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# Community assessment of benthic macroinvertebrates in fishponds in the presence and absence of fish

## Avaliação da comunidade de macroinvertebrados em viveiros escavados na presença e ausência de peixes

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**Abstract: Aim:** In the present study, the structure of the benthic invertebrates in ponds in the presence and absence of Nile tilapia was evaluated. **Methods:** The benthic macroinvertebrates and physicochemical parameters were analyzed for a period of eight weeks, every 15 days, in two ponds (one with fish and the other without fish). Benthic invertebrates were sampled with artificial substrate samplers, made with cheap and common materials, like expanded clay, loofah leaves and gravel. **Results:** With the exception of turbidity, the other physical and chemical variables of water quality were within the ideal limits for tilapia farming. Despite that, there was a significant difference for all physical-chemical parameters monitored, and the average turbidity was much higher in pond with fish. The use of artificial substrate samplers for biomonitoring was effective in the colonization of several taxa in a short period of time (15 days). For benthic community metrics, organism richness, diversity and evenness were significantly higher in the pond without fish. On the other hand, the dominance of Chironomidae and the abundance of Glossiphonidae were much higher in the pond with fish. For Chironomidae, this family was dominant in both treatments, but reached a higher frequency in the pond with fish (98.49%) than in pond without fish (92.87%). The presence of sensitive families, like Leptoceridae (Order Trichoptera), was higher in the pond with no fish, as well as the metric of Ephemeroptera, Plecoptera and Trichoptera families (% EPT). **Conclusions:** The presence of fish altered the macrobenthic community, since metrics of richness and diversity presented lower values in pond with fish.

**Keywords**: artificial substrate; biomonitoring; Brazil; fishponds; Nile tilapia.

**Resumo: Objetivo:** No presente estudo, avaliou-se a estrutura de invertebrados bentônicos em viveiros escavados na presença e ausência de tilápia do Nilo. **Métodos:** Os macroinvertebrados bentônicos e os parâmetros físico-químicos foram analisados por um período de oito semanas, a cada 15 dias, em dois viveiros (um com peixes e outro sem peixes). Os invertebrados bentônicos foram coletados com amostradores de substrato artificial, feitos com materiais baratos e comuns, tais como argila expandida, folhas de taboa e brita. **Resultados:** Com exceção da turbidez, as demais variáveis físicas e químicas da qualidade da água estiveram dentro dos limites ideais para o cultivo de tilápia. Apesar disso, houve diferença significativa para todos os parâmetros físico-químicos monitorados, sendo que a turbidez média foi bem maior no tanque com peixes. Para as métricas dos macroinvertebrados bentônicos, riqueza, diversidade e uniformidade de organismos foram significativamente maiores no tanque sem peixes. Por outro lado, a dominância de Chironomidae e a abundância de Glossiphonidae foram muito maiores no tanque com peixes.

Para Chironomidae, esta família foi dominante em ambos os tratamentos, mas atingiu maior frequência no viveiro com peixes (98,49%) do que no viveiro sem peixes (92,87%). A presença de famílias sensíveis, como Leptoceridae (Ordem Trichoptera), foi maior no viveiro sem peixes, assim como a métrica das famílias Ephemeroptera, Plecoptera e Trichoptera (% EPT). **Conclusões:** O uso de amostradores de substrato artificial para biomonitoramento mostrou-se eficaz na colonização de diversos táxons em um curto período de tempo (15 dias). Por fim, a presença de peixes alterou a comunidade macrobentônica, já que as métricas de riqueza e diversidade apresentaram valores mais baixos no viveiro escavado com peixes.

**Palavras-chave:** substrato artificial; biomonitoramento; Brasil; viveiros; tilápia do Nilo.

## 1. Introduction

Ponds are still the main production sites for freshwater fish in Brazil (Kubitza, 2012; FAO, 2022), although production in net cages have also grown in recent years (Valenti et al., 2021). Fishponds have some advantages, such as greater control over water quality, availability of complementary natural foods (phytoplankton) and greater security against theft (Trombeta et al., 2017). In 2020, Brazilian aquaculture production was almost 802,930 tons, and the production of Nile tilapia (*Oreochromis niloticus* Linnaeus,1758) increased by 12,5%, reaching 486,185 tons (PEIXE BR, 2021). While aquaculture puts less pressure on natural fish stocks, the rapid growth of the activity raises concerns about its sustainability. Uneaten food and fish faeces can accumulate in the bottom sediment, increasing the concentration of organic matter and making the environment more anaerobic (Boyd et al., 2020), and promote the growth of diatoms and heterotrophic/mixotrophic dinoflagellate (Yoonja et al., 2021). Thus, it is important to adopt Best Management Practices (BMP), which involve nutritional aspects of fish, control of population density, monitoring of water and sediment quality, among other factors (FAO, 1995; Queiroz & Rotta, 2016).

The classic physical-chemical analysis of water quality has a limitation in terms of detecting long-term impacts, in addition to not considering important ecosystem compartments, such as the bottom sediment and the surroundings. The sediment of lentic aquatic ecosystems is generally the compartment that accumulates pollutants in greater quantity, and the ultimate repository of anthropogenic contaminants (Moura e Silva et al., 2021). Benthic macroinvertebrates are widely used in water quality assessment and are recommended for environmental monitoring (Fonseca–Gessner & Guereschi, 2000), as they have several characteristics that make them excellent bioindicators (Hellawell, 1986; Rosenberg & Resh, 1993). The macrobenthic community has its composition determined by several factors, and one of the most important is the type of substrate.

The use of artificial substrates to sample benthic macroinvertebrate communities is usual and not new. It has the advantage of standardizing the samples, that is, as the substrate used is the same for all samples, the differences observed between the samplers will be attributed to the impacts observed, and not to factors related to the type of substrate found at the site (Loke et al., 2010). On the other hand, artificial substrates are selective, that is, only a few groups will be able to colonize them, due to the choice of the type of substrate placed, which are usually inorganic (stones of different sizes and brick, for example) (Modde & Drewes, 1990). Carvalho & Uieda (2004) used leaf bags when studying the diet of benthic macroinvertebrates in a tropical stream, in order to compare forested and deforested areas, at dry and rainy seasons. Braccia et al. (2014) investigated macroinvertebrate colonization rates related to algal accumulation in a perennial stream; they used artificial substrates to simulate bare patches after small-scale disturbances (flow generated disturbances).

The aim of this study was to compare the benthic macroinvertebrates community composition in fishponds with and without fish (Nile tilapia – *Oreochromis niloticus*).

## 2. Material and Methods

### *2.1. Study area*

The studied fish farm is located in the municipality of Itapira, in the State of São Paulo, Brazil (22°28’02” S; 46°47’45” W). The area has a subtropical or high-altitude tropical climate, according to the Köppen climate classification. Two markedly different seasons characterize this region: a dry season with low humidity (April to September) and a rainy season (October to March), which is common in a semi-humid tropical climate system (Nimer, 1989) and suitable for development of a variety of agricultural activities.

In the selected fish farms, all fishponds were disinfected with quicklime. To promote fertilization, tanned manure or other organic fertilizer was used in all ponds still without fish. This practice is a way of enriching the water that supplied the ponds in order to stimulate the growing plankton, mainly beneficial algae that will give it an emerald green color. This procedure was performed in all fishponds, therefore, the only variable that changed between ponds was the presence/absence of fish.

Two ponds with similar area and management practices were selected for the present study, being monitored for two months (from September to November 2016). Before the beginning of the study, both had fish, with the same age. So, to start the experiment, a total harvest was carried out in one of the ponds (all fish were removed). In pond with fish, the stocking density was 1.48 kg $m^2$ and the pond area was 1,200 $m^2$.

*2.2. Experimental design and laboratory processes*

Physical and chemical variables were monitored in the field during the morning with a multiparameter probe (Horiba® U52) for the following parameters: dissolved oxygen (DO) (mg $L^{-1}$); pH; electrical conductivity (EC) (μS $cm^{-1}$), water temperature (Temp) (°C), oxygen saturation (%DO); turbidity (NTU) and Total Dissolved Solids (TDS) (mg $L^{-1}$). This procedure was repeated six times (from 8th September to 11th November - sampling occasions 1 to 6) during a 60-day monitoring period.

The artificial substrate samplers used for colonization of benthic organisms were composed of nylon bags with a capacity of 5 kg (35 cm x 20 cm and 6.8 mm mesh), which were filled with 250 g of gravel, 250 g of expanded clay and 50 $cm^2$ of loofah. At the bottom of each tank, nine traps with artificial substrate were installed: three near the water inlet, three in the center of the reservoir (middle) and three near the water outlet. The bags remained in the water for 15 days, then they were collected and replaced by another set of artificial substrates, according to Henriques de Oliveira (2002), Queiroz et al. (2007) and Kochersberger et al. (2012). This procedure was repeated four times (from 23th September to 11th November - sampling occasions 2, 4, 5 and 6) during a 60-day monitoring period.

Every 15 days, during collection, the samples were fixed in 70% ethanol. In the laboratory, bags were washed in a 250 μm mesh sieve to remove the coarse material and perform the first screening of the organisms. After washing, the substrate was fixed again in 70% ethanol and screened for organisms in a transilluminated tray. The remaining material was observed under a stereomicroscope (80 x magnification) for final screening and taxonomic identification to the lowest possible taxonomic level or operational taxonomic units (OTUs) that correspond to the taxonomic category under study (species, genus, family, etc.), following identification keys (Mugnai et al., 2010; Simone, 2006; Trivinho-Strixino & Strixino, 1995).

*2.3. Data analysis*

The four sampling occasions were considered as blocks. As the water in the tanks was continuously renewed, the blocks were considered independent and not a repetition in time. Thus, the trial consisted of a randomized block experiment with two treatments and four replications. The position within each tank was considered as a covariate.

As the samplers were changed each time and the pond water was continuously renewed, the repeats were considered independent and not treated as a time series experiment. All samplers recovered after 15 days, from each pond, were grouped and considered as a single sample. Thus, the experiment was considered as randomized blocks with two treatments and four replications.

From the number of individuals of each OTU in each treatment and on each sampling date, the following biodiversity metrics were calculated: macroinvertebrate abundance; Shannon diversity index; Pielou evenness index; Simpson dominance index; and Hillsenhoff richness index.

We adapted the algorithms described by Montagna (2014) to calculate the metrics, using SAS program (SAS Institute INC, 2013). The four field campaigns were considered as replicates and used for analysis of variance (ANOVA) and to test means of all metrics. Where the F-test showed significance ($p < 0.10$), we applied the Tukey mean test to compare ponds with and without fish, using the procedure GLM (SAS Institute INC, 2013).

## 3. Results

During the study period, the water temperature, pH and total suspended solids for all fish farms were within the ideal range for tilapia indicated by Boyd & Tucker (1998). The pond with fish had a pH slightly more acidic than the pond without fish. The major difference between treatments was for turbidity values, where fishpond with fish showed values around five times higher than ponds without fish throughout the study period (Table 1).

As the adjusted models were all significant at 1% or 0.1%, the means comparison test (Tukey) was performed with the usual significance level of 5%. The tests, however, were only applied to compare treatments for variables in which the treatment was a cause of significant variation, which did not happen in the case of temperature and ORP. All physical-chemical parameters were significantly different between treatments, but for turbidity the difference was very evident, since its mean value was five times greater in pond with fish compared to pond without fish (Table 1).

In total, 40 taxa of benthic macroinvertebrates were observed in all the fishponds. The most abundant taxa were the families of insects Chironomidae, Leptoceridae and the families' leeches Glossiphonidae and Hirudinidae. The frequency of OTUs varied between treatments. Chironomidae family was dominant in both treatments but reached a higher frequency of 98.49% in the pond with fish and 92.87% in pond without fish. The Leptoceridae family contributed with 2.07% in pond without fish, against 0.06% in the pond with fish. Other taxa were exclusive to ponds without fish, such as Mollusca families (Physidae, Ampullaridae, Planorbidae); the Hyalellidae crustacean; and insects (Hydroptilidae, Coenagrionidae, Dytiscidae) (Table 2).

Some differences in benthic community metrics were observed between treatments. OTU richness was higher in ponds without fish (32 OTUs) than in ponds with fish (25 OTUs), but the abundance in ponds with fish was three times the number sampled in ponds without fish (62,952 and 20,930 respectively). The percentage of EPT families was much higher at fishponds without fish (2.3%) when compared with fishponds with fish (0.13%).

ANOVA showed that only abundance was not significantly different at the level of 0.05, but all of them were significantly different at 0.10 between treatments (Table 3).

Although diversity index was low in both treatments, being below 1,0, Shannon diversity index (H') was higher in ponds without fish, as was Pielou evenness. Simpson index was greater in ponds with fish. The analysis of variance of the model was only significant at 10% for all biological variables. So, the means comparison test (Tukey) was performed with the same level of significance. According to Tukey's test, all the metrics were significantly different. According to ANOVA performed with the number of individuals from each OTU, only two showed a significant fit of the analysis of variance model at 5%. They are: Chironomidae and Glossiphonidae. The abundance of Chironomidae was significantly higher in pond with fish (15.501,8) than in pond without fish (4.859,3); and for Glossiphonidae, the abundance was significantly higher at ponds without fish (73.0) than in pond in pond with fish (19.5) (Table 3).

## 4. Discussion

Despite the great dominance of Chironomidae in both treatments, some families were more abundant in ponds without fish, which means that this pond had a greater biodiversity of aquatic fauna when compared to pond with fish, mainly for more sensitive families, like Leptoceridae and Hydroptilidae (Trichoptera). Furthermore, the physical and chemical parameters of water quality, especially turbidity, also demonstrated the difference in the presence of fish in water quality.

Considering community composition parameters, it is clear that the presence of tilapia reduced the diversity (H') and evenness (Pielou) of macrobenthic community (Table 3). It is also evident the difference in the total abundance of macroinvertebrates, which was much higher in the pond with fish, which can be explained by the extremely high frequency of Chironomidae in this fishpond.

**Table 1.** Mean, standard deviation (SD), minimum and maximum values for physical-chemical parameters of water quality (September 8 to November 11, 2016). N = 9. EC – electric conductivity; Dissolved Oxygen (DO); Oxygen Saturation (%DO); TDS = Total Dissolved Solids.

| Parameter | Mean | | SD | | Min | | Max | |
|---|---|---|---|---|---|---|---|---|
| | Without | With | Without | With | Without | With | Without | With |
| Temp (°C) | 24.40 a | 24.27 a | 2.02 | 2.08 | 21.28 | 21.12 | 27.66 | 27.53 |
| pH | 7.53 a | 7.15 b | 0.14 | 0.16 | 7.30 | 6.70 | 7.79 | 7.30 |
| EC (mS $cm^{-1}$) | 0.063 a | 0.058 b | 0.002 | 0.002 | 0.060 | 0.054 | 0.065 | 0.061 |
| Turbidity (NTU) | 5.24 b | 28.64 a | 1.44 | 6.43 | 3.80 | 19.20 | 8.60 | 39.20 |
| DO (mg $L^{-1}$) | 6.33 a | 5.83 b | 0.46 | 0.94 | 5.90 | 4.40 | 7.35 | 7.72 |
| % DO | 75.72 a | 69.05 b | 6.23 | 10.10 | 66.00 | 54.30 | 88.00 | 93.40 |
| TDS (mg $L^{-1}$) | 0.040 a | 0.038 b | 0.001 | 0.002 | 0.039 | 0.035 | 0.042 | 0.040 |

Means followed by the same letter horizontally are not significantly different by Tukey's test at 5%.

**Table 2.** Operational Taxonomic Units (OTUs) and abundances at each sampling occasion.

| OTU | With Fish | | | | Without Fish | | | |
|---|---|---|---|---|---|---|---|---|
| | Sep 23th | Oct 14th | Oct 28th | Nov 11th | Sep 23th | Oct 14th | Oct 28th | Nov 11th |
| Chironomidae | 18176 | 11815 | 13436 | 18580 | 3605 | 5002 | 5503 | 5327 |
| Ceratopogonidae | 0 | 1 | 0 | 2 | 1 | 2 | 1 | 19 |
| Empididae | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| Simuliidae | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 |
| Baetidae | 0 | 1 | 0 | 7 | 8 | 7 | 6 | 4 |
| Polymirtacyidae | 12 | 4 | 5 | 0 | 0 | 0 | 0 | 0 |
| Caenidae | 3 | 1 | 3 | 0 | 3 | 4 | 3 | 0 |
| Leptophlebidae | 1 | 1 | 2 | 0 | 0 | 0 | 0 | 1 |
| Leptoceridae | 2 | 16 | 14 | 9 | 250 | 87 | 61 | 35 |
| Hydroptilidae | 0 | 0 | 0 | 0 | 2 | 3 | 1 | 0 |
| Hydrobiosidae | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| Polycentropodidae | 0 | 2 | 0 | 0 | 0 | 0 | 1 | 0 |
| Coenagrionidae | 0 | 4 | 0 | 2 | 57 | 34 | 19 | 22 |
| Libellulidae | 0 | 0 | 2 | 0 | 1 | 3 | 0 | 3 |
| Perilestidae | 0 | 0 | 1 | 0 | 2 | 6 | 0 | 0 |
| Megapodagrionidae | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 4 |
| Dytiscidae | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 0 |
| Dicteriadidae | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |
| Pleidae | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 |
| Gyrinidae | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 2 |
| Velidae | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 1 |
| Belostomatidae | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| Glossiphonidae | 7 | 6 | 33 | 32 | 36 | 76 | 94 | 86 |
| Pyralidae | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |
| Hirudinidae | 24 | 6 | 21 | 60 | 28 | 86 | 100 | 86 |
| Corbiculidae | 0 | 5 | 0 | 0 | 1 | 0 | 0 | 0 |
| Hidrobiidae | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| Oligochaeta | 125 | 22 | 28 | 64 | 38 | 6 | 5 | 42 |
| Crustacea | 0 | 0 | 0 | 0 | 10 | 0 | 0 | 1 |
| Amphipoda | 0 | 0 | 0 | 0 | 16 | 0 | 0 | 0 |
| Planorbidae | 2 | 1 | 1 | 1 | 20 | 20 | 13 | 35 |
| Thiaridae | 64 | 37 | 101 | 114 | 0 | 2 | 0 | 1 |
| Bivalvia | 18 | 0 | 11 | 1 | 0 | 2 | 0 | 0 |
| Physidae | 0 | 0 | 0 | 0 | 20 | 0 | 0 | 0 |
| Gastropoda | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| Ampullaridae | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 |
| Hyallelidae | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| Copepoda | 32 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |
| Colembola | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| Ostracoda | 25 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

**Table 3.** ANOVA and means for macroinvertebrate metrics at each treatment comparing ponds with and without fish. Pielou – Pielou evenness index; H' – Shannon diversity index; Simpson – Simpson dominance index; OTU – Operational Taxonomic Unit.

| | | ANOVA | | Means | |
|---|---|---|---|---|---|
| | | F | p value | Without Fish | With Fish |
| **Metrics** | Pielou | 7.48 | 0.065 | 0.139 a | 0.039 b |
| | H' | 7.10 | 0.070 | 0.400 a | 0.102 b |
| | Simpson | 5.48 | 0.097 | 0.858 b | 0.971 a |
| | Abundance | 7.90 | 0.060 | 5,233 b | 15,740 a |
| | OTU Richness | 6.66 | 0.076 | 17.750 a | 14.750 b |
| **OTU** | Chironomidae | 8.17 | 0.058 | 4,859 b | 15,502 a |
| | Glossiphonidae | 12.80 | 0.032 | 73.0 a | 19.5 b |

Means followed by the same letter horizontally are not significantly different by Tukey's test at 10%.

In order to increase productivity in fish farms, fish producers generally enhance fish stock densities and feed, which can promote eutrophication inside the ponds. So, the renewal of water and the control of physical-chemical parameters in adequate levels are important to maintain fish welfare (Bueno et al., 2015). In the present study, considering water quality, physical-chemical parameters presented adequate values for tilapia rearing (Boyd & Tucker, 1998; Conselho Nacional do Meio Ambiente, 2005). Nevertheless, all parameters showed significant difference between treatments. It is worth noting the higher dissolved oxygen (DO) values found in ponds without fish. The DO concentration below the value recommended by Brazilian legislation at pond with fish can cause stress in tilapia (Barcellos, 2022), and when exposed at this condition for a long time, can led to fish diseases (Tucker & Hargreaves, 2009).

Turbidity was much higher in ponds with fish, which can be related to fish movement itself, but also due to not consumed feed and feces in water. When studying the effects of turbidity in shallow lakes on macroinvertebrate communities, Van de Meutter et al. (2005) observed that turbidity affected the macroinvertebrate assemblage structure, that is, taxa colonizing open-water lakes were different from lakes with more turbidity. In general, open water shallow lakes usually have higher submerged macrophyte cover, which in turn promote a higher number of taxa than turbid lakes. Our study is in accordance with this, insofar as diversity and evenness indices were significantly higher in ponds without fish. Hoess & Geist (2021) highlight the deleterious effects of high turbidity in mussel populations and their fish hosts. These authors discuss the impacts of sediments in receiving streams and propose practices of sediment harvesting and sedimentation structures to minimize it.

The Chironomidae (Diptera) family is widely distributed and often the most abundant in freshwater environments. In the present study, this family was dominant in all treatments, representing almost 99% of the community in ponds with fish. Carvalho & Uieda (2004) also observed the dominance of Chironomidae and Oligochaeta in streams when using artificial substrate samplers in the interior of São Paulo State. In the work by Bertoncin et al. (2022), 90 taxa belonging to Chironomidae, Ephemeroptera and Oligochaeta were counted in artificial substrates, including natural lotic and lentic environments.

The artificial substrate samplers possess an intrinsic selectivity, which generally reduces the number of taxa capable of colonizing them. In artificial lentic systems, such as fishponds, an even lower taxonomic richness is expected, due to the homogeneity of the sediment (Loke et al., 2010). The high turbidity of water in ponds with fish may have harmed more sensitive families, such as Leptoceridae (Trichoptera), which abundance was lower than at pond without fish. Similarly, Akamagwuna et al. (2019) revealed that fine sediment and turbidity stress had severe negative effects on the taxonomic richness and diversity of EPT taxa in the studied river systems across the study period. Ponds with fish received feed, which sometimes may not be completely consumed, precipitating in the sediment, and consequently causing an anoxia situation. Fish feces also contribute to a more eutrophic environment, so only the taxa that are most tolerant of organic load remain and increase in number. On the other hand, Stephens & Farris (2004) observed an enhancement of abundance of Ephemeroptera, Plecoptera and Trichoptera (EPT) families in receiving streams below aquaculture facilities, possibly related with water of known quality from well-aerated ponds, among other reasons.

The number of individuals from Ephemeroptera, Plecoptera and Trichoptera (EPT) families is a well-known metric used in biomonitoring programs to assess the level of impacts in freshwater resources (Barbour et al., 1999). So, the higher the percentage of EPT in relation to the total number of organisms collected, the higher the level of preservation of the site. In fishponds, the richness of EPT is naturally low, when compared with lotic systems. Moura e Silva et al. (2016) also reported significantly lower values for richness and diversity in macroinvertebrate communities colonizing artificial substrate samplers near net cages for tilapia production. But even so, the percentage of EPT in ponds without fish was almost twenty times higher than in ponds with fish (2,3% and 0,13%, respectively), mainly represented by Baetidae (Ephemeroptera) and Leptoceridae (Trichoptera). The reduced number of EPT in pond with fish could be related with the increased turbidity of water, which can damage the gills of EPT organisms. Other impacts are associated with the reduction of EPT, like pesticides (Liess & von der Ohe, 2005) and changes of land use (Suren, 1994).

In natural ecosystems, benthic invertebrates supply food for both aquatic and terrestrial vertebrate consumers (e.g., fish, turtles, and birds) (Covich et al., 1999). In fishponds, the benthic invertebrate community acts as a natural food source for fish in fishponds (Solimini et al., 2003). In the present study, metrics such as OTU richness, Shannon diversity and evenness were higher at pond without fish, which could be related to the absence of predators (Nile tilapia). The tilapia is omnivorous, feeding on insects, microcrustaceans, seeds, fruits, roots, algae, plankton (mainly juveniles) and small fish. The presence of a greater number of mollusks in fishless pond could be explained by the greater supply of food, since these animals are herbivores (ex: Ampullaridae, Physidae). The absence of fish may have contributed to the establishment of taxa, mainly insect larvae, which had their presence or abundance limited when in the presence of predators (tilapia). Thus, this would partly explain the greater richness and diversity of macroinvertebrates in fish-free ponds. The significant differences obtained for benthic metrics suggest that the presence of fish in ponds is changing the macrobenthic community, by reducing its richness, diversity and evenness. Minoo et al. (2016) registered alterations in ecosystem function and integrity in tributaries downstream aquaculture facilities, and also an increasement of filter feeders (including Chironomids) and deposit feeders.

The impact of cage aquaculture on benthic macroinvertebrates was studied by Egessa et al. (2018). Among other results, they found a greater abundance of Oligochaete annelids within the farm area than at the upstream and downstream sites. In the present study, the Oligochaetes had twice the abundance in the ponds with fish compared to the pond without fish. In literature, it is well known the high tolerance of earthworms to moderate to high organic loads (Atanackovic et al., 2020). Glossiphonidae is a leech family associated with high nutrient concentrations, agrochemical impacts, and is considered a tolerant taxon (Arias et al., 2020). However, in the present study, they were more abundant in pond without fish, what is unexpected, as TDS, turbidity and dissolved oxygen presented greater values at pond without fish. However, the resuspension of sediment by fish due to feeding activity may enhance turbidity in water. This, in turn, can reduce foraging or oviposition microhabitats and refuges for many macroinvertebrate taxa (Bazzanti et al., 2009; Miller & Crowl, 2006). In the present study, in pond with absence of fish, the turbidity in water is low, which could partially explain the greater abundance of macroinvertebrates, including Glossiphonidae.

## 5. Conclusion

We conclude that the presence of fish in ponds has modified the macroinvertebrate benthic community, by reducing taxa richness, diversity and enhancing the dominance of tolerant taxa (mainly Chironomidae). Besides, the occurrence of sensitive families was reduced in ponds with fish, which can be associated with elevated turbidity levels. Finally, it is important to better understand the macrobenthic community, at lower taxonomic levels, as a way to implement biomonitoring in aquaculture.

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