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Zooplankton community dynamics in response to water trophic state in integrated multitrophic aquaculture

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ABSTRACT

Integrated multitrophic aquaculture (IMTA) is an alternative means to optimize feed usage in aquaculture which combines species of different trophic levels. The addition of substrate to IMTA has also been used to promote a lower release of phosphorus, thus minimizing eutrophication and impacts of effluents. In these aquaculture systems, the zooplankton community is important because it acts as a link in trophic chains. This study aimed to verify the dynamics and the structure of the zooplankton community in IMTA (tilapia-prawn), in response to trophic conditions in earthen ponds with different substrates. The object of the study was 12 earthen ponds organized in three treatments: no substrate (control), geotextile substrate, and bamboo substrate. Zooplankton samples were taken biweekly through a water bilge pump. Rotifers and microcrustaceans were identified and counted to determine changes in community diversity during the experiment. Eutrophication was determined through phosphorus and chlorophyll water concentrations. There were no differences in zooplankton communities among treatments, even though increases in levels of eutrophication of the system heavily influenced this community, by altering its diversity and abundance. Small organisms were the most representative ones under polyculture eutrophic conditions.

Keywords: Cladocera, Copepoda, Integrated multitrophic aquaculture, Rotifera, Substrate, Water trophy.

Dinâmica da comunidade de zooplâncton em resposta à trofia da água em aquicultura integrada multitrófica

RESUMO

Aquicultura multitrófica integrada (IMTA) é uma alternativa para otimizar a utilização de alimentos na aquicultura, combinando espécies de diferentes níveis tróficos. A adição de substrato no IMTA também tem sido utilizada para promover menor liberação de fósforo, minimizando a eutrofização e os impactos dos efluentes. Nesses sistemas de aquicultura, a comunidade zooplanctônica é importante porque desempenha papel de elo nas cadeias tróficas. Este estudo teve como objetivo verificar a dinâmica e a estrutura da comunidade zooplanctônica em IMTA (tilápia-camarão), em resposta a condições tróficas em viveiros de terra com diferentes substratos. O objeto de estudo foram 12 viveiros escavados, organizados em três tratamentos: sem substrato (controle), substrato geotêxtil e substrato de bambu. Amostras de zooplâncton foram coletadas quinzenalmente por meio de uma bomba de sucção de água. Rotíferos e microcrustáceos foram identificados e contados para determinar as mudanças na diversidade da comunidade durante o experimento. A eutrofização foi determinada mediante as concentrações de fósforo e clorofila na água. Não houve diferenças nas comunidades zooplanctônicas entre os tratamentos, embora o aumento dos níveis de eutrofização do sistema tenha influenciado fortemente essa comunidade, alterando sua diversidade e abundância. Organismos pequenos foram os mais representativos nas condições eutróficas de policultivo.

Palavras-chave: Cladocera, Copepoda, Aquicultura multitrófica integrada, Rotifera, Substrato, Trofia da água.

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INTRODUCTION

The intensification of the fish production chain has had a positive impact on socio-economic results. However, within the scope of the environmental impact generated, this activity has shown unsatisfactory results in studies on aquaculture sustainability. In these intensification systems, there is a constant input of feed for the animals, and a nutrient enrichment process takes place in the pond water (Sipaúba-Tavares et al., 2010; Sipaúba-Tavares and Santeiro, 2013), increasing the primary production and consequent eutrophication of the system. Since the ponds used in aquaculture are remarkably like natural environments, they comprise a greater diversity of ecological compartments, including the sediment in which many of the nutrients accumulate (Valenti et al., 2010).

Excess nutrients may also accumulate in the sediment or even reach and impact other aquatic environments. An alternative means to optimize feed usage is integrated multitrophic aquaculture (IMTA), which combines species of different trophic levels in the same pond, thus diversifying compartment exploitation (Chopin et al., 2001). By combining different species, the residues of one may serve as food for another (Chopin et al., 2012), promoting greater cycling of organic matter. This is what happens in polycultures of prawn and fish, where the prawns are benthic detritivores and feed on other animals' feces and excess food (Marques et al., 2016). The other organism may be fish like tilapia (*Oreochromis niloticus*), which feeds on zooplankton (Ibrahim et al., 2015), occupying a niche in the water column, as well as several species of carp, with traditionally used in polyculture (Ling, 1969), and more recently tambaqui (*Colossoma macropomum*) (Dantas et al., 2020; Franchini et al., 2020).

The contribution of zooplankton to feeding fish in aquaculture has been vastly studied (e.g., Oliveira et al., 2006; Menezes et al., 2010; Ibrahim et al., 2015; Taipale et al., 2018; Mischke et al., 2019), which also substantiate its important role as a link in the trophic chains in fish farming ponds. In addition to connecting energy between trophic levels, the zooplankton community is a water quality bioindicator in the system (Millan and Sipaúba-Tavares, 2014). Because of its trophic connection with phytoplankton, if there is a change in primary production, it will directly affect its quantity and composition. These changes may occur by excess nitrogen and phosphorus in the water (Eskinazi-Sant'Anna et al., 2007; Conley et al., 2009), caused by climate and by fish culture (Sipaúba-Tavares et al., 2011; Favaro et al., 2015). Therefore, analyzing this community is mandatory for assessing water quality and nutrient concentrations (Sipaúba-Tavares et al., 2010). To minimize nutrient accumulation and improve trophic conditions, the use of substrates has become traditional in cultivation ponds for periphyton colonization. This community promotes *in situ* cycling of toxic nutrients (Azim et al., 2003; Saikia, 2011), improving the water and effluent quality by releasing smaller amounts of phosphorus (David et al., 2017b) and, consequently, improving variety in the local community.

Based on Barica's (1993) statement, which says that the instability of the aquatic environment, with increased eutrophication and changes in planktonic structure, tends to decrease efficiency in fish production, and knowing the influence of the cultivation of fish and prawn on water quality and, consequently, on the local zooplankton community, this study aimed to verify the dynamics and the structure of the zooplankton community (rotifers and microcrustaceans) in IMTA (tilapia-prawn), in response to trophic conditions in earthen ponds with different substrates, which can aid in making decisions concerning the impact of this activity on the environment and on production.

MATERIAL AND METHODS

The experiment was conducted in the Crustacean Sector of the Aquaculture Center, Universidade Estadual Paulista "Júlio de Mesquita Filho" (CAUNESP), Jaboticabal, São Paulo, Brazil (21°15'22"S, 48°18'48"W). This study was part of an experiment using IMTA with prawn and fish, which was carried out to verify the efficiency in the production of these organisms in association, and which also evaluated water quality, using different substrates.

Experimental design

The experiment lasted 17 weeks, and 12 earthen ponds were filled with water from two reservoirs that receive effluents from aquaculture. Each pond has an area of approximately 100 m^2 and 1-m water depth, and a base was added of 0.1 kg $CaCO_3/m^2$ to stabilize the pH of the water. There was no water renewal in the ponds. Only the water lost by seepage and evaporation was replaced, by adding more water from the mentioned reservoirs. Aerators were only used when dissolved oxygen in the water declined to 1.5 mg/L.

The experimental design was completely randomized, with four replications for each of three treatments:

- TrCon = control treatment (no substrate);
- TrGeo = geotextile fabric substrate;
- TrBam = bamboo substrate.

With dimensions of approximately 7 m long and 1 m wide, the substrates were arranged in the ponds in a quantity to increase the pond surface area by 50% (see David et al., 2017a for details).

First, only *Macrobrachium amazonicum* juveniles (0.03 ± 0.01 g) were stocked in the ponds at a density of 21.5 prawns/m^2. They were fed a commercial pellet diet with 35% crude protein, at a rate of 10% of body weight twice daily. After a five-week period for adaptation of the prawns and to prevent their predation, male *O. niloticus* juveniles (29 ± 1.1 g) were introduced in the ponds (1.16 individual/m^2), thus beginning the integrated culture period (IMTA). After the tilapias had been introduced in the ponds, the prawns were no longer offered their diet, and they fed on detritus and other sources of nutrients from the ponds. Only the tilapias were fed with a commercial pellet diet with 40% crude protein twice daily (until individual weight of 150 g); a 28% crude protein diet was used from the second month. These commercial diets represented the allochthonous organic matter.

The polyculture lasted 140 days. At the end, the ponds were drained, the fish and prawns were weighed and measured, and survival and productivity were determined.

Trophic state

The water parameters were monitored, remaining within the standards for this practice (Table 1) (see Rodrigues et al., 2019 for more details). For dissolved oxygen, pH, and temperature, a multiparameter probe was used (YSI professional plus model). The water transparency was measured using a Secchi disk (Boyd, 1979). Water samples were collected to determine the concentrations of ammonia, nitrite, and nitrate (APHA, 2005).

To verify the trophic state of ponds throughout the experiment, the total phosphorus and chlorophyll-*a* concentrations were evaluated monthly. One liter of water was collected at 30-cm depth from each pond, using a horizontal Van Dorn bottle. For total phosphorus analysis, the sample was analyzed by stannous chloride method and colorimetric analysis (APHA, 2005). For chlorophyll-*a* analysis, a sample of approximately 300 mL of water collected from each pond was vacuum-filtered through a glass fiber membrane. The spectrophotometric method of American Public Health Association (APHA, 2005) was applied to this filtrate. Based on the results, Carlson's trophic state index (TSI) (Carlson, 1977) modified by Lamparelli (2004) was used to determine the pond's trophic state by nutrient enrichment (phosphorus and chlorophyll-*a*) (Eqs. 1, 2 and 3).

$$\text{TSI} = [\text{TSI(P)} + \text{TSI(Cl)}]/2 \quad (1)$$

$$\text{TSI(P)} = 10\text{x } (6\text{-}(1.77\text{-}0.42\text{x } (\ln \text{P})/\ln 2)) \quad (2)$$

$$\text{TSI (Cl)} = 10\text{x } (6\text{-}((0.92\text{-}0.34\text{x } (\ln \text{Cl}))/\ln 2)) \quad (3)$$

In which: P = total phosphorus concentration measured in the pond water, in µg/L^1; Cl = chlorophyll concentration measured in the pond water, in µg/L^1.

After determining the index, the trophic state was assigned according to the categories in Lamparelli (2004) (Table 2).

Table 1. Means (± standard deviation) of the water quality variables in integrated multitrophic aquaculture system with *Oreochromis niloticus* and *Macrobrachium amazonicum* obtained from the treatments: TrCon = control treatment (no substrate), TrGeo = geotextile fabric substrate, and TrBam = bamboo substrate*.

Parameters	Period	Treatment		
		TrCon	TrGeo	TrBam
Dissolved oxygen (mg/L)	Morning	4.5 ± 1.3	4.0 ± 1.5	4.1 ± 1.2
	Afternoon	11.4 ± 0.3	10.4 ± 0.4	10.9 ± 0.3
pH	Morning	7.9 ± 0.4	7.9 ± 0.1	7.7 ± 0.2
	Afternoon	9.3 ± 0.1	9.1 ± 0.1	9.2 ± 0.6
T (°C)	Morning	27.1 ± 0.9	27.1 ± 0.9	27.1 ± 0.9
	Afternoon	30.1 ± 1.0	29.9 ± 0.9	29.9 ± 0.9
Transparency (cm)		35 ± 2	39 ± 5	39 ± 5
Ammonia (µg/L)		138 ± 35	143 ± 30	143 ± 30
Nitrite (µg/L)		7.4 ± 3.3	10.2 ± 2.9	10.2 ± 2.9
Nitrate (µg/L)		36.5 ± 19.2	68.8 ± 18.0	68.8 ± 18.0

*Means did not differ by analysis of variance. Source: adapted from Santos et al. (2016).

Table 2. Ranking of the trophic state for reservoirs according to Carlson's index modified by Lamparelli (2004).

Ranking of Trophic state – Reservoirs	
Trophic state	TSI values
Ultraoligotrophic	TSI ≤ 47
Oligotrophic	47 < TSI ≤ 52
Mesotrophic	52 < TSI ≤ 59
Eutrophic	59 < TSI ≤ 63
Supereutrophic	63 < TSI ≤ 67
Hypereutrophic	TSI ≥ 67

TSI: Carlson's trophic state index.

Zooplankton community

Zooplankton samples were taken biweekly through a water bilge pump (Model 34,600-0000, Jabsco ITT). A total of 150 liters of water was filtered through a 40-µm plankton mesh net in each pond. Organisms were narcotized with 5 mL of carbonated water with the intention of reducing the degree of contraction of organisms, and later fixed with 4% formalin. In the laboratory, rotifers and planktonic microcrustaceans were identified to the smallest possible biological classification unit under an optical microscope, using specific identification keys. To evaluate the population densities, three 1-mL sub-samples were obtained with Stempel pipette, placed in square glass Sedgewick-Rafter chambers, and counted under an optical microscope with 10x and 40x magnification.

At the end of the experiment, phytoplankton samples were obtained for qualitative algae identification. Cyanobacteria identification was performed based on morphological characters, following taxonomic keys.

Data analysis

To determine the frequency of occurrence of species (FO), the constancy index (Dajoz, 1983) of each taxonomic group was determined. Species with FO below 25% were considered accidental; species with FO between 25 and 50% were accessory; and species with FO above 50% were considered constant.

The Jaccard similarity index was applied to analyze the proportion of species shared among the communities in the different treatments and weeks. The diversity of zooplankton species was analyzed using species richness (S), Simpson's dominance (D), Shannon-Wiener's diversity index (H') and Pielou's equitability (J).

All data were tested for normality through the Shapiro-Wilk test. In order to verify the variation among treatments and weeks, the densities and diversity index were compared through the Kruskal-Wallis non-parametric test, with a post hoc multiple comparison test of the mean scores for all groups (Siegel and Castellan, 1988) (Statistica 12).

Correlations were made between the density of each taxon of zooplankton and the physical-chemical parameters which were representative of eutrophication (total phosphorus and chlorophyll-*a*), by using the non-parametric Spearman correlation test (Statistica 12).

RESULTS

The total phosphorus and chlorophyll-*a* concentrations increased during the polyculture period (Fig. 1). As a result, the TSI also increased in the ponds, changing from mesotrophic in the first months to supereutrophic in the last two months (Table 3).

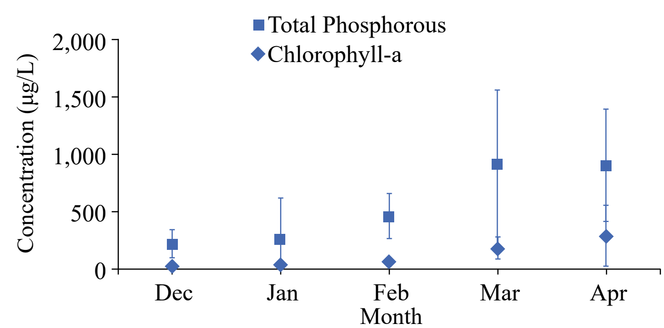


Figure 1. Average of the total phosphorus (µg/L) and chlorophyll-*a* (µg/L) of pond water during polyculture.

Table 3. Trophic state index (TSI) of the pond in each month and the last week of integrated multitrophic aquaculture.

Week	**TSI (µg/L)**	**Trophic state**
4	55.34	Mesotrophic
8	56.07	Mesotrophic
12	58.96	Mesotrophic
16	63.25	Supereutrophic
17	64.16	Supereutrophic

Due statistical analyses revealed that the zooplankton community did not present significant differences between the treatments, and so the results of the temporal variation will be presented regardless of the treatments. The only parameter that presented a significant difference among treatments was the richness (S) ($H = 6.60$, $P = 0.04$); the number of species was higher in the mantle substrate treatment than in the control treatment (without substrate).

Twenty-seven taxonomic groups of zooplankton were identified during polyculture. Rotifera was the most representative of them (15). Among the microcrustaceans, cladocerans had more species than copepods (six and four, respectively); however, copepods were an abundant group maintaining recruitment (nauplii and copepodites) in all periods (Table 4).

The density of total zooplankton, rotifera, cladocera and copepoda showed a difference during polyculture, as well as for some of their respective species (Fig. 2). The total zooplankton density was high during the experiment and increased considerably at the end (Fig. 2). This increase was due to the contribution of rotifers, mainly *Brachionus havanaensis*, followed by *Asplanchna* sp., *Keratella americana*, *Lecane elsa*, and *Lecane bulla*. In turn, *Brachionus falcatus* and *Polyarthra* sp., which occurred at high densities at the beginning, declined sharply in the second sampling and were no longer recorded at the end of the experiment. Bdelloidea, *K. tropica* and *Trichocerca* sp. increased and decreased their respective densities over the weeks, with the most pronounced variation for *Keratella tropica* (Fig. 2). *Brachionus calyciflorus* density showed growth pattern peaks followed by a decrease in the number of individuals (Fig. 2). The cladocerans also increased throughout the experiment, represented mainly by *Moina minuta*, which presented higher densities than *Diaphanosoma spinulosum* in the last weeks. Statistically significant increases in copepoda were reflected in increases at all stages of development – nauplii, copepodites, and adults. However, adults of Calanoida *Notodiaptomus iheringi* showed higher density than *Argyrodiaptomus furcatus* and *Thermocyclops decipiens* (Fig. 2).

In general, over 70% of the taxa were considered constant during the experiment, and five species were classified as accidental, all of them microcrustaceans. Temporal analysis showed that there were changes in the species' frequency of occurrence throughout the experiment (Table 5). The number of taxa classified as constant decreased from 13 at the beginning to 11 at the end of the experiment. The most constant rotifer taxa throughout the experiment were *Asplanchna* sp., *B. calyciflorus*, *L. elsa*, and *Trichocerca* sp. The rotifers *B. falcatus* and *Polyarthra* sp. were constant at the beginning of the experiment, but they were not found at the end (Table 5). Among cladocerans, although with less constant taxa (only *D. spinulosum* and *M. minuta* were found throughout the experiment), the number of species increased at the end of the experiment, with the accidental cases of *B. longirostris*, *C. cornuta*, and *M. spinosa*. Copepodites, nauplii, and adult copepods *N. iheringi* and *T. decipiens* were constant throughout the experiment. The other two species of copepods were unstable, most of the time occurring as accidental species (Table 5).

The Jaccard similarity index pointed to high species sharing (> 60%) among the zooplankton communities in the different treatments and during the polyculture (Fig. 3).

Table 4. Statistical analysis applied to the total density and densities of each zooplankton taxon during the experiment[#].

Taxon	H	*P*
Total zooplankton	32.80	0.00*
Rotifera	46.27	0.00*
Asplanchna sp.	19.40	0.01*
Bdelloidea	29.49	0.00*
Brachionus calyciflorus	11.10	0.20
Brachionus falcatus	43.36	0.00*
Brachionus havanaensis	52.36	0.00*
Filinia longiseta	7.37	0.50
Filinia opoliensis	12.71	0.12
Filinia terminalis	6.55	0.59
Keratella Americana	41.33	0.00*
Keratella cochlearis	14.59	0.07
Keratella tropica	34.10	0.00*
Lecane bulla	18.31	0.02*
Lecane elsa	30.47	0.00*
Polyarthra sp.	30.94	0.00*
Trichocerca sp.	21.47	0.01*
Cladocera	25.65	0.00*
Alonella dentifera	8.00	0.43
Bosmina longirostris	5.15	0.74
Ceriodaphnia cornuta	8.00	0.43
Diaphanosoma spinulosum	11.63	0.17
Macrothrix spinosa	6.12	0.63
Moina minuta	41.52	0.00*
Copepoda	44.30	0.00*
Argyrodiaptomus furcatus	18.40	0.02*
Copepodites (Calanoida and Cyclopoida)	32.31	0.00*
Mesocyclops meridianus	10.41	0.24
Nauplii (Calanoida and Cyclopoida)	38.41	0.00*
Notodiaptomus iheringi	39.38	0.00*
Thermocyclops decipiens	10.88	0.21

[#]Kruskal-Wallis and post hoc comparison of the mean scores between weeks (Siegel and Castellan, 1988) (N = 12, degrees of freedom = 8); *statistically significant.

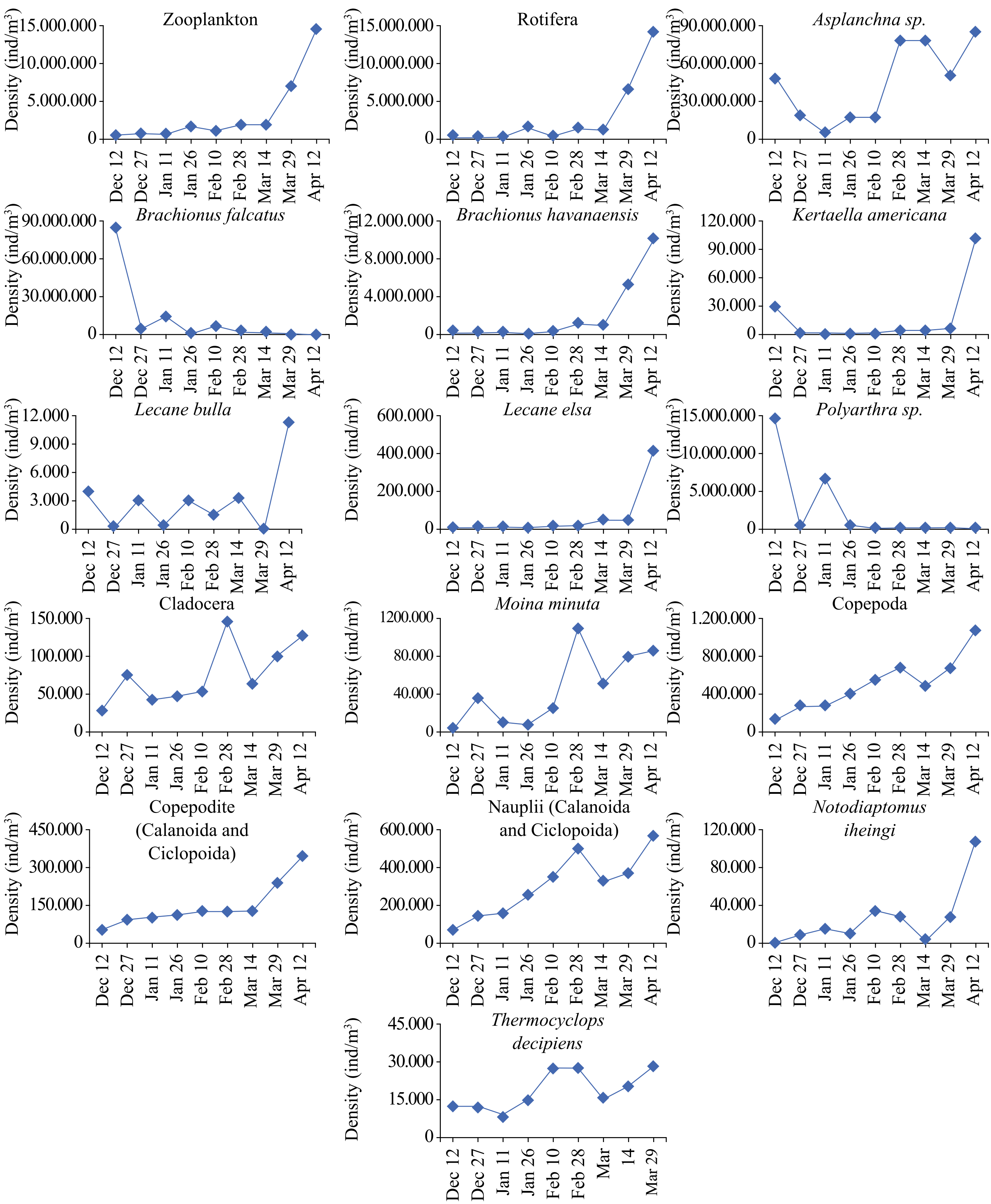
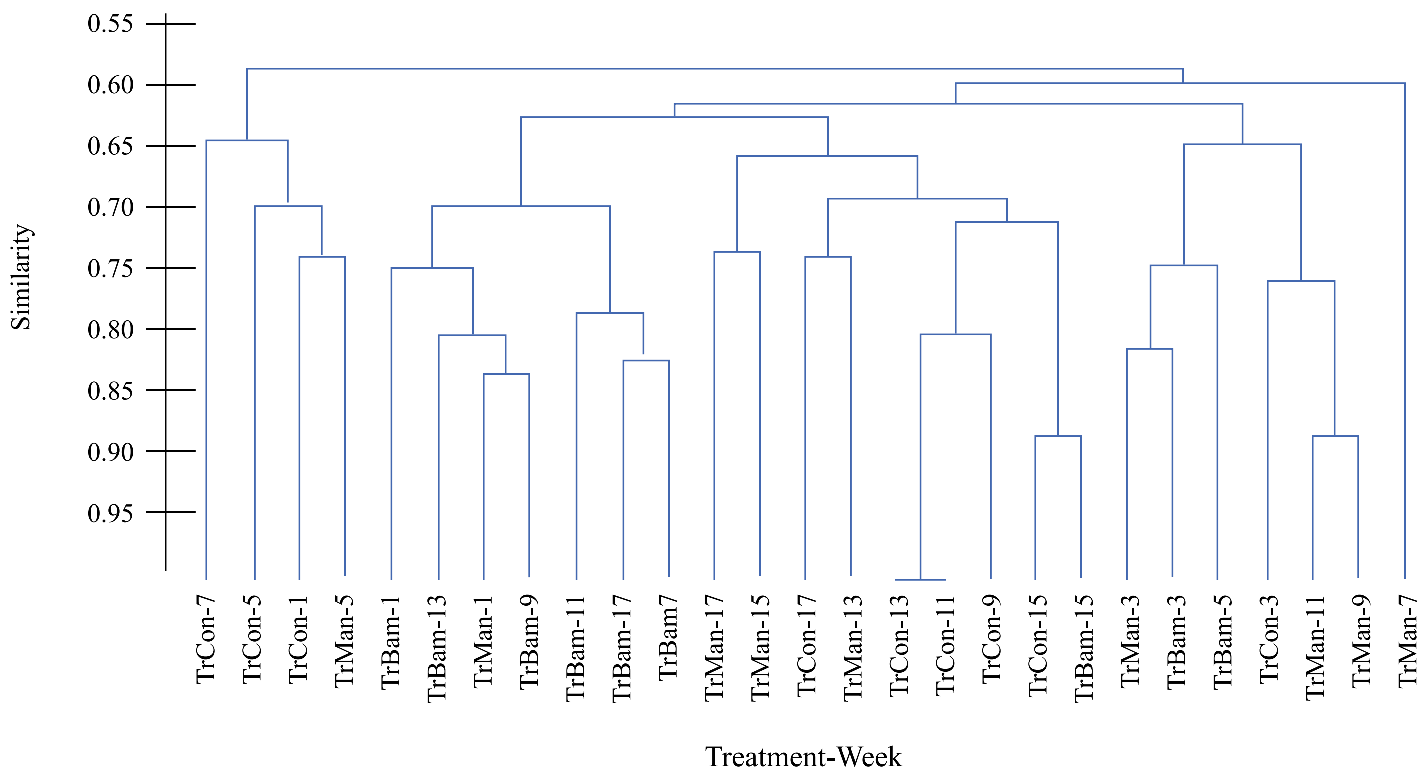


Figure 2. Average density of each zooplankton taxon during the experiment.

Table 5. Zooplankton groups: 17 weeks of experiment with the respective constancy index of Dajoz [+++ constant species (> 50%), ++ accessory species (25 to 50%), + accidental species (< 25%), - absent species].

Taxon	Constancy index of Dajoz								
	Wk-1	**Wk-3**	**Wk-5**	**Wk-7**	**Wk-9**	**Wk-11**	**Wk-13**	**Wk-15**	**Wk-17**
Rotifera									
Asplanchna sp.	+++	++	+	+++	+++	+++	+++	+++	+++
Bdelloidea	+++	+++	+++	+++	+++	++	++	+	++
Brachionus calyciflorus	+++	++	+++	+++	++	+++	+++	+++	+++
Brachionus falcatus	+++	++	+++	+	++	+	+	-	-
Brachionus havanaensis	++	+	+	++	+++	+++	+++	+++	+++
Filinia longiseta	+	-	+	+	+	-	+	+	-
Filinia opoliensis	++	++	+++	++	++	++	++	+++	+++
Filinia terminalis	+	+	++	+	++	++	++	++	++
Keratella americana	+++	-	+	+	+	+	+	+	++
Keratella cochlearis	+	-	+	+	-	-	-	++	+
Keratella tropica	+++	+	+++	+	+	+	++	+	+
Lecane bulla	+++	-	++	+	++	+	++	-	+
Lecane elsa	++	+++	+++	+	+++	++	+++	+++	+++
Polyarthra sp.	+++	+	++	+	-	-	-	-	-
Trichocerca sp.	+++	+++	+++	+++	+++	+++	+++	++	+++
Cladocera									
Alonella dentifera	-	-	-	-	-	-	+	-	-
Bosmina longirostris	-	+	+	+	+	-	-	-	+
Ceriodaphnia cornuta	-	-	-	-	-	-	-	-	+
Diaphanosoma spinulosum	+++	+++	+++	+++	+++	+++	+++	+++	+++
Macrothrix spinosa	-	-	-	-	-	-	+	+	+
Moina minuta	+	+++	+	+++	+++	+++	+++	+++	+++
Copepoda									
Argyrodiaptomus furcatus	+	++	++	-	+	+	-	++	+
Copepodites (Calanoida and Cyclopoida)	+++	+++	+++	+++	+++	+++	+++	+++	+++
Mesocyclops meridianus	-	+	-	+	+	+	-	-	+
Nauplii (Calanoida and Cyclopoida)	+++	+++	+++	+++	+++	+++	+++	+++	+++
Notodiaptomus iheringi	+	++	+++	++	+++	+++	+++	+++	+++
Thermocyclops decipiens	+++	+++	+++	+++	+++	+++	+++	++	++



TrMan: mantle substrate treatment; TrBam: bamboo substrate treatment; TrCon: control treatment; *the numbers following the treatments represent the sample week.

Figure 3. UPGMA dendrogram of the Jaccard similarity index for the composition of 27 samples collected in polyculture nurseries, analyzed for 27 zooplankton groups (R Global = 0.321; $P = 0.002$)*.

The diversity of the zooplanktonic community showed differences among the months. The species richness decreased significantly from the first to the third week, but they remained constant in other weeks (Table 6). Dominance increased at the end of the experiment, coinciding with a decrease in the diversity and equitability of species (Table 6).

Among seven rotifera species that correlated with studied variables, four species presented a significant positive correlation with chlorophyll-*a* (Table 7). *Brachionus falcatus* was the only species negatively correlated with phosphorus. Among cladocerans, *D. spinulosum* presented negative correlation with chlorophyll-*a*. All stages of copepods presented positive correlations with chlorophyll-*a*, whereas only the larval stages did with phosphorus (Table 7).

Three well-known phytoplankton classes were found: Cyanobacteria, Chlorophyceae, and Bacillariophyceae. Cyanobacteria were predominant in polyculture with the species *Aphanocapsa annulata* and *Microcystis aeruginosa*, which were the most constant and abundant ones.

Table 6. Mean values of species richness, Simpson dominance, Shannon-Wiener diversity, and Pielou equitability of the zooplankton community during the experiment*.

Week	Richness	Simpson dominance	Shannon-Wiener diversity	Pielou equitability
1	10.75^{a}	0.27^{b}	1.72^{a}	0.74^{ab}
3	7.17^{b}	0.34^{ab}	1.42^{ab}	0.73^{abc}
5	10.83^{a}	0.27^{b}	1.69^{a}	0.72^{abc}
7	8.17^{ab}	0.38^{ab}	1.36^{ab}	0.65^{abc}
9	10.50^{ab}	0.25^{b}	1.76^{a}	0.75^{ab}
11	8.92^{ab}	0.45^{ab}	1.25^{abc}	0.59^{abc}
13	10.50^{ab}	0.36^{ab}	1.48^{abc}	0.63^{abc}
15	8.67^{ab}	0.60^{a}	0.92^{bc}	0.43^{bc}
17	9.42^{ab}	0.67^{a}	0.78^{c}	0.34^{c}
H	27,74	28.09	30.10	25.85
P	0,00	0.00	0.00	0.00

*Kruskal-Wallis and post hoc comparison of the mean scores between weeks (Siegel and Castellan, 1988) (N = 12, degrees of freedom = 8).

Table 7. Values of r and P for Spearman correlations between zooplankton species density and total phosphorus and chlorophyll-*a* concentrations.

Taxon		Total phosphorus	Chlorophyll-*a*
Rotifera			
Brachionus falcatus	r	-0.37	-
	P	0.00	
Brachionus havanaensis	r	0.52	0.39
	P	0.00	0.00
Filinia opoliensis	r	0.31	0.30
	P	0.02	0.02
Keratella Americana	r	0.28	-
	P	0.03	
Keratella cochlearis	r	0.34	0.31
	P	0.01	0.02
Keratella tropica	r	0.30	-
	P	0.02	
Lecane elsa	r	0.36	0.32
	P	0.01	0.01
Cladocera			
Diaphanosoma spinulosum	r	-	-0.27
	P		0.03
Copepoda			
Notodiaptomus iheringi	r	-	0.27
	P		0.04
Copepodites (Calanoida and Cyclopoida)	r	0.32	0.36
	P	0.01	0.00
Nauplii (Calanoida and Cyclopoida)	r	0.29	0.40
	P	0.02	0.00

DISCUSSION

The zooplankton communities in polyculture were similar regardless of the type of substrate in the pond, which indicates that the presence and type of substrate do not significantly influence zooplankton communities. Statistical changes in zooplankton were observed over time and associated with the increase in eutrophication, which in turn did not differ among treatments. According to Sipaúba-Tavares et al. (2010), the constant input of organic matter in aquaculture ponds leads to the destabilization of planktonic communities. Therefore, it is not the type of substrate used in the cultivation that changes zooplankton communities, but it is the accumulation of organic matter that leads to eutrophication.

Although the total density of zooplankton presented a substantial increase during the experiment, the group that most influenced this increase was rotifera. Density changes in a taxon throughout the experiment are an indicator that they occurred as a response to changes in environmental variables. Zooplankton with higher trophic plasticity tends to develop in eutrophic environments, feeding on cyanobacteria, abundant organisms in these waters (Claps et al., 2011; Azevêdo et al., 2015).

According to Jeppesen et al. (2000), under experimental conditions, the richness of zooplanktonic species decreased as the environment became more eutrophic. In their experiment, cladocerans were the most negatively affected, followed by copepods; the number of rotifer species decreased little or not at all. In the present study, we did not observe a decrease in richness, but in diversity, thus indicating the community changes in which there was an increase in the dominance of a few species, mainly rotifers.

The population density of rotifers was always higher than microcrustaceans, with special prominence for *B. havanaensis* and the genus *Lecane*, which significantly increased their densities as the environment's eutrophication increased. As observed in the experiment conducted by Sipaúba-Tavares et al. (2011), the rotifer *Trichocerca* sp. was the most constant group, thus proving its high plasticity. Because the rotifers present a quick response to environmental changes, they are considered a good bioindicator of the aquatic environment among the zooplankton, such as *B. calyciflorus* (Pal et al., 2015). In the present experiment, this species showed population growth peaks followed by a sharp drop in the number of individuals, which can be considered as an example of an r-strategist species. We found prevalence of the rotifers from the order Ploima, which was also reported in other reservoirs with high nutrient concentration (Kuczyńska-Kippen and Pronin, 2018; Smaoune et al., 2021). In contrast, the orders Flosculariaceae and Collothecacea, which are more frequent in oligotrophic reservoirs (Wærvågen and Andersen, 2017), were represented by only three species of *Filinia*, which were classified as accidental or accessory during the experiment.

The genus Brachionus has been associated with eutrophication in several studies and is considered an indicator of the trophic status of water (Sládeck, 1983; Lougheed and Chow-Fraser, 2002; Vázquez-Sánchez et al., 2014; Silva et al., 2020; Smaoune et al., 2021). *Brachionus calyciflorus* and *B. havanaensis* stood out for having high population densities in the present study and have been frequently observed at high densities in other environments with high trophic levels (Branco et al., 2002; Almeida et al., 2009; Pal et al., 2015; Smaoune et al. 2021). Because it is considered cosmopolitan, the *B. havanaensis* population has the capacity to develop and even increase in density in the presence of excessive cyanobacteria (eutrophication), because it is resistant to the toxins released by cyanobacteria after their ingestion (Fulton e Pearl, 1988). Although some studies associate the high abundances of *B. falcatus* with the increase in the trophic status of the aquatic environment (Sládeck, 1983; Houssou et al., 2018), this species decreased in the ponds throughout the experiment, showing a negative correlation with the concentrations of phosphorus. In another study carried out in the neotropical region, *B. falcatus* was also reported as a species associated with oligotrophic conditions (Arruda et al., 2017), which raises questions about the actual capacity of the species to benefit from the organic matter available in ponds for growing fish. Additionally, according to the literature, *B. havanaensis* and *B. falcatus* are not found in abundance in the same place (Eskinazi-Sant'Anna et al., 2007; Santangelo et al., 2007; Azevêdo et al., 2015), which suggests that they are species competing for the same resource. This can also be inferred from the fact that in the study by Houssou et al. (2017), which guided the study with *B. falcatus* by Houssou et al. (2018), there was no *B. havanaensis* in the sampled locations.

Lecane is another genus frequently reported species present in eutrophic environments (Vázquez-Sánchez et al., 2014; Arruda et al., 2017; Smaoune et al., 2021). *Lecane bulla*, one of the most abundant species in our study, was an indicator of fish culture environments in the study by Arruda et al. (2017) in Brazil, a eutrophication indicator in the study by Yağcı (2016) in Turkey and was present in a eutrophic lake in Mexico in the studies by Moreno-Gutiérrez et al. (2018). For *L. elsa*, the positive correlation with the phosphorus and chlorophyll-*a* variables in the present study also characterizes its role as an indicator of nutrient-dense environments.

The constancy of cladoceran species, such as *D. spinulosum* and *M. minuta*, occurred throughout the study period. These species have already been found coexisting in aquatic environments (Keppeler, 2003; Brito et al., 2013). The low density of cladocerans may be due to the presence of the cyanobacteria *M. aeruginosa*, since these microcrustaceans are a characteristic group in oligo-mesotrophic environments (Jeppesen et al., 2000; Sipaúba-Tavares et al., 2010; Briland et al., 2020). According to Leonard and Pearl (2005), the presence of these algae may lead to the replacement of large cladocerans by smaller zooplankton due to feeding difficulties. This substitution may occur by rotifers or even small cladocerans, such as *M. minuta*, which increased at the end of the experiment. Furthermore, as reported by Vieira et al. (2011), *D. spinulosum* grows better in nutrient-poor environments, which explains the negative correlation of this species with chlorophyll-*a* in the present experiment. Although there was no correlation between the physical and chemical variables and *M. minuta*, it had a marked increase in abundance at the end of the experiment. In previous studies, *M. minuta* was also found in reservoirs classified as eutrophic (Sendacz et al., 2006), considered a tolerant species to high turbidity and with high parthenogenetic reproduction, reaching high population densities (Negreiros et al., 2009).

The constancy of the other microcrustacean groups during the experiment was also high, especially for larval and young copepod forms, as well as for *N. iheringi* and *T. decipiens* species, which are respectively Calanoida and Cyclopoid species. This constancy rate shows that survival conditions for larvae and young were favorable so they could reach adulthood. Although only the species *N. iheringi* showed a positive correlation with chlorophyll-*a*, both species showed a high density of individuals throughout the experiment, demonstrating their high plasticity in a eutrophic environment. This feature has been confirmed in several experiments across Brazil, under eutrophication and drought conditions (Silva and Matsumura-Tundisi, 2005; Landa et al., 2007; Perbiche-Neves et al., 2007; Silva et al., 2020).

Copepods also became more abundant as the experiment progressed. In general, the groups that most increased were nauplii, copepodites, and adults of *N. iheringi* and *T. decipiens*. In contrast to our results in tropical eutrophic environments, some studies have concluded that immature forms of copepods do not develop well in temperate eutrophic environments (Leandro et al., 2006; Abou Zaid et al., 2014). On the other hand, according to Rietzler et al. (2002) and Landa et al. (2007), *N. iheringi* and *T. decipiens* tend to adapt in eutrophic environments with cyanobacteria blooms, since *Microcystis* are part of the cyclopoid diet (Landa et al., 2007). Rietzler et al. (2002) reported an increase in *N. iheringi* density, and the disappearance of *A. furcatus* coincided with increases in environmental eutrophication, with *Microcystis* cyanobacteria being the most abundant genus in the studied environment. As reported by Sipaúba-Tavares et al. (2014), the high concentration of cyanobacteria occurred at the end of the experiment due to an increase in phosphorus in the water and consequently increased eutrophication. Lower densities of *T. decipiens* were associated with the oligomesotrophic conditions of the Volta Grande Reservoir, in Minas Gerais state, Brazil (Landa et al., 2007). In an eutrophic environment, Sipaúba-Tavares et al.

(2017) also found high concentrations of *T. decipiens*, besides rotifers. Therefore, the presence and density increase of these two copepod species (*N. iheringi* and *T. decipiens*) in the present study are in accordance with those findings. In accordance with literature data, it can be inferred that the high density of these species, as well as their larval stages, may be indicative of a eutrophic environment.

Jaccard's similarity to the zooplankton composition was relatively high (> 60%) for all treatments and data, due to most species' constancy during the experiment. However, the decrease in diversity during the experiment can be explained by the dominance of rotifers, which has been reported as a typical strategy of this r-strategist group; they have rapid reproduction under stressful conditions, besides generalist feeding habits (Xi et al., 2002; Claps et al., 2011). As reported by Millan and Sipaúba-Tavares (2014), zooplankton communities become more diverse when the environment presents a lower concentration of allochthone organic matter. Species of the genus *Brachionus* and *Lecane* found in the present study have this characteristic, which can explain their constant presence in polyculture ponds. The increase in total phosphorus and chlorophyll-*a* concentrations denotes the eutrophication of the water in the ponds, and the statistically significant correlation between phosphorus and chlorophyll-*a* and some species densities indicates that eutrophication is a process with the potential to change the whole zooplankton community. In this study, this event changed the diversity of the community by increasing the dominance of a few species. As this number is reduced in comparison to the general number of zooplankton species, only the most tolerant ones will develop, thus diminishing the diversity of the local community. According to our data, the lower diversity found in the last months of the experiment expresses this premise, showing that, by enriching the environment with nutrients, only species capable of tolerating that change will develop and dominate the site.

CONCLUSION

The zooplankton community is not influenced by the types of substrates used in polyculture ponds. On the other hand, increases in the eutrophication levels of the system have a significant influence on this community, altering its density and diversity. Smaller zooplankton organisms with a shorter life cycle tend to be better adapted to the supereutrophic conditions of polyculture, and this group is an important representative of the total density of the zooplankton community.

CONFLICT OF INTERESTS

Nothing to declare.

DATA AVAILABILITY STATEMENT

Data will be available by upon request.

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AUTHORS' CONTRIBUTION

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