Research article

Zooplankton Community as Indicator of Trophic Status of Lake Tadlac in Los Baños, Laguna, Philippines

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Abstract

Keywords

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water quality;
zooplankton

Tadlac Lake was heavily used for aquaculture until the late 1990s which resulted in a deterioration of its water quality. Aquaculture operations were banned to rehabilitate the lake after a massive fish kill in 1999. However, no thorough assessment of the rehabilitation's effectiveness has been conducted. In order to assess the success of the rehabilitation efforts, this study evaluated the lake's trophic status through study of its zooplankton community and physical-chemical parameters. Zooplankton samples and physical-chemical parameters were collected once a month from October 2017 to March 2018. Overall, 25 zooplankton species were documented during the sampling period. Rotifera dominated the zooplankton community with a relative density of 46.19%, followed by Copepoda (36.70%) and Cladocera (17.11%). Eutrophic indicator species *Brachionus forficula* had the highest density among the zooplankton taxa, followed by *Keratella tropica* and *Brachionus havanaensis*. Significant variation in zooplankton density was documented over the sampling period ($p<0.05$). Cluster analysis and analysis of similarities (ANOSIM) showed variation in the zooplankton density across the sampling months. Similarity percentage (SIMPER) revealed that the differences in plankton density were due to the densities of the most abundant taxa. Canonical correspondence analysis (CCA) showed that the zooplankton community was highly influenced by dissolved oxygen, conductivity, pH, biological oxygen demand, and temperature. The high density of the eutrophic indicator zooplankton species and the nutrient concentration of the lake indicated that Tadlac lake was still under eutrophic conditions. Biotic indices further confirmed that the lake was experiencing eutrophic conditions with moderate organic pollution. Therefore, additional approaches are necessary to effectively control the nutrient enrichment in the lake to improve its condition.

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1. Introduction

One of the major problems in lake ecosystems is eutrophication [1]. Lake and river eutrophication is a gradual process that happens whenever there is external input of nitrogen and phosphorus into a water system from multiple sources such as fluvial, atmospheric, and groundwater sources [2]. Cultural (man-made) eutrophication has accelerated this process significantly [3]. These activities alter the balance of the aquatic communities, affecting plankton, benthos, and nekton [4, 5]. Hence, evaluating the trophic state of an aquatic body is critical for gaining a better understanding of how to manage and conserve the aquatic body.

Zooplankton are small, heterotrophic animals that inhabit and drift with the water currents on the surface of rivers, lakes, and oceans [6]. These organisms are frequently utilized as environmental condition and trophic status indicators due to their distinctive characteristics [7, 8]. Zooplankton are capable of responding promptly to changes caused by algal blooms, as well as bottom-up process or top-down controls, such as factors control and determine their abundance and composition [9-11]. Furthermore, zooplankton communities react swiftly to variations in physical-chemical parameters, which can impact their species richness, increase or decrease their abundance, and induce changes in their diversity [12, 13].

Lake Tadlac is a small, closed lake in Barangay Tadlac Los Baños, Laguna, Philippines, and is one of the eight crater lakes of Laguna de Bay (Laguna Lake), the largest lake in the Philippines. Lake Tadlac has a surface area of 248,000m^{-2}, an average depth of 27 m, and an annual turnover from December to February [14]. It was classified as an oligotrophic lake before the introduction of *Oreochromis* (Tilapia) aquaculture in 1986. The Tilapia farming caused eutrophication in the lake. Despite Philippine legislation dictating that only 10% of the lake's surface area be used for aquaculture, the fish cages filled about 90% of the lake's surface area. A massive fish kill occurred in December 1999 when its yearly turnover occurred, resulting in the massive mortality of all fishes in the lake in February 2000 and loss of around \$100,00 aquaculture investment. Government agencies decided to rehabilitate the lake by prohibiting aquaculture in the lake [15]. The aquaculture ban was still in place when this study was undertaken. Despite the rehabilitation effort, there has been no thorough evaluation of the lake's trophic state or water quality after rehabilitation. This information is empirical because it serves as the main indicator of a lake's recovery following rehabilitation. Thus, this study aimed to assess the trophic state of Tadlac lake by describing its zooplankton community and physical-chemical parameters. The present study might help shed light on the current state of Tadlac lake after its rehabilitation following a period of eutrophication caused by aquaculture. This evaluation is crucial in determining whether the rehabilitation activities have been successful and whether further restorative measures are necessary.

2. Materials and Methods

2.1 Study site

Samples were taken from seven stations, five in the littoral zone and two in the limnetic zone (Figure 1). Stations 1 (14.1843306°, 121.2051167°) and 2 were non-residential areas with vegetation cover (14.1816667°, 121.2042500°). The commercial zone (resort) was represented by station 3 (14.1802917°, 121.2059222°), station 4 covered residential areas (14.1818528°, 121.2085194°), while station 5 covered the poultry farm and residential areas (14.1847861°, 121.2082778°). The limnetic zone is represented by stations 6 (14.1817222°, 121.2055556°) and 7 (14.1847861°, 121.2068889°). Every sampling station was divided into three sampling points with a 100-meter

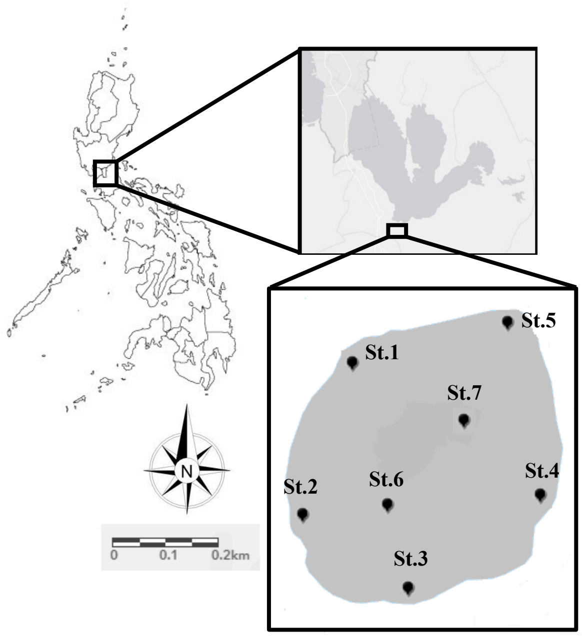

Figure 1. Map of Lake Tadlac showing the seven (7) sampling stations. Littoral Zone: Station 1-2 non-residential area; Station 3 commercial area (resort); Station 4 residential area; Station 5 poultry farm and residential area. Limnetic Zone: Station 6 and Station 7.

spacing between them, and then further divided into three sub-points with at least a 10-meter interval between them. Because of the local government's rehabilitation efforts, the lake was currently closed to aquaculture activities when the collection was done.

2.2 Sample collection

The study was conducted in Tadlac Lake (14º10′57″N, 12º112′23″E), which is situated in Brgy, Tadlac Los Baños, Laguna, Philippines. Los Baños experiences Philippines Type I climate, with two pronounced seasons: dry from November to April and wet during the rest of the year [16]. Sampling was conducted on the 30th of each month from October 2017 to March 2018; wherein October falls within the wet season and the remaining sampling months fall in the dry season. Zooplankton samples for the littoral and limnetic zones were collected using a 20-mesh plankton net by vertical towing (10 m depth) at the identified stations. Three replicates of water samples were obtained for each sampling location. All water samples were preserved with 10% buffered formalin and stored in 1000 mL PET bottles.

Eight physicochemical parameters were measured as possible factors that could influence plankton species composition and distribution in the sampled sites: (1) temperature (°C), (2) pH, (3) electrical conductivity (µS·cm^{1}), and (4) dissolved oxygen concentration (mg·L^{1}) were measured on-site with PASPORT multi-parameter water quality meter (PASCO PS-2230-PHT); (5) transparency (meters) was measured using Secchi disk; (6) biochemical oxygen demand (BOD) (mg·L^{1}) was measured using BOD 5 day method, and (7) phosphate (mg·L^{1} PO_4-P) and (8) nitrate (mg·L^{1} NO_3-N) concentrations were measured spectrophotometrically with Hach PhosVer and NitraVer reagent powder pillows, respectively.

2.3 Specimen processing and identification

A 20-meter mesh net was used to filter water samples, separating the zooplankton from the water. After that, the residuum (what was left after the water was removed) was diluted with 20 mL of water filtrate. To homogenize the material, the container was vigorously shaken, and a 1 mL aliquot was taken out with a pipette [17]. The aliquot was dispensed in a Sedgewick rafter and the zooplankton present was then counted using an electric compound microscope at 100x magnification. Counting was done three (3) times per sample. Zooplankton was identified to the lowest possible taxon using the identification keys of Mamaril *et al*. [18], and Petersen [17], and the density of individual species (individuals·m^{-3}) was calculated.

For the Copepod species, Calanoida and Cyclopoida species present from the aliquot were sorted and then dissected to isolate the P5 to identify to the genus or species level. The density of zooplankton was determined using the formula:

$$\text{density} = \frac{\text{number of individuals} * \text{ volume of water concentrate}}{\pi r^2 d}$$

where:
π = 3.1416
r = is the radius of the plankton net used
d = depth of the vertical towing

2.4 Biotic index

The saprobic index for zooplankton was also used to determine the trophic state of the lake. Saprobic indices (S) were calculated using the formula proposed by Pantle and Buck [19]:

$$\text{SI} = \frac{\sum s \times h}{\sum h}$$

where:
SI = is the Pantle and Buck (1955) saprobic index;
s = is the saprobic index of species “i” [19];
h = is the relative frequency (1, uncommon; 3, common; 5, abundant)
The saprobic index S is based on the following ranking scale: ≤1.5, oligosaprobic with low organic pollution; 1.6-2.6, mesosaprobic with moderate organic pollution; ≥2.7, eusaprobic with high to very high organic pollution [20].

Wetland Zooplankton Index (WZI) was calculated using the equation of Lougheed and Chow-Fraser [21] to further assess the status of the lake:

$$\text{WZI} = \frac{\sum \text{Yi} \times \text{Ti} \times \text{U}}{\sum \text{Yi} \times \text{Ti}}$$

where:
Yi =Abundance of species i,
Ti = Tolerance value (1-3) of species i,
Ui = Optimum value (1-5) of species i

The WZI values, which are used to indicate the level of water quality or eutrophication, ranged between 1.0 and 5.0. A value of 1.0 indicated low water quality (high eutrophication) whereas a value of 5.0 indicated high quality (low eutrophication). Tolerance values, represented by T, are used to measure the range of distribution or niche breadth of a species. As suggested by Lougheed and Chow-Fraser [21], species with a narrow distribution range were assigned a low tolerance score.

2.5 Data analysis

The normality of the data was first assessed through Shapiro-Wilk normality test. Due to significant deviation from normal distribution ($p<0.01$) of the zooplankton density data, a log10 (x+1) transformation was applied to improve homoscedasticity and normality. The differences in physicochemical parameters across sampling periods and zooplankton density across different sampling periods were examined using one-way analysis of variance (ANOVA) with Tukey's honest significant difference (HSD) as a post hoc analysis. To assess the similarity of zooplankton abundance between sampling months, cluster analysis using the Bray-Curtis similarity index was conducted. The zooplankton community structure was evaluated using Analysis of similarities (ANOSIM) to test for statistical differences among sampling periods. Absolute partitioning among assemblages was ascertained using ANOSIM pairwise R-values, with R values higher than 0.25 denoting separated groups [22, 23]. Similarity percentage (SIMPER) was applied to quantify the contribution of each species to the dissimilarity among sampling periods [24]. Canonical correspondence analysis (CCA) with type II scaling was employed to determine the physicochemical parameters that contributed to the variation of zooplankton density throughout the sampling period. All statistical analyses, except for ANOVA and post hoc tests, were conducted using Paleontological Statistics Software [25]. The ANOVA and post hoc tests were analyzed via Statistical Program for Social Science (SPSS) version 20.

3. Results and Discussion

3.1 Environmental parameters

All the physicochemical parameters that were measured showed statistical variance (Table 1). October (31.00°C), November (32.00°C), February (30.78°C), and March (31.74°C) had the highest mean surface water temperatures. December (26.30°C) and January (29.00°C) had lower temperatures. Similarly, in December (2.48 mg L^{-1}) and January (0.51 mg L^{-1}), DO dropped dramatically. The highest DO value was 10.43 mg L^{-1} with a mean of 6.17 mg L^{-1}. In December and January, the lowest Biological Oxygen Demand (BOD) was observed. The highest BOD, on the other hand, was recorded in November at 3.20 mg L^{-1}. October (8.95) and November (10.00) had the highest pH readings. On the other hand, the lowest pH (7.70) was documented in December. The average Secchi disk transparency (SDT) measurements were between 1.17 and 2.58 m. The highest SDT values were 2.58 m and 1.92 m in October and March, respectively, while the lowest SDT values were 1.17 m and 1.29 m in November and December, respectively. The electrical conductivity (EC) of the samples ranged from 847.00 to 1,048 µS cm^{-1}. The greatest EC value (1,048 µS cm^{-1}) was recorded in January, while the lowest was recorded in October (847 µS cm^{-1}). December had the highest nitrate concentration (1.55 mg L^{-1}) and January had the lowest (0.46 mg L^{-1}). The highest phosphorus concentration was in December (0.68 mg L^{-1}), while the lowest was in November (0.23 mg L^{-1}). Water quality classifications based on the Philippines' Department of Environment and Natural Resources [26] range from public water supply class 1 (Class AA) to industrial water quality class II supply (Class D). The mean values of the observed environmental parameters fell into the "Class C Water" category, which was appropriate for fish and other aquatic resource propagation and growth. Furthermore, the reported nutrient levels met the criteria for a eutrophic body.

Table 1. Summary of the measured physicochemical parameters during the six-sampling period

Environmental Parameter	Sampling Period						
	October	November	December	January	February	March	Mean
Temperature (°C)	31.00±0.27[c]	32.00±0.10[b]	26.30±0.05[a]	29.00±0.11[c]	30.7±0.01[c]	31.74±0.17[c]	29.97
pH	8.95±0.04[b]	10.00±0.01[c]	7.70±0.02[a]	8.73±0.02[b]	8.36±0.04[b]	8.55±0.01[b]	8.87
Transparency (m)	2.58±0.05[c]	1.17±0.01[a]	1.29±0.04[a]	1.62±0.02[b]	1.60±0.01[b]	1.92±0.01[c]	1.70
Conductivity (μS·cm^{-1})	847.00±4.9[a]	848.00±6.20[a]	939.00±12.60[b]	1048±8.21[c]	967.57±10.07[b]	865.48±3.97[a]	919.18
DO (mg·L^{-1})	7.58±0.07[c]	7.44±0.05[c]	2.48±0.12[b]	0.51±0.02[a]	8.51±0.01[d]	10.43±0.18[e]	6.17
BOD (mg·L^{-1})	2.74±0.15[c]	3.20±0.10[c]	1.09±0.07[b]	0.15±0.01[a]	2.23±0.18[c]	2.97±0.14[c]	2.06
Nitrates (mg·L^{-1})	1.00±0.05[b]	1.09±0.04[b]	1.55±0.06[d]	0.46±0.02[a]	0.99±0.18[c]	1.29±0.34[b]	1.06
Phosphates (mg·L^{-1})	0.26±0.02[b]	0.23±0.02[b]	0.68±0.02[a]	0.35±0.02[b]	0.23±0.01[b]	0.44±0.01[b]	0.37

*Values with the different superscript within the same row are not significant at $p<0.05$.

Eutrophic lakes have nitrate nitrogen levels of between 0.5 and 1.5 mg L^{-1} [27]. Lakes with phosphate values of less than 0.010 mg L^{-1} are categorized as oligotrophic, whereas those with concentrations greater than 0.020 mg L^{-1} are classified as eutrophic [28]. The mean nitrate concentration of the lake was 1.060 mgL^{-1}, while the mean phosphate concentration was 0.370 mg L^{-1}. According to the results of the nutrient analysis, Lake Tadlac is eutrophic. Domestic sewage effluent, urban run-off, and farm waste are the main sources of organic pollution and nutrient enrichment [29, 30]. Despite the absence of aquaculture, the lake's primary source of nutrients and organic matter was a poultry farm, and commercial and residential areas. These establishments could be a significant source of organic materials in the lake. Because the lake is a closed lake (no inlet or outflow), runoff from these sources might stay in the lake and accumulate over time.

Gonzales and Flavier [31] evaluated the physicochemical parameters of Tadlac Lake when the lake was heavily used for fish farming (Table 2). Compared to their findings, it can be deduced that the lake's water quality has improved, notably in terms of dissolved oxygen, transparency, biological oxygen demand, and nitrate. DO values were much higher in the present study, which reflects the improvement in the physical-chemical parameters of the lake. This could be attributed to the lower organic matter loading in the lake that needs DO during decomposition. This is also mirrored in the current lower BOD value recorded. Electrical conductivity was higher in the present study which could be ascribed to the higher temperature measurement. Bai *et al*. [32] state that electrical conductivity increases when the temperature increases. Even though parameters are within the accepted value for a lake system, the nitrate and phosphate concentrations of the lake clearly showed that the lake is still in eutrophic state.

3.2 Zooplankton species composition and distribution

The zooplankton composition of Lake Tadlac was represented by three major groups, namely Rotifera, Cladocera, and Copepoda, which constituted a total of 25 taxa. The taxa were distributed into 20 species of Rotifera, 3 species of Cladocera, and 2 species under Copepoda. Nauplius, the larval stage of copepod was not treated as separate taxa as it accounts for all larval form of copepod in the lake. Rotifera species are highly represented in Lake Tadlac with an overall relative density

Table 2. Comparison of physico-chemical parameters of Tadlac Lake before and after the aquaculture ban

Environmental Parameter	Present Study	Gonzales and Flavier [31]	Optimum Value based on DENR Water Quality Standard 2019 [26]
Temperature (ºC)	29.97	26.60	25-31
pH	8.87	7.97	6.5-9.0
Transparency (m)	1.70	1.14	*
Conductivity ($\mu S \cdot cm^{-1}$)	919.18	421.16	*
DO ($mg \cdot L^{-1}$)	6.17	2.64	5 $mg \cdot L^{-1}$ minimum
BOD ($mg \cdot L^{-1}$)	2.06	2.63	7 $mg \cdot L^{-1}$ maximum allowable limit
Nitrates ($mg \cdot L^{-1}$)	1.06	2.29	7 $mg \cdot L^{-1}$ maximum allowable limit
Phosphates ($mg \cdot L^{-1}$)	0.37	0.32	0.50 $mg \cdot L^{-1}$ maximum allowable limit

*No optimum values were set.

of 46.19%, followed by Copepoda (36.70 %), and Cladocera (17.11 %) (Figure 2). This can be attributed to their wide tolerance to physical and chemical factors like DO, salinity [33], and temperature [34]. Additionally, the diversity of rotifers could also be ascribed to some of their special characteristics, such as their high reproductive rate, frequent asexual reproduction, and a set of life traits that make them opportunistic and characteristic of r-strategists. These traits have enabled them to thrive in unstable and eutrophic environments [35, 36]. Furthermore, their ability to consume diverse food particles, including algae, detritus of various sizes, and bacteria could favor their abundance. These characteristics, in turn, make them highly adaptable to different ecosystems [37]. Furthermore, according to Leitao *et al*. [38], several species of Rotifera possess the capacity to adjust to environmental changes due to the formation of diapause stages as one of their strategies associated with increased resistance to environmental extremes [39]. Also, as compared to Cladocerans and Copepods, Rotiferan species are smaller in size; thus, they are not highly visible to fish predators allowing them to avoid predation [40].

In the littoral zone, Rotifera, Cladocera, and Copepoda had relative densities of 75.73%, 16.92%, and 7.35%, respectively. While for the limnetic zone, the relative densities of Rotifera, Cladocera, and Copepoda were 16.65%, 17.31%, and 66.04%, respectively (Figure 2). It was apparent that Rotifera and Cladocera had higher relative densities in the littoral zone while Copepoda had higher relative densities in the limnetic zone. Although the limnetic and littoral zones are connected, the two zones can produce microhabitats that can affect the distribution of zooplankton organisms. The presence of aquatic macrophytes in the littoral zone can create diverse environments with different levels of structural complexity [41, 42]. Vegetated beds with high levels of structural heterogeneity offer organisms refuge from predators, as well as ideal spawning and foraging grounds, which facilitate trophic interactions among a wide variety of organisms [43, 44]. Duggan *et al*. [45] and Smith [46] stated that around 75% of rotifers inhabit the littoral zone, and this is due to the microhabitat brought about by the macrophytes that prevent them from being eaten by their predators. Moreover, this area offers more food sources as compared to the limnetic zone [47]. Conversely, copepods can undergo nocturnal diel vertical migration to avoid predation. This could explain why copepods dominated the limnetic area even though they are exposed to predators [48].

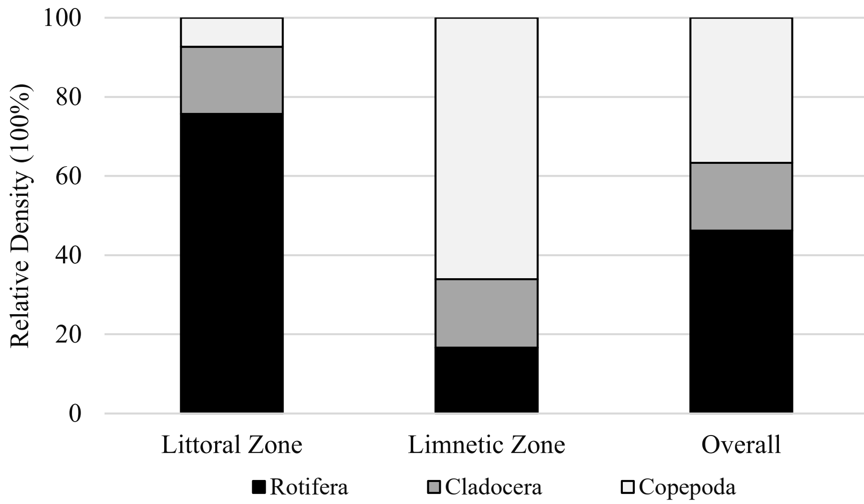

Figure 2. Zooplankton relative density between limnetic zone and littoral zone

One-way ANOVA revealed that there was variation in zooplankton density across sampling months ($p<0.05$) for all taxa (Table 3). The highest mean densities were recorded in October, November, and March. Likewise, the highest number of species present was also noted during this time, with 18 species documented in October and 21 and 22 species in November and March, respectively. A decrease in zooplankton density and species number was observed from December to February. *Brachionus forficula, Brachionus havanaensis, Conochilus dossuarius,* Copepod Nauplius, and *Keratella tropica* were consistently abundant throughout the sampling period. The rotifer community of Lake Tadlac is typical for water bodies with high nutrient levels (eutrophic body) which is evident in the abundance of *Brachionus* spp. among all rotifer taxa. This together with absence of *Trichocerca* spp. is an indicator of eutrophic lakes [49].

The top three taxa that obtained the highest mean density (MD) and relative density (RD) (Figure 3) were *Brachionus forficula* (MD: 2,691 indiv/m^3; RD: 61.50%), *Keratella tropica* (MD: 1,854 indiv/m^3; RD: 42.37%), and *Brachionus forficula* (MD: 1,731 indiv/m^3; RD: 39.56%) (Figure 4). These three zooplankton species are known as indicators of eutrophic conditions. Berzins and Pejler [50] suggested that the presence of *Keratella* and *Brachionus* species in a water body was an indication of high nutrient levels. Additionally, Sladecek [51] and Nogueira [52] identified *Brachionus* species as biological indicators of eutrophic conditions. Ceirans [53] stated that rotifers, particularly those belonging to the *Brachionus* genus, are better indicators of trophic conditions than crustaceans since they are less impacted by algal blooms. The high densities of *Brachionus* species in Lake Tadlac suggest that the lake is still experiencing eutrophication. Ismail and Adnan [54] conducted a similar study where they evaluated the distribution and abundance of zooplankton in Harapan and Aman Lakes, which were both experiencing algal blooms. Their findings indicated that both lakes were in a eutrophic condition, based on the abundance of zooplankton indicator species such as *Brachionus forficula* and *Brachionus nilsoni*.

Brachionus havanaensis, the second most abundant zooplankton species in Tadlac lake, is also an indicator of eutrophic conditions as evidenced in earlier research. Frutos *et al*. [55] compared the composition and abundance of zooplankton communities in a lake affected by domestic sewage (Soto Lake) and in an unaffected lake (Sanches Lake). One of the abundant zooplankton species

Table 3. Mean density (MD = no. of inds/m^{-3}), relative density (RD = %) of zooplankton species in Tadlac Lake during the sampling period

Taxa	Density (individuals/m^{-3})						MD	RD (%)	P value
	OCT	NOV	DEC	JAN	FEB	MAR			
ROTIFERA									
1. *Anuraeopsis coelata*	0	0	89	14	56	488	108	0.98	0.001
2. *Conochilus dossuarius*	2,769	2,587	208	143	165	172	1,007	9.13	0.001
3. *Asplanchna* sp.	1,043	869	61	14	39	400	404	3.66	0.001
4. *Collotheca* pelagica	251	89	0	0	243	383	161	1.46	0.010
5. *Brachionus forficula*	4,951	4,208	2,051	247	381	3,998	2,639	23.91	0.001
6. *Brachionus havaenesis*	2,332	4,135	475	24	0	344	1,218	11.04	0.001
7. *Filinia longiseta*	125	0	0	0	0	228	59	0.53	0.001
8. *Hexarthra* sp.	2,656	1,067	0	4	29	854	768	6.96	0.001
9. *Keratella tropica*	4,111	1,322	30	51	19	2,964	1,416	12.83	0.001
10. *Lecane bulla*	28	234	0	0	4	197	77	0.70	0.001
11. *Lecane closterocerca*	28	0	30	0	5	32	16	0.14	0.001
12. Lecane curvicornis	0	32	0	0	4	28	11	0.10	0.001
13. *Lecane decipiens*	0	32	0	0	4	6	7	0.06	0.001
14. *Lecane papuana*	28	30	30	0	0	38	21	0.19	0.001
15. *Lecane pyriformis*	28	58	0	0	0	20	18	0.16	0.001
16. *Lecane subtilis*	0	30	30	0	0	0	10	0.09	0.001
17. *Lepadella patella*	0	0	0	0	4	20	4	0.04	0.001
18. *Platyias quadricornis*	0	89	30	0	0	0	20	0.18	0.001
19. *Polyarthra vulgaris*	699	536	30	38	38	730	345	3.13	0.020
20. *Testudinella patina*	28	61	30	13	13	158	50	0.46	0.001
CLADOCERA									
1. *Ceriodaphnia cornuta*	0	61	59	17	105	154	66	0.60	0.001
2. *Diaphanosoma* sp.	61	91	150	30	77	378	131	1.19	0.001
3. *Moina micrura*	28	30	75	8	14	0	26	0.24	0.001
COPEPODA									
1. *Arctodiaptomus dorsalis*	2,130	327	0	0	70	1,434	660	5.98	0.001
2. *Thermocyclops* sp.	1,067	119	0	0	106	2,890	697	6.32	0.001
3. Copepod nauplius	2,405	566	15	0	451	3,145	1,097	9.94	0.001
SPECIES NUMBER	18	21	15	12	19	22			
OVER MEAN ZOOPLANKTON DENSITY	953[a]	637[b]	131[c]	23[d]	63[d]	331[e]			0.001

*Values with the same superscript are not significant at α=0.05.

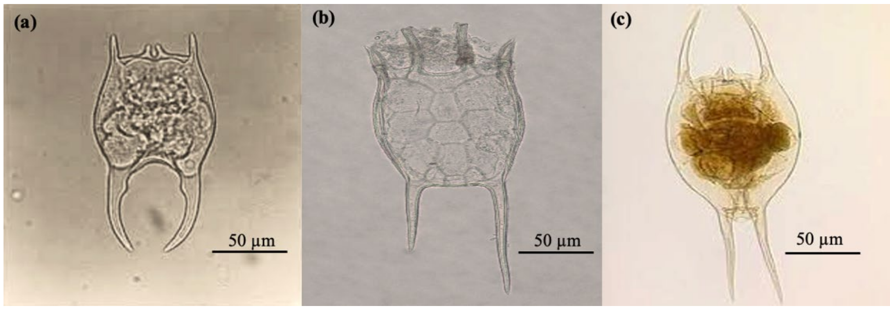

Figure 3. Top three zooplankton taxa with the highest density in Lake Tadlac during the study period. (a) *Brachionus forficula*, (b) *Keratella tropica*, and (c) *Brachionus havanaensis*

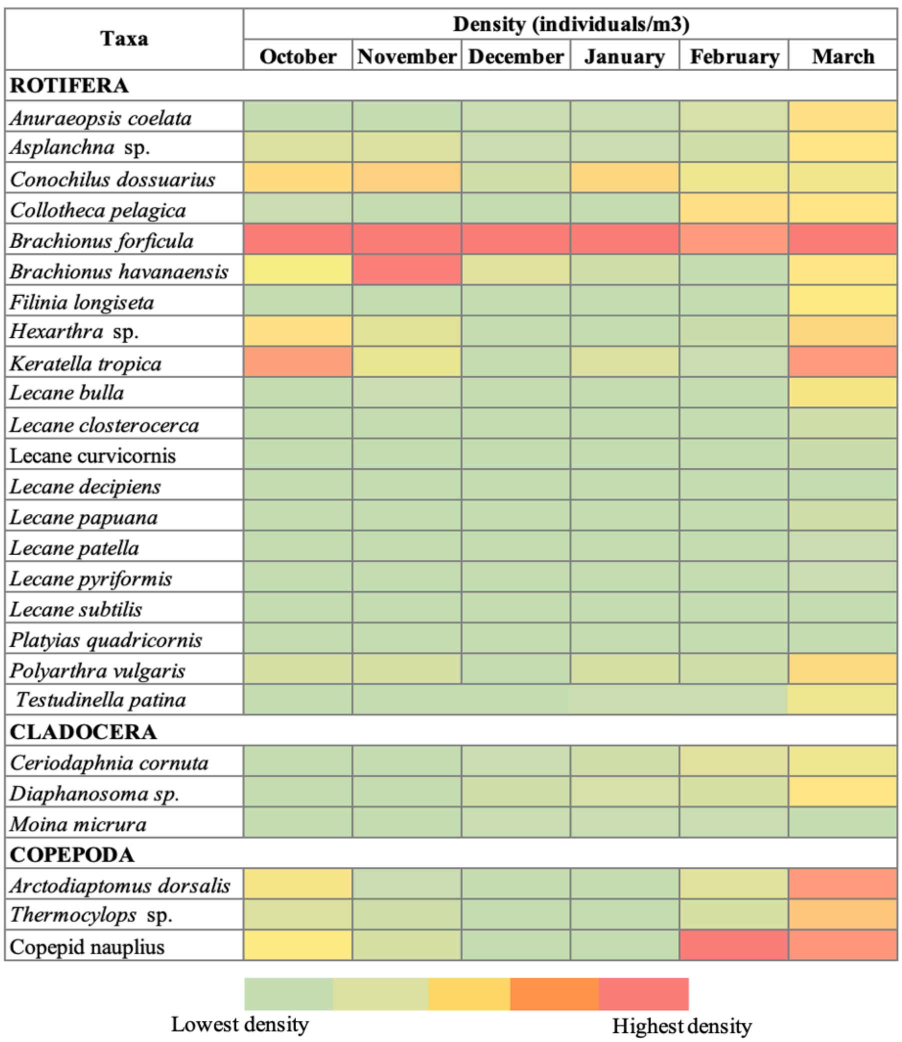

Figure 4. Heatmap showing the monthly densities of zooplankton species in Tadlac Lake during the 6-month sampling period.

documented in lake Soto was *Brachionus havanaensis* which suggests that the lake was eutrophic. Sendacz *et al*. [56] investigated the trophic status of 8 reservoirs in Sao Paulo Brazil. Th results of their study showed that the abundance of *Brachionus havanaensis* in all reservoirs indicated the eutrophic condition of the surveyed water body.

Cluster analysis with Bray-Curtis similarity index was used to determine the similarity of zooplankton abundances during the 6-sampling period (Figure 5). Two major clusters were formed based on the zooplankton abundance. The months of March, November, and October formed one cluster with November and October being highly similar in the clade while December, January, and February were grouped into one major cluster. January and February formed one tight clade due to the abundance of zooplankton during these months being more similar compared to that recorded in December. The peak of the zooplankton abundance was recorded during October, November, and March. Zooplankton abundance during October and November was comparable, which could explicate why these months were clustered tightly together. Analysis of similarities (ANOSIM) divulged that the clusters in the dendrogram were significantly different (R-value = 0.93, P<0.05). These findings are consistent with the previous results of ANOVA. This could suggest that there was temporal variation in the zooplankton density in Lake Tadlac.

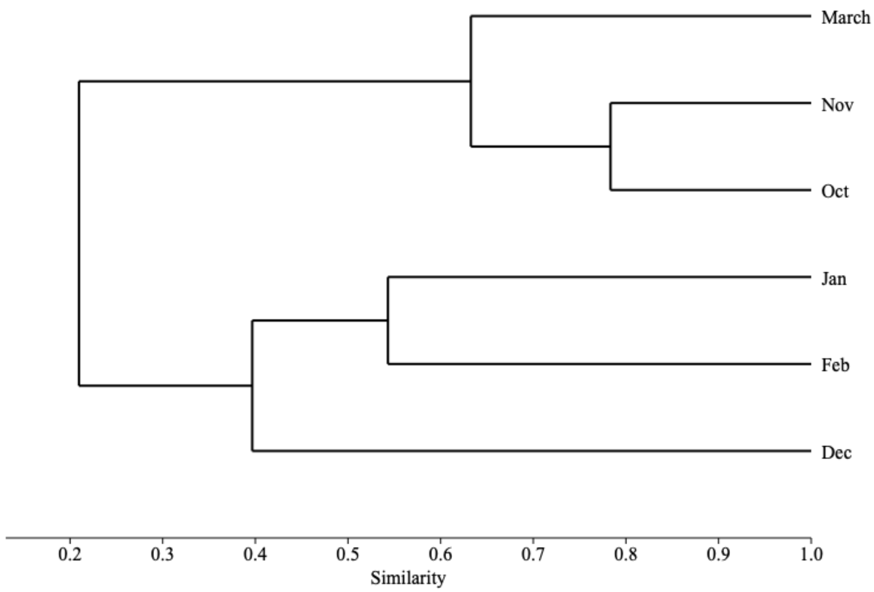

Figure 5. Cluster analysis of zooplankton abundance in the different sampling periods

Similarity percentage (SIMPER) was also done to determine which species contributed most to the differences in zooplankton abundance during the 6-month study period (Table 4). Findings in the SIMPER analysis further support the result of the ANOSIM where high dissimilarity in zooplankton abundances among months ranged from 18.32.% to 73.47%. The observed variations in the zooplankton abundance during the 6 sampling months were contributed to mainly by the abundance of the eutrophic indicator species *Conochilus dossuarius, Brachionus forficula, Keratella tropica, Brachionus havaenaensis,* and Copepod nauplius.

Table 4. Average percentage dissimilarities of the 6-month sampling period based on zooplankton abundance and the taxa responsible for the dissimilarities

Comparison of Sampling Months		Average Dissimilarity (%)	Leading Contributing Taxa for Dissimilarity	Percentage Contribution per Taxon
October	November	35.02%	*Keratella tropica*	19.35%
	December	79.03%	*Keratella tropica*	18.42%
	January	95.69%	*Brachionus forficula*	19.45%
	February	87.75%	Nauplius	69.32%
	March	32.41%	*Brachionus havanenensis*	18.32%
November	October	35.02%	*Keratella tropica*	19.35%
	December	69.02%	*Conochilus dossuarius*	26.92%
	January	93.20%	*Conochilus dossuarius*	26.02%
	February	92.15%	Nauplius	70.60%
	March	52.24%	*Conochilus dossuarius*	20.42%
December	October	79.03%	*Keratella tropica*	18.45%
	November	69.02%	*Conochilus dossuarius*	26.92%
	January	76.97%	*Brachionus forficula*	58.78%
	February	96.60%	Nauplius	73.47%
	March	73.98%	Nauplius	18.91%
January	October	95.69%	*Brachionus forficula*	19.45%
	November	93.20%	*Conochilus dossuarius*	26.02%
	December	76.67%	*Brachionus forficula*	58.78%
	February	99.47%	Nauplius	72.69%
	March	94.54%	*Brachionus forficula*	20.20%
February	October	87.75%	Nauplius	69.32%
	November	92.15%	Nauplius	70.60%
	December	96.60%	Nauplius	73.47%
	January	99.47%	Nauplius	72.68%
	March	86.47%	Nauplius	72.20%
March	October	32.41%	*Brachionus havaenensis*	18.32%
	November	52.24%	*Conochilus dossuarius*	20.42%
	December	73.98%	Nauplius	18.91%
	January	94.54%	*Brachionus forficula*	20.20%
	February	86.47%	Nauplius	72.20%

3.3 Environmental parameters as a factor of zooplankton assemblages

The parameters that can best explicate the observed variation in the zooplankton community were dissolved oxygen, conductivity, pH, biological oxygen demand, and temperature based on the CCA ordination (Figure 6). Axis 1 of the CCA triplot was positively correlated with pH but negatively correlated with dissolved oxygen (59.93% variance, an Eigenvalue value of 0.27). The second canonical axis, with an Eigenvalue of 0.09 and variance of 20.23%, was negatively correlated with transparency, temperature, and BOD. Most of the zooplankton taxa were not positioned on the decreasing value of dissolved oxygen (DO). Particularly, *Lecane subtilis, Moina micrura*, and *Platyias quadricornis* were positioned on the opposite direction of the DO vector. This would indicate that decreasing dissolved oxygen is not favorable for the abundance of zooplankton. DO is one of the major factors which affects the zooplankton abundance in Lake Tadlac. DO is very important for the aerobic respiration of aquatic organisms like the zooplankton [57], thus low DO during December and January greatly influenced the zooplankton community. Moreover, BOD,

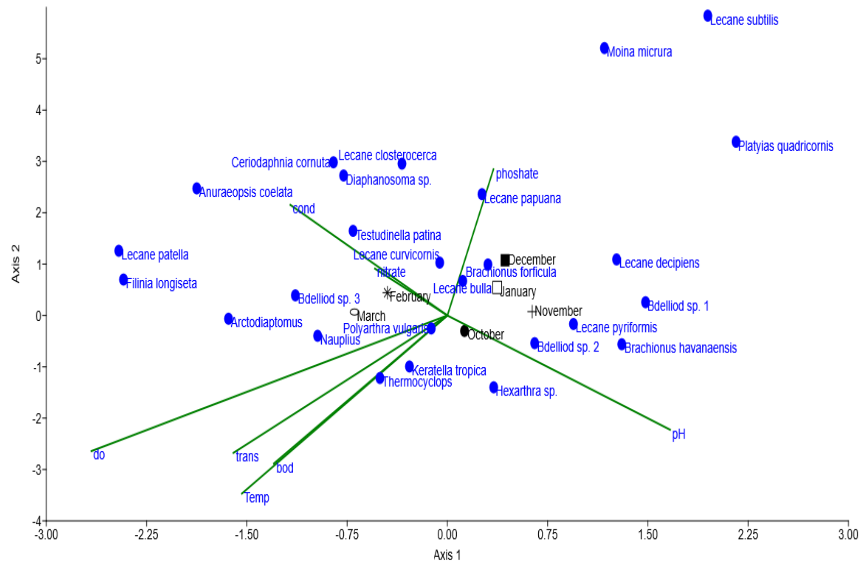

Figure 6. Canonical correspondence analysis based on zooplankton abundance and environmental parameters during the 6-month sampling period

which measures the amount of oxygen consumed by microorganisms when they decompose organic matter in water [58] was also an important parameter that affects zooplankton assemblages. If BOD is higher, high DO concentration is used for decomposition and thus lowers the available DO concentration for respiration of organisms.

Temperature is also known to have a direct effect on the zooplankton community by influencing reproductive activity, the rate of molting, and the rate of egg development. These activities increase as the temperature rises. Low temperature can initiate physiological changes which cause the adult zooplankton to produce resting stages [59]. This could explain why few zooplankton species were found at decreasing temperature vector in the CCA.

Water transparency was an important parameter for zooplankton abundance as well as conductivity and pH. An increase in turbidity may favor larger species like copepods and cladocerans since it hinders the visualization of the predator, allowing these zooplankton species to avoid predation. Furthermore, depending on the quality of suspended matter, the matter can serve as food for zooplankton organisms (e.g., phytoplankton), contributing to higher abundance values. The water pH has an indirect effect on zooplankton abundance. The pH level of the lake can stimulate phytoplankton blooms, which are one of the major foods of zooplankters [60-62].

3.4 Biotic indices

For the saprobic index (SI), the calculated values ranged from 1.65 to 1.84, with a mean value of 1.73 (Table 5). As classified by Battes and Momeu [20], SI values ≤1.5 indicate an oligosaprobic water body with low organic pollution, 1.6-2.6 signifies a mesosaprobic water body with moderate organic pollution, while SI value greater than or equal to 2.7 indicates eusaprobic water body with high to very high organic pollution. Based on the zooplankton saprobic index, Lake Tadlac is experiencing mesosaprobic conditions with moderate organic pollution as the SI values fall within the range of 1.6-2.6.

Table 5. Summary of calculated values for saprobic index and wetland zooplankton index

Sampling Month	Saprobic Index*	Wetland Zooplankton Index**
October	1.75	3.72
November	1.84	2.79
December	1.71	2.27
January	1.65	2.28
February	1.65	2.06
March	1.76	3.00
Mean	1.73	2.69

*Indication for saprobic index: ≤1.5 oligosaprobic zone with low organic contamination, 1.6-2.6 mesosaprobic with moderate organic pollution, ≥2.6 eusaprobic zone with high to very high organic pollution

**Indication for wetland zooplankton index: 1.0 hyper eutrophication, 3.0 mesotrophic, 5.0. oligotrophic

For the Wetland Zooplankton Index (WZI), the values computed range from 2.06 to 3.72, with a mean of 2.69. The lowest WZI was observed during February while the highest was recorded during October. According to Lougheed and Chow-Fraser [21], 1.0 WZI value is indicative of low water quality (high eutrophication), 5.0 indicates high water quality (low eutrophication), and a 3.0 value signifies mesotrophic conditions. The values for WZI indicated eutrophic conditions except for October and March, herein the calculated WZI value was 3.00 and 3.72 (mesotrophic), respectively. However, the mean WZI indicates that the lake is still in eutrophic condition (2.69). Overall, the result of the biotic indices suggests that despite the rehabilitation and a slight improvement in the physicochemical parameters of Lake Tadlac, it is still eutrophic and experiencing moderate organic pollution.

Effluent discharge from anthropogenic activities such as farm waste, domestic sewage, and urban run-off are the major sources of organic pollution [29, 30, 63]. These point sources were present around Tadlac lake. The point sources observed were residential areas, a poultry farm, and a resort (Laresio resort). These establishments were possible contributors of organic matter loading in the lake. The eutrophic conditions of the lake could be ascribed to these sources despite the total ban of aquaculture.

4. Conclusions

The zooplankton community of Lake Tadlac was assessed in this study. During the study period, a total of 25 taxa of zooplankton species were documented in Lake Tadlac, which could be classified into three major groups namely, Rotifera, Cladocera, and Copepoda. Rotifera was the most dominant among all zooplankton groups in Lake Tadlac in terms of density and number of species. Eutrophic indicator species *Brachionus forficula, Keratella tropica,* and *Brachionus havanaensis* showed the highest densities among the zooplankton species. Statistical variation in the zooplankton density was noted across the sampling period, which is generally influenced by dissolved oxygen, biological oxygen demand, temperature, pH, and conductivity. Water quality parameters particularly nutrient concentration, the abundance of eutrophic indicator species, and the calculated values of biotic indices revealed that the lake is still experiencing eutrophication despite the rehabilitation done. The local government must consider the mitigation of organic matter discharge into the lake. Moreover, periodic monitoring of the zooplankton community and physical-chemical parameters must be implemented to fully elucidate recovery of the lake in terms of trophic status and water quality. This could help improve the trophic status and water quality of the lake.

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