corvo river and Paranapanema river). According to the climatic classification of Köeppen, the Northwest of Paraná has climate Cfa - mesothermal, humid, without dry season and hot summers. The average temperature of the coldest month is below 18 °C and the average temperature warmest month is above 22 °C. (Maack, 1968).

The artificial substrate samplers were manufactured with square plastic nets of five kg (32.5 cm × 35.5 cm), using as substrate 15 g of cattail, 50 g of loofah, and 450 g of crushed stone and gravel, all together in one plastic bag. A nylon thread was tied to each sampler; they were dropped into the water to reach the bottom, and the upper end of the threads were tied to a signaling floater. We evaluated three sites upstream from tilapia cages, three sites near the cages and three sites downstream from aquaculture production (Figure 2). The distance of 300m from the production system is due to the buoys that limited our experimental area, from the right, left and front of the cages. Moreover, it is a safe distance as a reference point (without fish production). As the reservoir has a strong current flow close to the tilapia production area, artificial samplers were fixed at the buoys. The average depth of sites near the cages was 7.87 m, and for distant sites the average depth was 6.23 m. At each site three samples were evaluated, after 30 days of colonization, totaling

three samples per site. The physico-chemical parameters were measured with a Horiba U10® multiparameter probe. Samplers were removed from the reservoir after having remained submersed for 30 days, totaling 48 (16 sites in treplicate) collectors in each field campaign. After their removal, samplers were transported in plastic bags with local water to the laboratory, where they were washed under running water in steel sieves of 500 μm and then stored in containers with 80% ethanol. Benthic organisms were identified at family or subfamily levels, and quantified with the aid of a stereomicroscope and taxonomic keys (Fernández and Domínguez, 2001; Mugnai et al., 2010). We calculated the following metrics for macrobenthic communities: *taxa* richness as Operational Taxonomic Units (S_{OTUs}), composed by the sum of number of identified *taxa*; total number of individuals, relative abundance (%), expressed by the number of individuals of each taxon divided by the total number of individuals in the sample; Shannon diversity index, Pielou equitability index and Margalef richness index

Metrics averages were compared to assess the difference between near and far cage sites, using SAS/STAT 9.3 (SAS Institute, 2011). Randomized tests were also carried out (Manly, 2007) to evaluate the hypothesis that community metrics were different between near and far cage sites. As the number of samplers near the tank network was different from the number of samplers placed farther away, including loss of some sampler units, and due to the fact that diversity indices are dependent on the sample size, it was not possible a direct comparison of results between sites. In the case of community sampled in the dry season, three samplers were placed in ten locations per site in near
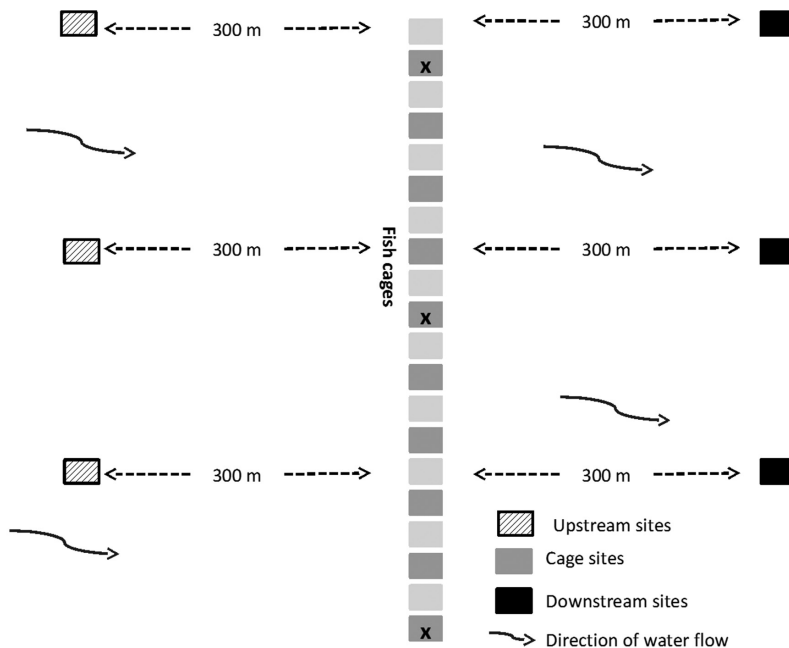

Figure 2. Schematic diagram of sampling sites distribution in the Corvo River, Paraná State, Brazil.

to the tanks and six samplers upstream or downstream of the tanks. After the colonization period, 26 samplers remained near the tanks and 12 samplers remained distant to the tanks. To enable comparison between the indices, we used the following procedure: for sites with less number of samplers (12) (distant from cages) the value of the indices was normally calculated. For sites with greater number of samples we used the bootstrap technique, using SAS software to extract 10,000 random samples of size 12, with replacement of the original population size 26 samplers. Indices of diversity and richness were calculated for each sample. It was obtained the difference between the ratios for the two sites: near or far, resulting in 10,000 values for the difference between them. The null hypothesis was that the position of the samples relative to the tanks did not affect the results of the indices. If it should prove true, the expected average difference would be zero. Thus, from the 10.000 values obtained of the differences, we applied a t-test to test whether or not the average was equal to zero.

3. Results

3.1. Physico-chemical parameters

Table 1 shows the mean value of physicochemical parameters in two field campaigns. Except for temperature, the measured parameters did not differed between the two sampled months.

3.2. Macroinvertebrate assessment for 30 days of colonization

In the dry season, when comparing data of near and distant sites as concerns the relative abundance of macroinvertebrate groups, it was observed a predominance of dipteran subfamily Chironominae (44.16%) in distant sites, and 38.85% in near sites (Figure 3). Considering the Chironomidae family, the most abundant subfamily was Chironominae, followed by Tanypodinae and Orthocladinae subfamilies. Class Oligochaeta was the third most frequent and most representative in numbers, in sites near the cages. The gastropods of Ancylidae also had expressive abundance with 37.9% in near sites, and 20.57% in distant sites.

During the rainy season (November), a high incidence of the subclass Oligochaeta (families Tubificidae and Glossiphonidae) was observed in sites near the cages, with about 78.31% and 15.6%, respectively (Figure 4). The Ostracoda were well represented in distant sites, with 33.13%.

Table 2 present the macroinvertebrate fauna observed in both field campaigns. Sites near net cages showed a greater total number of individuals than distant sites. In the rainy season, Subclass Oligochaeta was the most abundant taxon, with the presence of family Tubificidae representing 76,3% of macroinvertebrate community, unlike
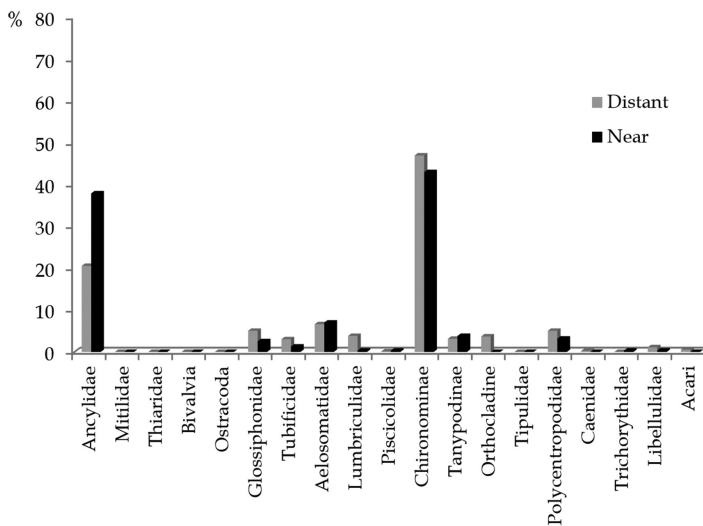

Figure 3. Relative abundance of benthic macroinvertebrates in dry season, for sites located near and distant from netcages, after 30 days of colonization in the Corvo River.

Table 1. Mean and standard deviation values for physicochemical parameters in the Corvo River in 2010.

Parameter	1st sampling (May –dry season)	2nd sampling (November – rainy season)
Temperature (°C)	23.37 ± 0.09	28.26 ± 0.06
Dissolved Oxygen (mg/L)	8.07 ± 0.05	8.36 ± 0.15
pH	7.5 ± 0.1	7.37 ± 0.15
Electric Conductivity (mS/cm)	43.8 ± 0.19	46.74 ± 0.08

738

Braz. J. Biol., 2016, vol. 76, no. 3, pp. 735-742

Table 2. Composition and abundance of macroinvertebrate (operational taxonomic units) present at sites near and far from net cages in the Corvo River after 30 days of colonization.

|	Number of individuals			
	Near Sites (10)		Distant sites (6)	
	Dry season	Rainy season	Dry season	Rainy season
Ancylidae	183	98	119	23
Mytilidae	0	8	0	0
Thiaridae	0	30	0	19
Bivalvia NI*	0	26	0	1
Ostracoda	0	0	0	221
Glossiphonidae	45	388	8	98
Tubificidae	27	1899	4	250
Aelosomatidae	59	0	22	0
Lumbriculidae	34	0	1	0
Piscicolidae	1	3	1	0
Chironomidae	479	28	147	65
Tipulidae	0	6	0	8
Polycentropodidae	45	7	10	3
Caenidae	2	0	0	0
Trichorythidae	0	0	1	0
Libellulidae	10	0	1	0
Acari	5	0	0	0
TOTAL	**890**	**2489**	**314**	**688**

*NI - non identified.
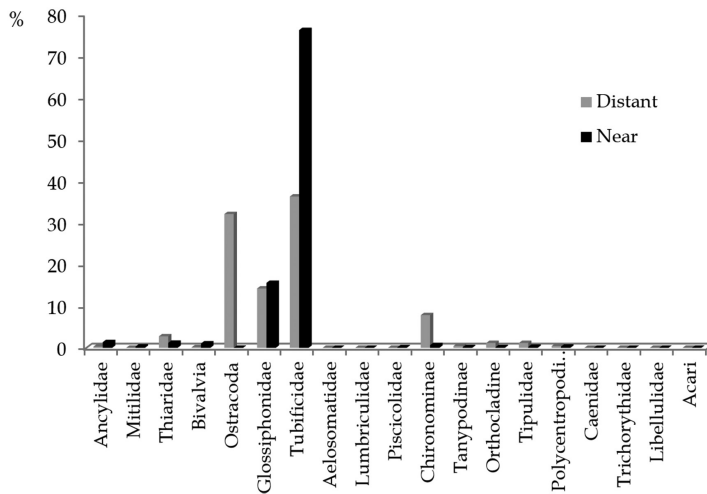
Figure 4. Relative abundance of macroinvertebrate *taxa* in rainy season, for sites located far from the netcages, after 30 days of colonization in the Corvo River.

the observed in the dry season period, when Aelosomatidae and Lumbriculidae families were also found (Table 2).

After Oligocheta, Chironomidae was the second major group (for all subfamilies) followed by Ancylidae and Polycentropodidae (Trichoptera).

3.3. Metrics comparison

Significant differences between near and far sites were observed for all the community metrics. In all cases, the null hypothesis was rejected with high significance (p <0.0001).

That is, the position of the samplers significantly affected the structure of macroinvertebrate community (Table 3). Furthermore, near sites showed higher values for these measures.

Similarly to what happened to the dry season, significant differences between near and far sites were observed for all the diversity and richness indices in the rainy season. In all cases, the null hypothesis was rejected with high significance (p < 0.0001). That is, the position of the samplers significantly affected the structure of macroinvertebrate

Table 3. Margalef Richness ($S_{Margalef}$), Shannon diversity index (H') and Pielou evenness index (J) for macrobenthic community in dry season at the Corvo river and difference between indices (near – distant samplers).

Indices	Near	Distant	Difference
Shannon (H')	1.780	1.510	0.270**
Pielou eveness (J)	0.705	0.608	0.097**
Richness ($S_{Margalef}$)	1.938	1.913	0.025**

** p < 0.0001.

Table 4. Margalef Richness ($S_{Margalef}$), Shannon diversity index (H') and Pielou evenness index (J) for macrobenthic community in rainy season at the Corvo river and difference between indices (near – distant samplers).

Indices	Near	Distant	Difference
Shannon (H')	0.776	0.894	−0.118**
Pielou eveness (J)	0.312	0.467	−0.155**
Richness ($S_{Margalef}$)	1.417	1.835	−0.418**

**p < 0.0001.

community. However, distant sites showed higher values for these measures (Table 4).

4. Discussion

Physicochemical parameters may influence both qualitatively and quantitatively a macrobenthic community. Changes in water quality resulting from processes of natural evolution or anthropogenic activities may cause disruption and are manifested by changes in the structure and dynamics of biological communities, as well as by the marked reduction in aquatic biodiversity. The presence of feces and feed scraps from aquaculture can increase the amount of nutrients in the sediment, mainly nitrogen and phosphorus. This contributes to the increase of the taxonomic richness and abundance of benthic macroinvertebrates. However, when the organic pollution is high, the benthic biodiversity decreases, leaving only the organisms tolerant to low concentration of dissolved oxygen in water.

Molozzi et al. (2011) observed higher taxonomic richness at the least impacted reservoir, where Chaoboridae (Diptera) represented the most abundant taxon. On the other hand, at the most impacted reservoir (urban impacts) the dominance was of *Melanoides tuberculatus* (Gastropoda: Thiaridae). In this study, although we have not assessed the extent of the reservoir eutrophication, the Chironomidae family was numerically important in the dry season, contributing with 52% of the fauna. In rainy season, the Chironomid larvae and pupa responded for only 2.8%.

The present study showed that the relative abundance of macrobenthic *taxa* is different depending on the season, as we can note by greater number of Chironomidae in the dry season (May), and Tubificidae in spring (November). High densities of Oligochaeta and Chironomidae are indicative of high levels of organic matter (Lang, 1998; Hepp and Santos, 2009), as these benthic invertebrates are more tolerant to pollution (Bubinas and Jagminiené, 2001). Our data showed that these groups presented greater relative abundances in sites near the cages, with an increased total number of individuals. However, in distant sites, due to a lower relative abundance of groups considered tolerant to organic enrichment, indices for diversity and evenness indices were higher. This was reinforced by statistical analyses that proved the difference between macrobenthic communities in distant and near sites, at least for the dry period.

Jorcin and Nogueira (2008) evaluated the composition, specific richness and abundance of benthic macroinvertebrates in eight reservoirs along the Paranapanema River (São Paulo State, Brazil), as well as in its major tributaries (Taquari, Pardo and Tibagi) and in the river mouth into the Paraná River, and they also noted the predominance of Diptera (family Chironomidae). These authors also observed a relationship between abiotic factors (increased trophic, less depth) with the further development of the benthic fauna in the study area.

Other studies have reported the prevalence of the Chironomidae group in different types of environments, as reported by Lucca et al. (2010) in a tropical lake (Northeast region, Brazil), and Reis et al. (2012), who evaluated 45 lotic sites in the Peixe Angical reservoir (North region, Brazil) and observed a greater number of genera in the subfamily Chironominae, although the subfamily Orthocladiinae (considered less tolerant to organic pollution) had been prevalent (genus *Cricotopus* was the most abundant one).

We also found that the position of the samplers (samplers located near and far from the tanks) strongly influenced the structure of macroinvertebrate community, according to diversity and richness indices. And this occurred independently of season.

However, some facts should be highlighted: for dry season, diversity and richness indices presented higher values than the wet season. So, this indicates that the community as a whole was more balanced in the dry season, with less dominance of some *taxa*. In fact, as observed by relative abundances of *taxa*, in May (dry season) the most frequent group was Chironominae (Diptera), with about 45% of total number of individuals; and for wet season, the Tubificidae (Oligochaeta) represented about 80% of all the macroinvertebrate community.

Ogbeibu and Oribhabor (2002) also observed the dominance of Oligochaeta (Family Naididae) in a reservoir.

740

Braz. J. Biol., 2016, vol. 76, no. 3, pp. 735-742

In that case, Naidid oligochaetes, the dominant group in the reservoir, showed a temporal trend that was the reverse of that of Chironomidae. They were generally more prominent in the rainy season than in the dry season. Although being a different Oligochaeta family, this trend was also observed in the present study, as the Tubificidae were more abundant at the rainy season (November). Despite the recognized relationship between Tubificidae and its tolerance to high concentrations of organic matter, it cannot be said that this occurred in this study. In the present study, although the Tubificidae have been more abundant in places close to the cages (where rests of fish feed and faeces are concentrated), the dissolved oxygen values were high in both periods. The same is truth for electric conductivity, which, in case of a large resuspension, would have to show marked differences between the dry and rainy season. The greater values of diversity and richness indices in the dry season could also be explained by the stability of the water column.

Menezes and Beyruth (2003) analyzed the impacts of fish tanks on benthic communities, and observed greater percentages of Oligochaeta in the sediment close to the tanks, probably due to the increased levels of organic matter and nutrients from the fish excreta. However, the authors argue that although the increase of organic matter near the tanks network has contributed to the increase of the benthic fauna, this did not result in changes regarding water quality. So, a positive environmental impact would happen, as the increase in the benthic fauna would benefit the environment in many ways, for example, expanding the area of food fish in the natural environment. The same thing was observed by Ansah et al. (2012) for effluents of fish farms in streams in Gana. In that case, the authors classified this as a possible subsidy-stress response of macroinvertebrates.

Other studies correlate the change in macrobenthic communities structure with fish production. Camargo et al. (2011) evaluated water quality before and after the installation of a trout farm in a river in the Spanish peninsula. Results indicated an increase in the percentage of Oligochaeta and Chironomidae, and a decrease in the numbers of EPT (Ephemeroptera, Plecoptera, and Trichoptera) due to the increase in the concentration of nutrients (N and P) from the effluents of fish farming. In the present study, organic nutrients were not measured, so, we cannot state that the presence of tolerant organisms to organic pollution is due to aquaculture.

The use of samplers with artificial substrate will not only house colonizations by benthic organisms, but also by the periphyton, since the materials used in the sampler (cattail leaves, loofah, crushed stone and gravel) serve as a basis for the development of periphytic algae, which indirectly also benefits herbivorous animals. This contributes to increase local biodiversity. So, it is possible that the artificial substrate sampler used in this work has contributed to enhance the diversity of benthic fauna as a result of the accumulation of feces and feed remnants in the bottom sediment, and also allowing for

opportunistic species (tolerant to increased organic matter) to increase their abundance at sites near the net cages.

We highlight that there was an important difference between communities colonizing sites near and distant from the cages, and this difference was related to the dominance of groups widely known as tolerant to organic enrichment in the sites near fish cages. The seasons (dry and rainy periods) also determined the structure of macrobenthic invertebrates in artificial substrate samplers. However, long term studies (taking one year at least) and studies that can monitor physicochemical parameters more frequently are necessary to warrant a better understanding of the association between bioindicators and water quality in fish production systems. After this, the proposition of biotic indices developed for pisciculture in reservoirs would be of great value to allow monitoring of the activity and its development based on Best Management Practices.

Acknowledgements

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742

Braz. J. Biol., 2016, vol. 76, no. 3, pp. 735-742