



Journal of  
**Fisheries and  
Aquatic Science**

ISSN 1816-4927



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## Research Article

# Levels of Available Nitrogen-Phosphorus Before and After Fish Mass Mortality in Maninjau Lake of Indonesia

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## Abstract

**Background and Objective:** Maninjau lake is one of the important locations for aquaculture activity for many local people in Indonesia. The objective of the current research was to estimate the Nitrogen (N), Phosphorus (P) and Total Organic Matter (TOM) before and after fish mass mortality in Maninjau lake. **Materials and Methods:** This research was conducted in February, 2017, four months after mass mortality occurred. Nile tilapia (*Oreochromis niloticus*) and Common carp (*Cyprinus carpio*) mortality were occurred in August and September, 2016. Data were collected from four stations on Maninjau lake (Muko-Muko, Pasa, Pandan and Sungai Tampang). Water samples were taken from the surface (depth 0.1 m) and under floating net cages (depth 30 m) at each station and analyzed for N, P and TOM content. Water quality data taken in February, 2016 was used as a comparison. The differences between N, P, TOM, Particulate Organic Matter (POM) and Dissolved Organic Matter (DOM) levels before and after fish mass mortality were analyzed using a student t-test. Any differences between stations were analyzed using one-way ANOVA was performed using SPSS computer software. **Results:** The levels of N and P before and after fish mass mortality were significantly different ( $p < 0.05$ ). The N levels at surface ranged from 1.83-2.30 mg L<sup>-1</sup>. At 30 m, N levels ranged from 2.11-2.60 mg L<sup>-1</sup>. The P levels ranged from 0.50-0.91 mg L<sup>-1</sup> and 0.81-0.92 mg L<sup>-1</sup> at 0.1 and 30 m depths, respectively. The N level tended to the limiting factor for algae growth at each station (all N/P < 16). The TOM levels before and after fish mass mortality ranged from (Mean  $\pm$  SD) 4.55  $\pm$  0.02-16.33  $\pm$  0.01 mg L<sup>-1</sup> and 6.97  $\pm$  0.72-19.04  $\pm$  0.04 mg L<sup>-1</sup> at 0.10 and 30 m depths, respectively. **Conclusion:** The availability of N, P and TOM in the water was significantly higher ( $p < 0.05$ ) after fish mass mortality and had a negative effect on the water quality of Maninjau lake.

**Key words:** Aquaculture, fish mass mortality, depth water, floating net cages, total organic matter

**Received:** March 22, 2017

**Accepted:** May 01, 2017

**Published:** June 15, 2017

**Citation:** Hafrijal Syandri, Azrita, Junaidi and Ainul Mardiah, 2017. Levels of available nitrogen-phosphorus before and after fish mass mortality in Maninjau lake of Indonesia. J. Fish. Aquat. Sci., 12: 191-196.

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**Competing Interest:** The authors have declared that no competing interest exists.

**Data Availability:** All relevant data are within the paper and its supporting information files.



## INTRODUCTION

Nitrogen (N) and phosphorus (P) are very important elements in water bodies because they serve as a source of nutrients for plant-based organisms<sup>1-3</sup>. In land water quality criteria are determined by the levels of N and P<sup>4</sup>. Increases in the levels of N and P in water bodies are largely related to aquaculture activity involving floating net cages<sup>5,6</sup>. Although increase in P load have made lakes more productive, the ratio of N to P is a limiting factor that can be used to analyze the growth of phytoplankton<sup>7,8</sup>. Compared to other micronutrients P plays an important role in the metabolism of lake biota<sup>9</sup>. The availability of P in water bodies (e.g., from PO<sub>4</sub>) can often be used directly by vegetative components<sup>2</sup>.

The surface area of Maninjau lake is 99.5 km<sup>2</sup><sup>10</sup>. The aquaculture activity of *Oreochromis niloticus* and *Cyprinus carpio* by floating net cages is very intensive<sup>6,11</sup>. In 2015, the total number of floating net cages at Maninjau lake was 20,608 units and the dominant species cultured was *O. niloticus*<sup>12</sup>. The aquaculture activity related to *O. niloticus* and *C. carpio* has the potential to release significant amounts of N and P into water bodies<sup>2,13</sup>, mainly from fish feed<sup>6</sup>. As a result, increasing aquaculture activity related to these fish species may lead to eutrophication and aquatic ecosystem changes<sup>3,14-17</sup>.

Determining the availability of N, P and total organic matter in Maninjau lake is very important considering the intensive level of fish aquaculture activity involving floating net cages. From August-September, 2016, as much as 600 t of fish drowned in Maninjau lake<sup>18</sup>. Briefly, the fish mass mortality was occurred due to upwelling condition in Maninjau lake. The aim of the current study was to analyze the availability of N, P and TOM before and after fish mass mortality in Maninjau lake in order to increase the knowledge regarding the effects of this even on water quality.

## MATERIALS AND METHODS

**Sampling location:** The present research was conducted in February, 2017, four months after mass mortality of *O. niloticus* and *C. carpio* occurred in Maninjau lake, West Sumatera, Indonesia. Fish mass mortality occurred in August and September, 2016. The N, P, TOM, Particulate Organic Matter (POM) and Dissolved Organic Matter (DOM) levels recorded in February, 2016, were compared with data take in February, 2017. The data were collected from four stations: Muko-Muko, Pasa, Pandan and Sungai Tampang. Water samples (250 mL) were taken at the surface (depth

0.10 m) and under floating net cages (depth 30 m) at each station using a Kemmerer Water Sampler (Wildco, USA) with diameter (D) 118 mm and sample volume 1200 mL. The sampler consists of a metal tube with stopper on each end that can be held open when the sampler is lowered by a line to a desired depth. After the stoppers close the ends of the tube, the sampler is retrieved with the desired samples of water being uncontaminated by other water. Then samples were placed in bottles and preserved with H<sub>2</sub>SO<sub>4</sub>.

**Nitrogen and phosphorous analysis:** The N and P levels were analyzed by (ultraviolet-visible) light spectrophotometry (UV 160 A. Japan) according to APHA<sup>19</sup>. The P was determined using the molybdovanadate method indicated by AOAC<sup>20</sup> at an absorbance of 400 nm. All samples were analyzed in triplicate. Redfield criteria were assessed by calculating the N/P ratio. An N/P<16 means that N is the limiting factor, while an N/P>16 means that P was the limiting factor, an N/P = 14-16 means that N and P were collectively at the limiting factor<sup>21</sup>.

**Water quality analysis:** Dissolved oxygen (O<sub>2</sub>) levels were determined using a Yellow Spring oxygen meter model 52-Yellow Spring Instrument Co., Yellow Springs, OH, USA). The TOM, DOM and POM were analyzed from surface water samples using the titrimetric method<sup>22</sup>. The POM was calculated as the difference between TOM and DOM.

**Statistical analysis:** The N, P, TOM, POM and DOM levels before (February, 2016) and after (February, 2017) fish mass mortality were analyzed using a student t-test. The N, P, TOM, POM and DOM levels between stations were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's HDS *post-hoc* test using SPSS software (Version 16.0 for Windows, SPSS Inc, Chicago IL)<sup>23</sup>. All samples were performed in triplicates and data presented as Means±Standard Deviation (SD). The treatment effects were considered to be significant at p<0.05.

## RESULTS

**Availability of N and P in surface water:** The N and P levels in 0.10 m depth water before and after fish mortality occurred are shown in Table 1.

**Availability of N and P in water under floating net cages:** The levels of N and P in 30 m depth water before and after fish mass mortality occurred are presented in Table 2.



Table 1: Levels of nitrogen (N) and phosphorus (P) in Maninjau lake surface water

Stations	N (mg L <sup>-1</sup> )		P (mg L <sup>-1</sup> )	
	February, 2016	February, 2017	February, 2016	February, 2017
Muko-Muko	0.85±0.015 <sup>aA*</sup>	1.83±0.015 <sup>bB</sup>	0.16±0.015 <sup>aA</sup>	0.91±0.010 <sup>bB</sup>
Pasa	1.16±0.025 <sup>bA</sup>	2.15±0.025 <sup>bB</sup>	0.23±0.030 <sup>bA</sup>	0.72±0.010 <sup>bB</sup>
Pandan	0.71±0.015 <sup>cA</sup>	2.09±0.020 <sup>cB</sup>	0.14±0.015 <sup>cA</sup>	0.50±0.020 <sup>cB</sup>
Sungai Tampang	1.13±0.030 <sup>dA</sup>	1.95±0.020 <sup>dB</sup>	0.53±0.020 <sup>dA</sup>	0.74±0.010 <sup>dB</sup>

Surface water samples were taken at a depth of 0.10 m, data are presented as the Mean±SD of triplicate samples, \*The difference between mean with different lower case letters in a columns and the difference between means with different upper case letters for each parameter are statically significant (p<0.05)

Table 2: Levels of nitrogen (N) and phosphorus (P) in water under floating net cages in Maninjau lake

Stations	N (mg L <sup>-1</sup> )		P (mg L <sup>-1</sup> )	
	February, 2016	February, 2017	February, 2016	February, 2017
Muko-Muko	0.96±0.017 <sup>aA*</sup>	2.11±0.035 <sup>bB</sup>	0.36±0.015 <sup>aA</sup>	0.72±0.010 <sup>bB</sup>
Pasa	1.23±0.015 <sup>bA</sup>	2.30±0.020 <sup>bB</sup>	0.37±0.030 <sup>bA</sup>	0.97±0.010 <sup>bB</sup>
Pandan	0.90±0.010 <sup>cA</sup>	2.60±0.030 <sup>cB</sup>	0.34±0.015 <sup>cA</sup>	0.81±0.020 <sup>cB</sup>
Sungai Tampang	1.43±0.010 <sup>dA</sup>	2.41±0.025 <sup>dB</sup>	0.63±0.020 <sup>dA</sup>	0.85±0.010 <sup>dB</sup>

Surface water samples were taken at a depth of 30 m, data are presented as the Mean±SD of triplicate samples, \*The difference between mean with different lower case letters in a columns and the difference between means with different upper case letters for each parameter are statically significant (p<0.05)

Table 3: Levels of TOM, DOM and POM in Maninjau lake

Stations	TOM (mg L <sup>-1</sup> )		DOM (mg L <sup>-1</sup> )		POM (mg L <sup>-1</sup> )	
	February, 2016	February, 2017	February, 2016	February, 2017	February, 2016	February, 2017
Muko-Muko	4.55±0.020 <sup>aA*</sup>	6.97±0.720 <sup>bB</sup>	3.65±0.030 <sup>aA</sup>	4.56±0.036 <sup>bB</sup>	0.90±0.015 <sup>aA</sup>	2.41±0.020 <sup>bB</sup>
Pasa	9.12±0.025 <sup>bA</sup>	12.55±0.030 <sup>bB</sup>	6.58±0.035 <sup>bA</sup>	8.66±0.010 <sup>bB</sup>	2.55±0.015 <sup>bA</sup>	3.89±0.015 <sup>bB</sup>
Pandan	16.33±0.015 <sup>cA</sup>	19.04±0.035 <sup>cB</sup>	2.55±0.040 <sup>cA</sup>	3.89±0.020 <sup>cB</sup>	6.50±0.014 <sup>cA</sup>	6.82±0.035 <sup>cB</sup>
Sungai Tampang	13.59±0.035 <sup>dA</sup>	15.31±0.020 <sup>dB</sup>	8.17±0.030 <sup>dA</sup>	9.48±0.015 <sup>dB</sup>	5.43±0.035 <sup>dA</sup>	5.83±0.010 <sup>dB</sup>

Data are presented as the Mean±SD of triplicate samples, \*The difference between mean with different lower case letters in a columns and the difference between means with different upper case letters for each parameter are statistically significant (p<0.05), \*TOM: Total organic matter, DOM: Dissolved organic matter, POM: Particulate organic matter

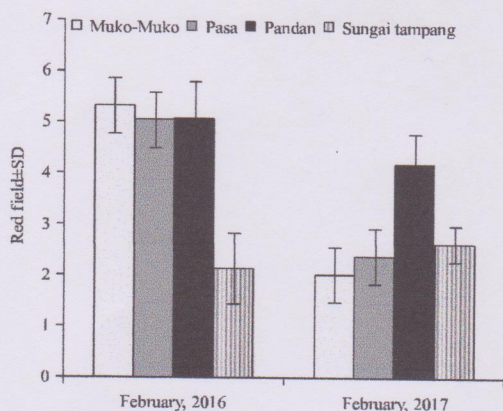


Fig. 1: Red field of nitrogen/phosphorus ratio (m mol<sup>-1</sup>) in Maninjau lake before and after fish mass mortality

**Red field criteria of the N/P ratio:** The N/P values reported in February, 2016 ranged from 2.13±0.68-5.31±0.83, whereas those calculated here in (February, 2017) ranged from 2.01±0.42-4.18±0.58 (Fig. 1). Interestingly all sampling stations had an N/P<16.

**TOM, DOM and POM levels before and after fish mass mortality:** The levels of TOM, DOM and POM before and after fish mass mortality in Maninjau lake are presented in Table 3.

## DISCUSSION

The current research showed the levels of N and P at 0.10 and 30 m depths significantly increased (p<0.05) after fish mass mortality (February, 2017). This increase was the result of as much as 600 t of *O. niloticus* and *C. carpio* drowning and sinking to the bottom of Maninjau lake. Previously, Asir and Pulatsu<sup>24</sup> reported that each ton of dead Rainbow trout (*Oncorhynchus mykiss*) released as much as 56.00 kg of N and 10.66 kg of P into water bodies. Other researchers have found similar result regarding *O. mykiss* production reporting 43.9 and 8.8 kg t<sup>-1</sup> of N and P, respectively<sup>16</sup>.

The N and P are end-products of fish load that can affect water quality in fish farming areas<sup>9</sup>. While the concentration of N and P in water can be estimated from the total amount of feed given to the fish<sup>24</sup>, it is possible that increases in N and P may also result from fish mass mortality.



For example, the main end-product of protein metabolism in teleost fish is ammonia ( $\text{NH}_3$ ) and most nitrogenous wastes from same fish species are excreted as urea<sup>25</sup>. Riche and Brown<sup>26</sup> also reported that the end-products of protein catabolism in fish were dissolved  $\text{NH}_3$  and urea. Meanwhile, the amount of N and P in fish feed and changing feed conversion ratios have been influenced the amount and types of nutrients excreted into the aquatic environment<sup>27</sup>. Furthermore, Mallekh *et al.*<sup>28</sup> found that the fish size, species and feed type affect the amount of digestive residue.

The levels of N and P at 0.10 and 30 m depths after fish mass mortality in Maninjau lake was significantly different between water sampling stations ( $p < 0.05$ ). The level of N ranged from 1.83-2.30  $\text{mg L}^{-1}$  at 0.10 m and 2.11-2.60  $\text{mg L}^{-1}$  at 30 m, while, P levels ranged from 0.50-0.91  $\text{mg L}^{-1}$  at 0.10 m and 0.81-0.92  $\text{mg L}^{-1}$  at 30 m. Fish mass mortality have an effect to N and P levels in Maninjau lake. The dead fish was decomposed by bacteria which can increase the level of N and P. The level of N and P at the surface and 30 m depth of Maninjau lake tend to higher than before fish mass mortality. In Toba lake, Indonesia, the N and P levels were 0,013-0,457  $\text{mg L}^{-1}$  and 0,005-0.116  $\text{mg L}^{-1}$ , lower compared than Maninjau lake. This condition was due to fish mass mortality occurred in this place<sup>1</sup>. The N and P levels at Maninjau lake based on the regulations of the Ministry of Environment of the Republic of Indonesia Number 28/2009<sup>29</sup>. The water status of Maninjau lake refer to hypereutrophic. Moreover, the levels of N and P at the bottom of Maninjau lake (under floating net cages, 30 m depth) was higher than at the surface (0.10 m depth). The P containing nutrients settle and accumulated in deeper parts of the lake most likely when orthophosphate in the epilimnion zone undergoes co-precipitation and absorption with particulates or macro metals such as iron, manganese, aluminum and/or other organic compounds<sup>1</sup>.

The amount of fish mass mortality observed in Maninjau lake was correlated with the number floating net cages at each station. The number of floating net cages was highest at Pasa (133 units/10,000  $\text{m}^2$ ) and the lowest at Muko-Muko (69 units/10,000  $\text{m}^2$ ). The N levels at 0.10 and 30 m depths at Pasa in February, 2017 were  $2.15 \pm 0.025$  and  $2.30 \pm 0.020$   $\text{mg L}^{-1}$ , respectively. While, the level of P was  $0.72 \pm 0.01$  and  $0.97 \pm 0.01$   $\text{mg L}^{-1}$ , respectively. Furthermore, the productivity of Maninjau lake is relatively high as indicated by the brightness of water, which ranges from 1.30-1.80 m. Compared to that of Toba lake which is lower water brightness 7.0-15.0 m<sup>1</sup>.

In general, orthophosphate is a P containing nutrient whose availability for growth of phytoplankton. The

orthophosphate level at the surface of Maninjau lake was relatively high, ranging from 0.055-0.125  $\text{mg L}^{-1}$ . The nutrient element of P is highly dependent on the dissolved  $\text{O}_2$  concentration. The levels of dissolved  $\text{O}_2$  at 0.10 m at each station after fish mass mortality were 5.89, 5.93, 6.17 and 5.85  $\text{mg L}^{-1}$ , respectively. However, the dissolved  $\text{O}_2$  levels at 30 m were 4.70, 4.92, 5.01 and 4.82  $\text{mg L}^{-1}$ , respectively. Largely an aerobic conditions are may be found at depths greater than 50 m and the average depth of Maninjau lake is 105 m<sup>10</sup>. More orthophosphate is found in particle form of dissolved  $\text{O}_2$  decreases below 50 m. According to Nomosatryo and Lukman<sup>1</sup> the accumulation of P at the bottom of Toba lake is related to higher levels of orthophosphate at the water's surface. Moreover, Kelly<sup>30</sup> stated that orthophosphate compounds liberated from sediment diffuse at the water's surface. The levels of N tended to be a limiting factor for algae growth in Maninjau lake because all stations had an  $\text{N/P} < 16$  (Fig. 1). This result was similar to that of Lake Sentani-Indonesia  $\text{N/P}$  10.24<sup>31</sup> but different from that of other Indonesia lakes. Both N and P levels were found to limit algae growth in Toba lake ( $\text{N/P}$  1.9-46.3)<sup>1</sup>, while P was the limiting factor in Panglima Besar Soedirman Reservoir ( $\text{N/P} = 19.13-65.82$ )<sup>8</sup>.

Analysis of different types of organic matter in Maninjau lake revealed that although DOM, POM and TOM levels increased. The DOM levels were dominant (Table 3). The level of TOM was higher after fish mortality, the TOM was highest at Pandan and Sungai Tampang stations. Similarly, Lukman and Hidayat<sup>32</sup> reported the level of DOM was higher than the POM in Toba lake. Analysis of the accumulation of DOM in aquaculture activity is very important<sup>33</sup>, because the process of sedimentation and mineralization may contribute to the decline of organic values, especially in Maninjau lake and both processes are easily to occur in lentic waters.

Interestingly the level of TOM at Pasa, Pandan and Sungai Tampang stations after fish mortality were relatively similar mean TOM 15.63  $\text{mg L}^{-1}$  or (0.01563  $\text{kg m}^{-3}$ ), whereas, that at Muko-Muko was 6.97  $\text{mg L}^{-1}$  (0.00697  $\text{kg m}^{-3}$ ). These difference in the level TOM also confirm that the increase in TOM found in February, 2017 were largely the result of fish mass mortality. This condition may be correlated with water retention time of lake. According to Syandri *et al.*<sup>10</sup>, Maninjau lake has an area 99.5  $\text{km}^2$  (75.38%) from the catchment area, has water volume 10,226,001,629.2  $\text{m}^3$ , average depth was 105 m, the average of outflow water was 12.86  $\text{m}^3/\text{sec}$  and the role of groundwater is quite large as a source of water at Maninjau lake. So Maninjau lake has water retention time during 25 years.



## CONCLUSION

The current research found clear evidence that mass mortality of *O. niloticus* and *C. carpio* related to floating net cages in Maninjau Lake is a significant source of N, P and TOM. The level of these parameters was significantly higher in deeper water (30 m depths) compared to that at the surface (0.10 m depths) after fish mortality. It is concluded that a major side effect of fish mass mortality is that it decreased the water quality. Fish mass mortality can be reduced by adjusting the timing and stocking density of aquaculture fish. This will help to reduce the downstream negative effects on the lake and surrounding and in turn positively effect to many local people, as improving labor conditions lead to increased economic welfare.

## SIGNIFICANCE STATEMENTS

This study discovered the increasing levels of N, P and TOM after fish mass mortality in Maninjau lake. This condition has negative effect on water quality which cause hypereutrophic condition. While N level tended to as a limiting factor for algae growth at Maninjau lake. This study is an emerging issue now days and require urgent action from the concerned authorities and awareness from fish farmers by adjusting rearing time and stocking density of aquaculture fish for the future.

## ACKNOWLEDGMENT

This study was supported by a study grant (Riset Unggulan Perguruan Tinggi) from the Directorate of Research and Community Service of the Ministry of Research Technology and Higher Education, Republic of Indonesia, (Grant No. SP.DIPA-042.06-0/2017).

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