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ORIGINAL RESEARCH

Can shrimp farming wastewater negatively affect water quality and zooplankton community structure of a Neotropical estuary? A case study during a productive cycle of *Litopenaeus vannamei*

Ítala Gabriela S. Santos . Glauber Pereira C. Santos . Carlos Yure B. Oliveira . Clarissa Vilela F. S. Campos . Luis Otávio Brito . Alfredo Olivera Gálvez

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Abstract Aquaculture wastewater can influence various communities' structures in both marine and freshwater environments. This study describes the zooplankton community structure and environmental variables in both Passos River estuary (Northeast Atlantic, Brazil) and commercial shrimp farming ponds associated with this estuary. Samples for water quality analysis and quantification and identification of zooplankton were taken during a shrimp culture cycle (July to September 2014) from eight sites (two across the main channel, downstream and upstream, and six shrimp farming ponds). The main water quality parameters showed difference between the shrimp farming ponds and the Passos River estuary – higher levels of total phosphorus and chlorophyll-a were observed in the shrimp ponds. The zooplankton structure at different environments was dominated by the copepods (53.3 to 83.7%) and rotifers (9.2 to 35.5%) but no significant difference was observed among the individual densities. Meanwhile, nutrients availability was a key for high plankton densities in shrimp ponds. These findings have suggested that wastewater from shrimp farming did not influence the zooplankton community on a Neotropical estuary on a short-time scale.

Keywords Aquaculture impact . Chlorophyll . Environment . Shrimp farming . Trophic level . Wastewater

Introduction

Aquaculture is one of the world's fastest growing food production industries and plays a key role towards food security and global nutrition. The aquaculture industry has the largest share in global fish production and handles around USD 230 billion annually (FAO 2018). Aquaculture can relieve fishing pressure on fish stocks without compromising seafood availability. On the other hand, although aquaculture is a recent technology for food production, some unsustainable practices are still present, for example: the use of fishmeal and fish oil in fish production (Galkanda-Arachchige et al. 2020), biological invasions caused by non-native cultured species (Forneck et al. 2020) and disposal of crude wastewater containing large amounts of nitrogen, phosphorus and organic material from undigested feed and feces (Oliveira et al. 2020). In particular, aquaculture wastewater may drive the eutrophication process in receiving water bodies, and this has negatively impacted several environments, including inland, estuarine and marine environments (Oliveira et al. 2019; Bohnes et al. 2019).

Ítala Gabriela S. Santos . Carlos Yure B. Oliveira (✉) . Clarissa Vilela F. S. Campos . Luis Otávio Brito . Alfredo Olivera Gálvez
Departamento de Pesca e Aquicultura, Universidade Federal Rural de Pernambuco, 52171-900, Brazil
e-mail: yureboliveira@gmail.com

Ítala Gabriela S. Santos . Glauber Pereira C. Santos
Instituto de Tecnologia de Pernambuco, Recife, Brazil

Estuaries are highly productive ecosystems that play an ecological role in supporting coastal commercial fisheries (Azhikodan and Yokoyama 2016; Ai et al. 2019). In addition, brackish waters from estuaries are considered the most appropriate for the production of one of the main aquaculture species – the Pacific white shrimp *Litopenaeus vannamei* (Gusmawati et al. 2018). The diverse and abundant microbiota of this environment is suitable as live food for shrimp (Herbeck et al. 2020). Meanwhile, estuaries are negatively impacted by several other human activities and shifts in climate (Cearreta et al. 2000; Statham 2012). Therefore, the increasing of aquaculture ponds in estuarine regions is a potential threat to ecosystem functioning.

Identifying changes in plankton structure is crucial for appropriately managing water resources. This is because they are the basis of the trophic web and environmental changes will affect the composition of communities. Based on both phytoplankton and zooplankton community structures it is possible to determine the trophic level of an aquatic environment, as it serves as an ecological bioindicator (Uriarte and Villate 2005; Batuello et al. 2016; Liang et al. 2020; Fernandes et al. 2020). Recently, trophic level determination and indirect phytoplankton estimation approaches have been successfully achieved using chlorophyll-*a* contents (Millette et al. 2019; Mourão et al. 2020). Thus, chlorophyll-*a* and zooplankton community structure together can provide valuable information on the lower trophic levels of aquatic environments.

Although the obvious effects of aquaculture wastewater on aquatic ecosystem functioning can be estimated, there is no abundant literature on this subject. Considering that estuaries are diverse environments and that they can have distinctive characteristics, an estuary can be more or less influenced depending on several oceanographic parameters. Thus, comparing zooplankton communities in both aquaculture ponds and adjacent estuaries can help to identify the possible trophic changes in these environments even in a short-time scale. In this context, this study aimed to determine the short-time influence of commercial shrimp ponds on a Neotropical estuary, by observing the spatial and temporal variation of zooplankton community structure during a cycle of shrimp production.

Materials and methods

Study and sampling area

The Passos River estuary (08° 38’ 28.7” S, 035° 04’ 58.6” W) is one of the main water bodies on the complex estuarine of Formoso River, and it presents estuarine characteristics with a high marine water influence (Honorato da Silva et al. 2009). This estuary is located in the south of the state of Pernambuco, northeastern Brazil and adjacent to a commercial shrimp farming. Eight sampling strategically points were analyzed: the Passos River estuary downstream (1) and upstream (2) during both high and low tide; four shrimp ponds in different culture stages: a grow-out pond (3) and three grow ponds (4-6), water catchment pond (7) and sedimentation pond (8) where wastewater was treated before being returned to the estuary (Fig. 1).

Samples were collected biweekly for monitoring of physical and chemical variables and zooplankton community between May and September (dry season) – comprising a shrimp farming culture cycle. Shrimp ponds (about 4 ha) were fertilized using $NaSiO_3$ (44 ± 25 Kg) and $Ca(NO_3)_2$ (2,175 ± 61 Kg) to promote phytoplankton growth. Shrimp were stocked under 74±3 shrimp m^{-2} and feed provided were commercial pellets (35% crude protein) resulting in a total of 33 ± 5 tones $cycle^{-1}$ supplied.

Water quality analyses

Water temperature (ºC), salinity (g L^{-1}) and pH were analyzed *in situ* with a YSI 6820–V2 multiparametric probe, Secchi transparency (m) was also measured. Nutrients, such as N-ammonia, N-nitrate, N-nitrite and Total phosphorus (µmol L^{-1}) were analyzed using specialized literature (Strickland and Parsons 1972; Grasshoff et al. 1983); total suspended solids (mg L^{-1}) level was determined according to APHA (2012). Water samples used for chlorophyll-*a* quantification were filtered through glass fiber microfilters (0.45µm porosity) and analyzed according to Jeffrey and Humphrey (1975).

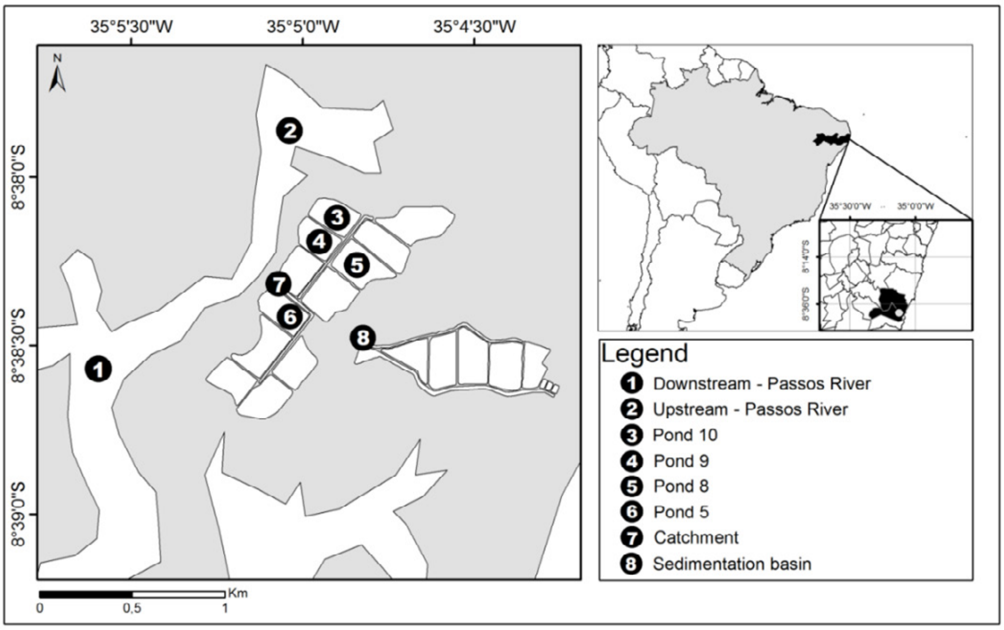

Fig. 1 Locations of sampling places on the Passos River estuary and shrimp commercial ponds in Northeast Brazil.

Zooplankton community and structure

For identification and quantification of zooplankton, 50 L of water from the points were filtered with a 68 µm mesh-size plankton net and stored in 100 mL bottles. The samples were then fixed in 4% formaldehyde and analyzed in a 1 mL Sedgewick-Rafter chamber (in triplicate) under an optical microscope (OLYMPUS CH30) with 100× magnification.

Taxonomic identification and grouping (Copepoda, Cladocera, Rotifera, Protozoa and Cirripedia) were performed based on Boltovskoy (1999). Density was also calculated from the organisms counting in Sedgwick-Rafter chambers, in three sub-samples based on Hensen-Stempel (APHA 2012). Absolute (ind mL^{-1}) and relative (%) abundance for each identified group were determined.

Statistical analysis

Environmental variables were standardized using the range function, while zooplankton data were log-transformed (log(x + 1)) to ensure the data normality (Lilliefors test) and homogeneity (Barlett test). A one-way analysis of variance was used to compare the environmental and zooplankton data. Pairwise comparisons were performed using the Tukey post-hoc test. Principal component analysis (PCA) was performed to determine possible patterns among environmental data from different collection locations, using a correlation to produce a cross product matrix. All statistical analyses were performed in the software Rstudio (version 1.2.5), with a significance level set at $P < 0.05$.

Results and discussion

Environmental variables

Fig. 2 and Table 1 show that the water quality parameters analyzed throughout the shrimp production cycle in shrimp ponds and the estuary (upstream and downstream points) have presented different patterns. Although certain parameters (such as temperature and salinity) have not shown significant differences ($P > 0.05$), the PCA ordination ($F = 5.59$, $P = 0.48$) shows a clear dispersion between the collection places. The collection points in the estuary (and also in the aquacultural sedimentation pond), in general, have presented lower levels of DIN and chlorophyll-*a*, however, Secchi transparency was higher at this place

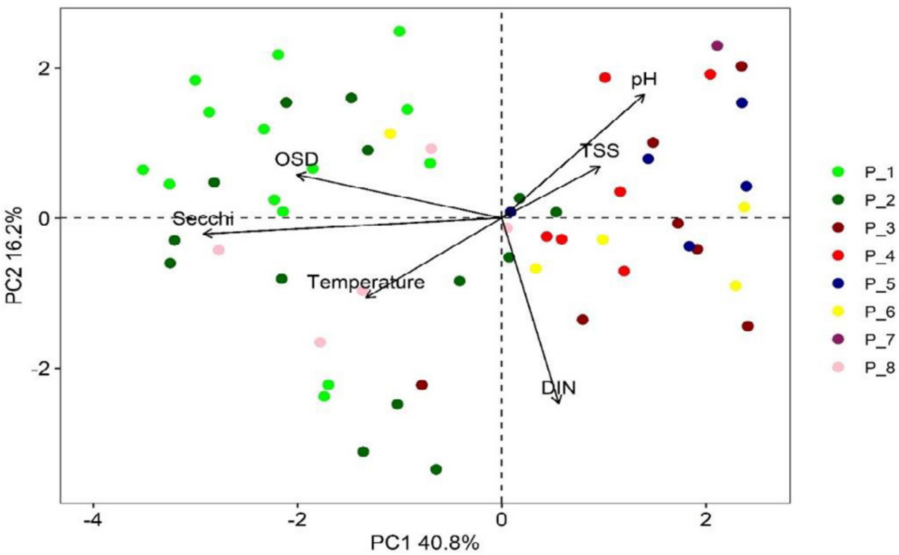

Fig. 2 PCA ordination for water quality parameters from Passos River estuary (places 1 and 2) and shrimp farming ponds (places 3 to 8). See Fig. 1 for more details on collection places.

Table 1 Physical and chemical characteristics of water from the Passos River estuary (places 1 and 2) and shrimp farming ponds (places 3 to 8). See Fig. 1 for more details on collection places.Data correspond to the mean ± SD. Results were analyzed by performing one-way ANOVA and a Tukey's test. Mean values in the same row with different superscripts differ significantly (P < 0.05).

Parameters	Place							
	1	2	3	4	5	6	7	8
Salinity	24.5±3.7	26.±3.6	22.3±3.7	23.8±1.9	23.9±1.7	24.33±1.1	22.0±2.4	24.0±2.9
Chlorophyll-*a* (mg m⁻³)	10.8 ± 4.4^{cd}	6.8 ± 1.3^{d}	40.4 ± 28.8^{ac}	53.07 ± 30.6^{b}	58.5 ± 30.6^{a}	75.9 ± 48.4^{a}	24.2 ± 9.5^{c}	14.4 ± 5.2^{bcd}
pH	8.0±0.2	8.1±0.2	8.5±0.4	8.4±0.4	8.5±0.5	8.2±0.3	8.1±0.2	8.18±0.2
Temperature (ºC)	27.0±0.9	26.9±0.9	27.0±1.1	26.9±1.1	27.5±1.1	26.9±0.9	27.3±1.0	27.1±0.9
Secchi (m)	0.8 ± 0.3^{b}	1.1 ± 0.2^{c}	0.4 ± 0.2^{ab}	0.3 ± 0.1^{a}	0.32 ± 0.1^{a}	0.4 ± 0.2^{ab}	$0.5^{abc}\pm 0.14$	0.8 ± 0.2^{bc}
N-Nitrite (µmol L⁻¹)	0.2 ± 0.4^{ab}	0.1 ± 0.1^{a}	0.1 ± 0.1^{ab}	0.2 ± 0.2^{ab}	0.1 ± 0.1^{a}	0.0 ± 0.0^{ab}	0.6 ± 0.7^{b}	0.1 ± 0.1^{ab}
N-Nitrate (µmol L⁻¹)	1.5 ± 1.5^{ab}	0.9 ± 0.6^{b}	2.2 ± 1.8^{ab}	1.6 ± 0.7^{ab}	1.3 ± 0.4^{ab}	1.70 ± 1.2^{ab}	4.2 ± 2.6^{a}	1.3 ± 0.9^{ab}
N-Ammonia (µmol L⁻¹)	0.1±0.1	0.0±0.0	0.1±0.1	0.0±0.0	0.1±0.2	0.00±0.0	0.0±0.0	0.0±0.0
TP (µmol L⁻¹)	1.1 ± 0.5^{b}	0.7 ± 0.3^{b}	4.2 ± 1.5^{a}	5.1 ± 2.1^{ac}	4.6 ± 2.0^{a}	4.84 ± 2.4^{a}	2.0 ± 1.2^{a}	1.1 ± 0.5^{c}
TSS (mg L⁻¹)	50.7±27.1	81.7±36.5	116.9±104.3	105.9±86.1	88.8±36.4	119.6±76.0	73.7±35.2	55.3±29.7

than at the shrimp ponds. It suggests that although the environmental conditions of the shrimp ponds and the estuary points were different, the sedimentation pond could improve water quality before returning it to the estuary (Gao et al. 2020). Meanwhile, aquaculture sedimentation ponds must be properly managed to avoid organic matter accumulation, which can favor the growth of toxic and harmful organisms (Chen et al. 2020; Möller et al. 2020; Hidayati et al. 2020). High oscillations in Secchi's transparency have already been reported in the Passos River estuary (Jorge Filho et al. 2013). This may suggest a high capacity for water renewal, which helps mitigate the impacts of aquaculture wastewater on the water quality of the estuary. Thus, inorganic compounds and TSS levels can be higher in upstream, depending on sea water flow. Finally, in the dry season higher concentration of salinity, pH and dissolved oxygen are favored by coastal waters influence which can result in greater remediation of water quality by the influence of sea water (Mourão et al. 2020).

Fig. 3 shows the temporal variation, throughout the shrimp production cycle, of chlorophyll-*a* (Fig. 3a and 3c) and total phosphorus (Fig. 3b and 3d). At the beginning of the production cycle, chlorophyll and phosphorus levels were similar both in the estuary and in shrimp ponds. Lower oscillations were observed at the points sampled in the estuary, on the other hand, relative higher oscillations have occurred in shrimp ponds in both parameters. Finally, at the end of the production cycle, mean values of chlorophyll-*a* and total phosphorus were higher in shrimp ponds than estuary points six and eight times, respectively.

The main reason for higher levels of phosphorus in aquaculture wastewater is due to feed offered daily

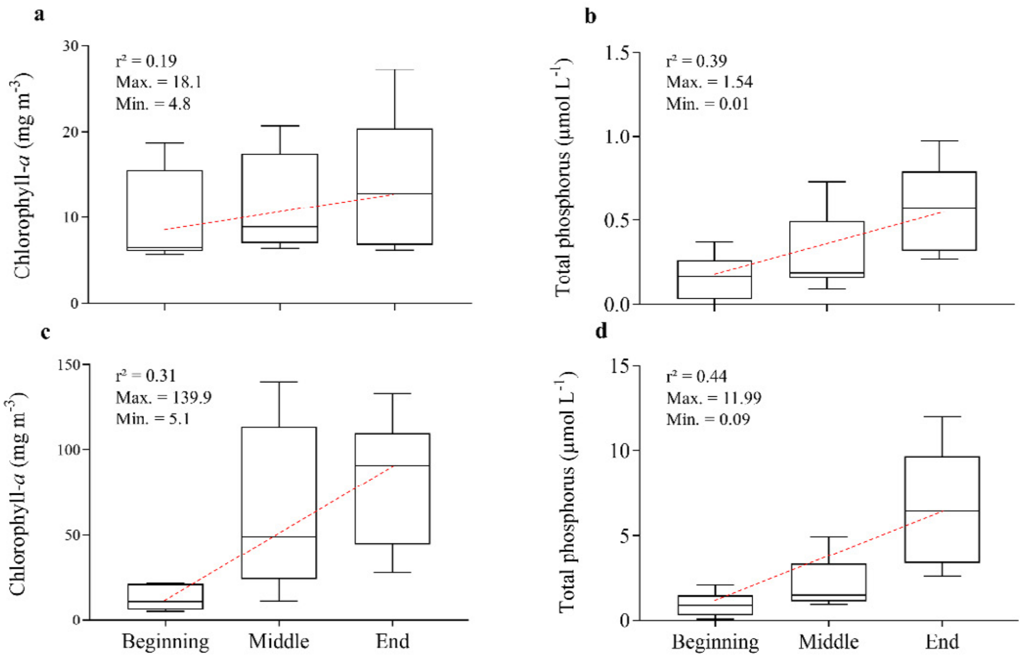

Fig. 3 Temporal variations of Chlorophyll-*a* and total phosphorus in the Passos River estuary (a and b) and the aquaculture ponds (c and d).

to shrimp ponds. Although phosphorus is a key nutrient in fish feed used in aquaculture, only about 40% is retained by animals (Sugiura 2018). Furthermore, microalgae uptake less phosphorus than nitrogen (Oliveira et al. 2020; Oliveira et al. 2021), and therefore, it is one of the main drivers for eutrophication (Amorim et al. 2020). Assessing phosphorus emissions in six estuaries from Northeastern Brazil, Lacerda et al. (2006) demonstrated that livestock (0.7 t km^{-2} $year^{-1}$) and agriculture (0.34 t km^{-2} $year^{-1}$) emit more phosphorus than aquaculture (0.23 t km^{-2} $year^{-1}$). It is worth noting that in recent years the feed used in aquaculture has increased its quality from the knowledge on the nutritional requirements of the shrimp (Guimarães et al. 2019). Thus, as the total phosphorus levels were up to seven times lower in the Passos River estuary and were within acceptable standards according to Brazilian legislation (CONAMA 357/05), it was not possible to observe a negative effect of shrimp farming wastewater on this parameter.

Chlorophyll-*a*, likewise as the phosphorus levels, is recognized as a good index of the productivity, and trophic condition of estuaries (Boyer et al. 2009; Millette et al. 2019) and they can estimate primary production and provide information on the trophic status of estuaries, inland, and seawaters. The levels of this parameter in the present study were according to common reported for tropical and neotropical estuaries that do not have influence of aquaculture activities (Otsuka et al. 2016; Pei et al. 2018; Mourão et al. 2020). The negative impact of wastewater (urban and agricultural) on water quality was visible in other neotropical estuaries, such as the Ipojuca and the Merepe rivers (Batista and Flores Montes 2014) and the Paraíba River (Correia et al. 2015), however, in the Passos River estuary, negative impacts may be mitigated by hydrodynamics. Precipitation, river flow and tidal regime are the main drivers that determine the water quality parameters, since the Passos River estuary is from small hydrographic basin, which possibly explains the low oscillation in the zooplankton community (Honorato da Silva et al. 2009; Aquino et al. 2012).

Zooplankton structure

In general, aquaculture ponds have presented zooplankton density higher than estuary places – only the aquaculture pond 9 had similar density to those found in the estuary (Fig. 4). The difference in zooplankton density among aquaculture ponds can be attributed to the different stages of shrimp culture that influence trophic composition and plankton density (Llario et al. 2019). Regarding the zooplankton structure,

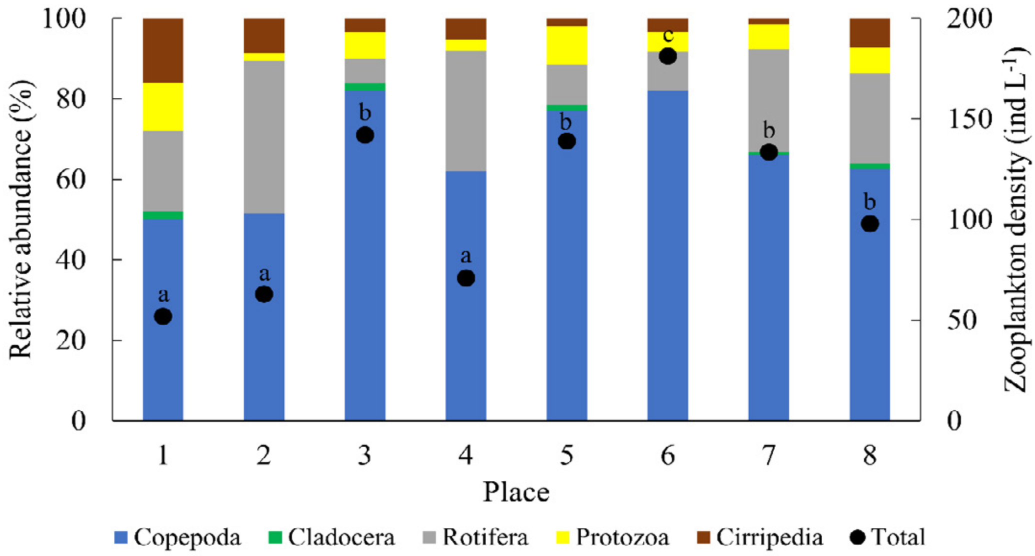

Fig. 4 Relative abundance (%) and density (individuals L^{-1}) of zooplankton in the Passos River estuary (places 1 and 2) and shrimp farming ponds (places 3 to 8). See Fig. 1 for more details on collection places.

Copepoda was the most abundant group in all environments analyzed. In upstream estuary, and also aquaculture ponds, higher Rotifers and lower Protozoa abundance were observed. On the other hand, in downstream place, Rotifers, Protozoa and Cirripedia were observed almost at the same proportion. The spatial variation of Rotifers on the estuary points may be attributed to the release of aquaculture wastewater as well as to salinity gradient. As suggested by Salvador and Bersano (2017), higher zooplankton diversity was generally found in the channels, depending on water circulation and salinity conditions. Herein, Copepoda (eleven taxa) and Rotifera (nine taxa) were the most diverse taxonomic groups (Table S1).

Generally, salinity is the major factor affecting the spatial variation of zooplankton structure community in estuaries (Wei and Xu 2014). This implies that the spatial pattern of rotifer communities could have been justified by salinity levels – but this parameter was not different in the studied points. In addition, the spatial and temporal dynamics of rotifers can be influenced by eutrophication level, driven mainly by food availability (Wen et al. 2017). In our study, although lower oscillations in environmental variables have been observed, interestingly the chlorophyll-*a* levels were slightly higher upstream than downstream which may explain the differences in the relative abundances of the zooplankton groups. The pattern of rotifers communities presented differences even in shrimp ponds, and it suggests that biotic factors, such as competition with Cladocera for food and predation by Copepoda (Nogrady 1993), has more than abiotic factors in the Passos River estuary. Our findings were in agreement with Arruda et al. (2017) that showed that habitat (aquaculture ponds vs water reservoir ecosystem) is more important than aquaculture activity in determining the rotifers occurrence.

The effects of phosphorus on zooplankton diversity are still debatable, and two hypotheses have been discussed: (1) a positive relationship (high phosphorus increases diversity) or (2) unimodal relationship (lower diversity at the both lower and upper extremes of phosphorus levels) (Abrams 1995; Rose et al. 2017; Wen et al. 2017). Considering that the levels of total phosphorus were up to eight times higher in the aquaculture ponds than in the estuary (Fig. 3), and that the highest densities of zooplankton were found in the shrimp ponds, our findings suggest that the positive correlation between the phosphorus content of zooplankton density, seems to be more suitable for the Passos River estuary.

Conclusions

This study has demonstrated that wastewater from shrimp farming did not influence the zooplankton community in a short time scale. Both zooplankton compositions and density were not influenced during a shrimp production cycle. Water quality also did not indicate a negative effect of aquaculture on the neotropical estuary, especially regarding phosphorus and chlorophyll levels, that are good indicators of artificial eutrophication. Although our study provides evidence that aquaculture does not negatively affect water quality and the zooplankton community in a neotropical estuary, there must be caution in decision making. That is important due to several factors, such as: (1) aquaculture wastewaters can have different composition depending on the culture system; (2) seasonal climate, estuary morphology and water flow regulation and; (3) environmental resilience and trophic structure of phytoplankton. To confirm this hypothesis, more data from various estuaries and long-time approaches on the relationship of environment-zooplankton based on their taxa and trait indices are also needed to better understand aquaculture wastewater effects on functioning of receiving aquatic ecosystems.

Data Availability Statement The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declaration of competing interests The authors declare that they have no conflict of interest.

Authors' contributions IGSS - data collect, water quality analysis and writing – review and editing; GPCS - formal analysis and writing – review and editing; CYBO - formal analysis and writing – original draft; CVFSC - formal analysis and writing – review and editing; LOB - writing – review and editing; AOG - writing – review and editing and supervision.

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