OPEN ACCESS Asian Journal of Scientific Research

ISSN 1992-1454
DOI: 10.3923/ajsr.2018.

Research Article
Nitrogen and Phosphorus Waste Production from Different Fish Species Cultured at Floating Net Cages in Lake Maninjau, Indonesia

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Abstract
Background and Objective: Aquaculture operations that use floating net cages have become one of the primary mean of intensive fish-culture in Lake Maninjau. The fish-culture species studied were *Cyprinus carpio* (*C. carpio*) (T_1), *Oreochromis niloticus* (*O. niloticus*) (T_2), *Osphronemus goramy* (*O. goramy*) (T_3) and *Clarias gariepinus* (*C. gariepinus*) (T_4). The objective of the research was to estimate the nitrogen (N) and phosphorus (P) loads into Lake Maninjau. **Materials and Methods:** The capacity of floating net cages was approximately 32 m^3 ($4\times4\times2$ m) with densities of 32 fish m^{-3} in triplicate groups. Approximately 1,500 kg of feed was used in each cage during the experiment. The difference of N and P loads from different fish species were analyzed using one-way ANOVA (SPSS 16.0) computer software. **Results:** The total N loads into the water bodies from T_1, T_2, T_3 and T_4 were estimated at 37.93 ± 2.59, 49.90 ± 5.17, 45.90 ± 4.18 and 20.35 ± 4.12 kg t^{-1} of fish production, respectively. The P load was estimated to be 18.30 ± 0.12, 20.01 ± 0.99, 22.60 ± 0.80 and 13.93 ± 1.47 kg t^{-1} of fish production, respectively. Every ton of feed consumed by each fish species will contribute as much as 38.26 ± 2.55, 35.68 ± 1.69, 32.12 ± 0.39 and 48.99 ± 2.35 kg N load into the water bodies, respectively. The P load was 11.45 ± 2.43, 9.11 ± 0.21, 8.34 ± 0.04 and 12.51 ± 0.30 kg, respectively. **Conclusion:** The *C. gariepinus* species is preferred for aquaculture operations at Lake Maninjau, because it minimizes N and P load releases into water bodies which can maintain sustainable aquaculture operations.

Key words: Aquaculture, floating net cages, N and P mass balance, FCR, environmental factors

Received: Accepted: Published:

Citation: Hafrijal Syandri, Azrita and Ainul Mardiah, 2018. Nitrogen and phosphorus waste production from different fish species cultured at floating net cages in Lake Maninjau, Indonesia. Asian J. Sci. Res., CC: CC-CC.

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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

The high levels of nutrients and suspended solids released into water bodies from aquaculture operations is one of the major environmental problems causing water pollution[1-5]. Intensive aquaculture operations can result in the release of dissolved organic and inorganic nutrients, such as nitrogen (N) and phosphorus (P)[6-8]. N and P levels from intensive aquaculture operations can cause or accelerate eutrophication in natural water systems[9-11]. In addition, N and P levels released into water bodies depend on diet composition[12,13], type of feed[10], fish species[14], feed conversion ratio[15], stocking densities, feed quality[16], fish mass mortality[17] and the local environment[18]. Lazzari and Baldisserotto[19] state that N and P are the primary end products of fish loading, which has had an effect on fish rearing waters and the environment.

Aquaculture around the world has grown at a rapid rate in recent years, including in Indonesia[20,21]. Medium and large scale freshwater aquaculture operations in Indonesia were conducted in the lake, reservoir and river[21-23]. Fish-cultured species in this location were *Cyprinus carpio*, *Oreochromis niloticus*, *Osphronemus goramy*, *Hemibagrus nemerus*, *Clarias gariepinus*, *Pangasius* spp. and *Leptobarbus hoevenii*[20,24,25].

Lake Maninjau is tecto-volcanic with a surface area of 99.5 km^2, both features that serve very important roles to many Indonesians for aquaculture operations of the *O. niloticus* and *C. carpio* in floating net cages[8,22]. Total number of floating net cages in the years of 2013, 2014 and 2015 are 16,120, 16,580 and 20,608 U, respectively[8,22,24].

In the past decade, the water quality of Lake Maninjau has been decreasing due to the loading of organic matter from aquaculture operations of *C. carpio* and *O.niloticus*[22,26]. However, upwelling that has occured at Lake Maninjau every year has caused a lack of oxygen in the water which is a result of mass fish mortality[16]. Furthermore, since the year 2014, aquaculture operations of *O. goramy* and *C. gariepinus* have been successful in floating net cages. Both species were resistant to poor water quality and had a wide market in Indonesia, because they are favored by consumers. These species also have high prices and a high demand in the market.

The aim of the study was to estimate the quantitative, values of N and P loads released from each species cultured in floating net cages on Lake Maninjau. The results of this study were used to increase the scientific understanding of the effects of N and P load releases of different fish species into the water at Lake Maninjau.

MATERIALS AND METHODS

Study area: The experiment was conducted in Lake Maninjau of West Sumatera Province, Indonesia. The geographical position is S:00°12'26.63"-S:00°25'02.80" and E:100°07'43.74"-E:100°16'22.48" and it is located at an altitude of 461.50 m above sea level[24]. Based on the Schmidth Ferguson climate classification, Lake Maninjau has characteristics of climate types A and an annual rainfall of 3,490 mm.

In this study, the *C. carpio*, *O. niloticus*, *O. goramy* and *C. gariepinus* fingerlings were designated T_1, T_2, T_3 and T_4, respectively. All species were collected from a private hatchery in the Luak District, Lima Puluh Kota Regency, West Sumatera Province. The fingerlings were transferred by truck to the Research Center of the Faculty of Fisheries and Marine Science, Bung Hatta University near the Lake Maninjau. Each fish species was treated with a prophylactic formalin bath (100 mg L^{-1}) for 1 h to remove external parasites and acclimatized in a floating net cage (4×4×2 m) for 1 month prior to the experiment. The average initial weight of the T_1, T_2, T_3 and T_4 fingerlings were 56.79±4.45, 53.08±1.60, 55.33±1.14 and 51.18±1.59 g, respectively.

This study was conducted between March, 2017 and June, 2017 (100 days). All species were cultured in the floating net cages. Each floating net cage had a capacity of approximately 32 m^3 (4×4×2 m) and was constructed using a 10 mm mesh size sieve. The fingerlings were fed by a commercial feed (pelleted) with drowned type during 100 days of the experiment. The approximate composition of the feed was 12% moisture content, 29% crude protein, 6% crude lipid, 12% crude fiber and 6% crude ash.

The stocking density was 130 fish m^{-3} (4,160 fish/cage). There were 3 replicates for each fish species in each experiment. During the experiment, 1,500 kg of feed was used. The fish were fed daily at a rate of 4% of their biomass at 9:00, 14:00 and 18:00. All fish mortalities were removed and weighed daily. Fish mortality was replaced in each treatment. The amount of feed provided was adjusted according to temporal changes of biomass and growth of fish in the floating net cages.

Measurements parameters: To determine the growth performance of the fish, the following parameters were calculated based on Aryani *et al.*[35]:

$$\text{Final mean weight, feed conversion ratio (FCR)} = \frac{\text{Total feed fed (g)}}{\text{Total wet weight gain (g)}}$$

and

$$\text{Survival rate (SR\%)} = \frac{\text{Number of fish survived}}{\text{Number of fish stocked}} \times 100$$

Water quality: The water transparency was measured with a Secchi disc. The water samples were collected at a depth of 10 cm from each floating net cage to determine the dissolved oxygen (DO) levels. An oxygen meter (YSI model 52, Yellow Spring Instrument Co., Yellow Springs, OH, USA) was used *in situ* and pH values were determined using a pH meter (Digital Mini-pH Meter, 0-14 pH, IQ Scientific, Chemo-science [Thailand]) Co., Ltd, Thailand). Water temperature was measured using a thermometer (Celsius scale). The levels of alkalinity and hardness of the water in each replication were measured according to standard procedures[27]. The water quality parameters were measured once every month.

Analytical methods: Nitrogen (N) concentrations (as % of dry weight) of feed and fish were determined by standard methods of the Association of Official Analytical Chemists[28]. The P concentrations were determined using a spectrophotometer (UV 160 A, Japan) and the molybdate-ascorbic acid method indicated by the Association of Official Analytical Chemists[28] at the Chemistry Laboratory of the University of Bung Hatta Padang, Indonesia. The results were expressed as absorbance at 400 mm. All samples were performed in triplicate.

Estimation of N and P loads: The levels of N and P loads from fish-culture was estimated according to Ackefors and Enell[29]. The following parameters were analyzed according to the formulas given below:

$$\text{N load (kg of N)} = [(\text{Feed}\times\text{Feed}_N)\text{-}(\text{Fish}\times\text{Fish}_N)]$$

$$\text{P load (kg of P)} = [(\text{Feed}\times\text{Feed}_P)\text{-}(\text{Fish x Fish}_P)]$$

where, Feed is total feed used during the experiment, Fish is wet weight of fish produced per harvested, Feed_N is N content of feed. Feed_P is P content of feed (expressed as % of dry weight). Fish_N is N content of fish and Fish_P= P content of fish (expressed as % of wet weight).

$$\text{N and P loads from the production of 1 t of fish} = (\text{1 t feed}\times\text{FCR}\times\text{Feed}_{\text{N and P content}})\text{-}(\text{1 t fish}\times\text{Fish}_{\text{N and P content}})$$

$$\text{N and P loads from 1 t of feed consumption} = \frac{(\text{1 t feed}\times\text{N or P content of feed})}{(\text{FCR})}$$

Statistical analysis: The mean values for final weight, feed conversion ratio, mortality parameters of different treatments and monthly variations of water quality parameters, were subjected to a one-way ANOVA test followed by Duncan's new multiple range test[30]. All statistical analyses were performed using SPSS software (version 16.0 for Windows, SPSS Inc., Chicago, IL). The standard deviation of each parameter and treatment was determined and expressed as the Mean±SD. The treatment effects were considered to be significant at p<0.05.

RESULTS

The results for certain growth parameters, FCR, mortality and chemical analyses, from each feed and fish species are presented in Table 1. The difference in fish species has a significant (p<0.05) effect on the final mean weight, FCR and mortality. The N and P content of the feed were 5.52±0.29 and 1.41±0.03%, respectively. The N and P content of each fish species is presented in Table 1. Monthly variations in the water quality parameter in Lake Maninjau are as indicated in Table 2. There was no significant difference in the water transparency, water temperature, dissolved oxygen, pH, alkalinity or hardness values for months of March, April, May and June, of 2017.

Table 1: Growth parameters and chemical analysis of different fish species

	Species			
Parameters	T_1	T_2	T_3	T_5
Initial mean weight (g)	56.79±1.77	53.08±1.60	55.33±1.14	51.18±1.59
Final mean weight (g)	182.45±2.00^a	175.10±2.30^b	148.55±7.53^c	233.30±7.51^d
Feed conversion ratio (FCR)	1.44±0.02^a	1.55±0.03^b	1.69±0.03^c	1.13±0.10^d
Mortality (%)	12.46±0.93^a	10.92±0.36^b	8.18±0.28^c	5.20±0.30^d
N content of fish (%)	4.17±0.05	3.55±0.05	4.59±0.07	4.13±0.04
P content of fish (%)	0.20±0.02	0.18±0.02	0.13±0.03	0.29±0.02
N content of feed (%)	5.52±0.29	5.52±0.29	5.52±0.29	5.52±0.29
P content of feed (%)	1.41±0.03	1.41±0.03	1.41±0.03	1.41±0.03

Values are Mean±SD, *Values in the same row with a different superscript are significantly different (p<0.05), T_1: *C. carpio*, T_2: *O. niloticus*, T_3: *O. goramy* and T_4: *C. gariepinus*

Table 2: Monthly variations in physicochemical and water quality parameters

	March, 2017	April, 2017	May, 2017	June, 2017
Water transparency (m)				
Mean	1.96[a]	1.98[a]	1.99[a]	1.91[a]
Standard deviation	0.14	0.07	0.10	0.10
Median	1.85	1.98	2.01	1.94
Minimum-Maximum	1.80-2.10	1.90-2.08	1.85-2.10	1.90-2.00
Temperature (°C)				
Mean	28.20[a]	27.20[a]	27.00[a]	27.00[a]
Standard deviation	0.83	0.83	28.00	1.22
Median	28.00	27.00	0.70	27.00
Minimum-Maximum	27.00-29.00	26.00-28.00	27.00-29.00	26.00-29.00
Dissolved oxygen (mg L^{-1})				
Mean	6.21[a]	5.78	6.11[a]	6.00[a]
Standard deviation	0.12	0.44	0.05	0.04
Median	6.24	5.44	6.13	5.96
Minimum-Maximum	6.00-6.31	5.40-6.24	6.03-6.13	5.96-6.05
pH				
Mean	7.67[a]	7.45[a]	7.41[a]	7.68[a]
Standard deviation	0.05	0.32	0.35	0.07
Median	7.69	7.65	7.51	7.67
Minimum-Maximum	7.62-7.76	7.08-7.71	6.90-7.68	7.62-7.79
Alkalinity (mg L^{-1})				
Mean	83.79[a]	78.30	79.12	79.88[a]
Standard deviation	4.37	1.48	2.44	5.14
Median	80.70	78.50	77.97	83.74
Minimum-Maximum	80.51-88.90	76.00-80.00	76.68-82.00	76.15-84.34
Hardness (mg L^{-1})				
Mean	64.84[a]	65.28[a]	70.64[a]	70.20[a]
Standard deviation	3.31	2.10	5.93	5.95
Median	67.00	66.50	73.71	68.70
Minimum-Maximum	61.64-68.50	62.80-67.01	63.83-74.91	64.59-79.30

*Values in the same row with the same superscript are not significantly different (p>0.05)

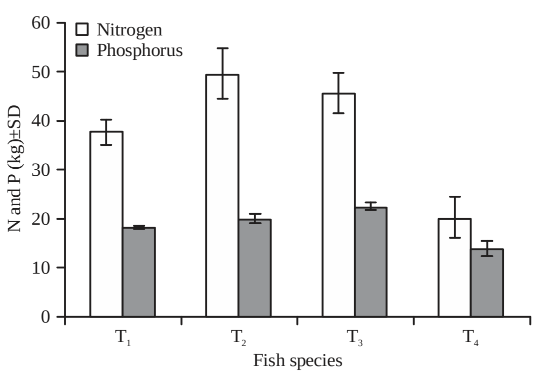

Fig. 1: Nitrogen and phosphorus loads from the production of 1 t of fish in Lake Maninjau

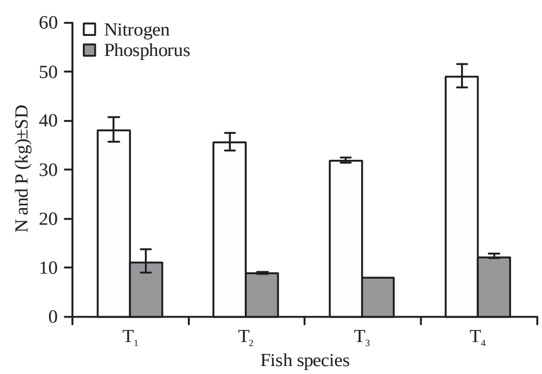

Fig. 2: Nitrogen and phosphorus loads released into Lake Maninjau from 1 t of feed consumption

Table 3 presented the summaries of the mass balance of N and P content from four fish species, while Fig. 1 and 2 provide an estimation of N and P loads from the production of 1 t of fish and 1 t of feed consumption.

DISCUSSION

The present study was conducted to report the N and P loads introduced by floating net-cages in Lake Maninjau. These results indicated that species T_4 had a better growth rate compared to those of T_1, T_2 and T_3. The differences in growth rate might be due to the specific growth rate of each fish species. The specific growth rates (SGR, % day^{-1}) for T_1, T_2, T_3 and T_4 used with the feeding rate of 4% were 1.16, 1.19, 0.98 and 1.52, respectively. Numerous studies elsewhere have shown that the specific growth rate (SGR) of each species of fish is different. The SGRs (% day^{-1}) are

Table 3: Mass balance of nitrogen (N) and phosphorus (P) of different fish species

Species	N from feed (kg)	N retained in fish (kg)	N load (kg)	P from feed (kg)	P retained in fish (kg)	P load (kg)	N and P retained in fish (%)	N and P load (%)
T_1	60.72±3.19^{a}	31.78±0.83^{a}	28.93±2.36^{a}	15.51±0.33^{a}	1.53±0.17^{a}	13.90±0.12^{a}	52.39±1.42^{a}N	47.60±1.42^{a}N
							9.83±0.88^{A}P	90.62±0.59^{A}P
T_2	60.72±3.19^{a}	25.25±0.53^{b}	35.46±3.12^{b}	15.72±0.18^{a}	1.28±0.17^{b}	14.43±0.34^{b}	42.87±0.41^{b}N	58.34±2.13^{b}N
							8.17±1.17^{A}P	91.83±1.17^{A}P
T_3	60.72±3.19^{a}	29.87±0.22^{c}	30.85±2.12^{c}	15.50±0.30^{C}	0.84±0.18^{c}	13.67±0.15^{c}	50.06±.00^{c} N	49.94±2.00^{c}N
							4.87±0.75^{C}P	94.60±1.05^{C}P
T_4	60.72±3.19^{a}	40.33±0.84^{d}	20.39±3.27^{d}	15.51±0.33^{d}	2.83±0.17^{d}	12.68±0.17^{d}	66.53±3.62^{d}N	33.46±3.62^{d}N
							18.23±0.78^{D} P	81.77±0.78^{D} P

Data are presented as the (Mean±SD) of triplicate samples, *The difference between means with different lower case letters in a column and the difference between means with different capitalized letters for each parameter (N, P) are statistically significant (p<0.05), T_1: *C. carpio*, T_2: *O. niloticus*, T_3: *O. goramy* and T_4: *C. gariepinus*

1.63 for *C.carpio*[31], 2.14 for *O. niloticus*[32], 2.47 for *C.gariepinus*[33] and 1.66 for *O. goramy*[34].

In this study, the water quality in each floating net cage during the months of March, April, May and June showed no significant differences. The growth of the fish species depends not only the water quality[35-38] but also on the fish species[39,40]. Although each fish species used the drowned feed type with a feeding rate of 4%, the feed conversion ratio (FCR) for each species was significantly different ($p<0.05$) (Table 1). The FCR is usually used to estimate the efficiency of converting feed into body mass. In this study, the lowest FCR value was observed in T_4 (1.13), while the highest was observed in T_3 (1.69). The differences among the FCR values are caused by differences in fish species (T_1, T_2, T_3 and T_4) and possibly also by food habits. Conversely, a lower FCR value indicated that the efficiency of feed utilization was better. An FCR value that is less than 2.0 or very close to 2.0 is considered "good" in the aquaculture industry[41]. In contrast, the FCR for Tilapia fish cages in Lake Malawi are between 2.1 and 3.9 and FCR values tended to be higher in recent production cycles. Production cycles during the study period were on average (±SE), 376±9 days long[14].

Negative environmental impacts of cage aquaculture operations have been reported in many parts of the world[20,42-46]. In this study, the difference in fish species has a significant effect ($p<0.05$) on the mass balance of N and P (Table 3). N and P retention (kg) was significantly higher in T_4 compared to that of T_1, followed by T_3 and T_2, while N and P load (kg) was significantly higher for T_2 compared to that of T_1, T_3 and T_4. Although the same feed was applied at an equal ratio, the mass balance of N and P differed significantly among all fish species. The reason for this difference might be due to the differences in FCR for each species and there was less difference in genetic improvement for feed consumption. The N loads in T_1, T_2, T_3 and T_4 were 47.60, 58.34, 49.94 and 33.46%, respectively, while the P loads in T_1, T_2, T_3 and T_4 were 90.62, 91.83, 94.60 and 81.77%, respectively. For *Oreochromis karongae* and *O. shiranus* in Lake Malawi, N loads were 59 and 80%, respectively and P loads were 85 and 92%, respectively[14]. In addition, for Rainbow Trout (*Oncorhynchus mykiss*) in the Kesikköprü Dam Lake, N loads were 54.37% and P loads were 70.00%[10]. According to Yogev *et al.*[47] fish only use 20-30% of the N in feed and 50% of P in feed, while the remainder is released into the water.

In this study, total N and P load releases into water bodies were different for each ton of fish production in T_1 (37.93±2.59 and 18.30±0.12), T_2 (49.90±5.17 and 20.01±0.99), T_3 (45.90±4.18 and 22.60±0.80) and T_4 (20.35±4.12 and 13093±1.47) kg t^{-1}, respectively (Fig. 1). These differences could be caused by the FCR and the N and

P content of feed and fish. There was a strong linear relationship between FCR and N (r^2 = 0.87) and P (r^2 = 0.99) loads for the floating net cages. In comparison other values in the literature, show that N released into water bodies (t^{-1} of live-weight fish) for Tilapia, Black Pacu and Trout were 34.7, 25.8 and 66.1 kg, respectively. Alternatively, the P was 3.0, 9.7 and 9.6 kg, respectively[43]. Other research found that 56.0 kg of N and 10.66 kg P were released for Trout[10] and 64.0 kg of N and 4.6 kg of P were released for Tilapia[47]. The N loading values also varied considerably with fish species with Rainbow trout having the lowest values of 47.3-124.2 kg t^{-1}, while values given by other fishes ranged from 103.5-320.6 kg t^{-1}[15].

In this study, total N and P load releases into water bodies were different for each ton of feed consumed by T_1, T_2, T_3 and T_4 (Fig. 2). This difference is also caused by FCR and feed composition. The FCRs for T_1, T_2, T_3 and T_4 were 1.44$\pm$0.02, 1.55$\pm$0.03, 1.69$\pm$0.03 and 1.13$\pm$0.10, respectively. There was a strong linear relationship between FCR and N (r^2 = 0.99) and P (r^2 = 0.87) loads for the floating net cages. The N and P levels in the feed used and the FCRs in the farms directly affected N and P loads for each ton of pelleted feed used. Every ton of feed consumed by each fish species (T_1, T_2, T_3 and T_4 will contributes N loads as much as 38.26$\pm$2.55, 35.68$\pm$1.69, 32.12$\pm$0.39, 48.99$\pm$2.35 and P loads as much as 11.45$\pm$2.43, 9.11$\pm$0.21, 8.34$\pm$0.04 and 12.51$\pm$0.30 into Lake Maninjau. Furthermore, releases of N and P t^{-1} into the Kesikköprü Dam Lake for *Oncorhynchus mykiss* were 44.78 and 8.60 kg, respectively[10]. The feed composition and feed conversion of aquaculture operations primarily had a negative effect on the environment. In addition, the aquaculture integrated model, recirculating aquaculture systems, site selection, feeding rate, size of the farm and species of cultivated fish should also be considered important factors[32,43,45,47,48].

CONCLUSION

The present study observed clear evidence that different species of fish-cultured in floating net cages can release different levels of total N and P into water bodies of Lake Maninjau. The estimated N and P loads from the production of 1 t of fish were significantly lower for *Clarias gariepinus* compared to those for *O. goramy*, *C. carpio* and *O. nilaticus* while the estimated N and P loads from 1 t of feed consumption were significantly lower for *O. goramy* compared to other species. Based on the research, the appropriate species cultured in Lake Maninjau was C. *Gariepinus* because this species had low N and P loads into water bodies. Alternatively, N and P loads can be reduced by adjusting the stocking densities and feed regime timing of *C. gariepinus.* This approach will help to reduce the downstream negative effects on the lake and in turn positively affect the water quality.

SIGNIFICANCE STATEMENTS

This study analyzes the different levels of N and P load releases into water bodies of Maninjau Lake from each fish-culture species. The N and P loads were significantly lower for *Clarias gariepinus* compared to other fish species. Fish-culture of *Clarias gariepinus* in Lake Maninjau is an important consideration for fish farmers and authorities in the future due to the lower N and P load releases into water bodies of Lake Maninjau. This fish species is also resistant to poor water quality, has a higher growth rate and is favored by consumers.

ACKNOWLEDGMENTS

This study was funded 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 (No. SP.DIPA-042.06-0/2017).

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