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**DETERMINATION OF THE TROPICAL STATUS OF FLOATING
NET CAGE WATER BASED ON THE DISTRIBUTION OF
NITROGEN, PHOSPHORUS AND CHLOROPHYLL-A**

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Abstract

The dominant source of pollutants for floating net cages are fish feed and feces. They cause phosphorus and nitrogen in water increase, trigger eutrophication, marked by the appearance of algae. Algae are green plants, contain chlorophyll-a. The content of phosphorus, nitrogen and chlorophyll-a can be used to determine the tropical status of water. The objective of this research is to determine the tropical status of marine cage water. The research method was descriptive laboratory. Nitrogen content is measured as nitrite according to SNI 6989.9-2004, nitrate according to APHA Section 4500-NO₃, ammonia according to SNI 19-1655-1989. Phosphorus analysis according to SNI 06-6989-31: 2005. Chlorophyll-a analysis used the Strickland & Parson method by spectrophotometry. Laboratory data were analyzed for tropical status based on nitrogen, phosphorus and chlorophyll-a content. The results showed that the tropical status of the KJA water of Gajah Mungkur Reservoir Wonogiri in the rainy season had eutrophic status, containing high levels of phosphorus and nitrogen elements. The eutrophic status indicated that the water had been polluted by an enhancement of nitrogen levels by 18.345 µg/L and phosphorus by 420.65 µg/L. These nitrogen and phosphorus pollutants increased the growth of chlorophyll-a by 12.70 µg/L.

Keywords: Nitrogen; Phosphorus; Chlorophyll-a; Tropic status; KJA

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INTRODUCTION

Gajah Mungkur Wonogiri Reservoir (WGM) is used for the cultivation of red tilapia in floating net cages (KJA), developed by fish farmers and PT Aquafarm Nusantara. Feed form of pellets, 70% of the feed is eaten by fish, the remaining 30% is released into the water as pollutants (Marganof *et al.*, 2007), a source of nitrogen and phosphorus pollutants from indigenous activities (Pujiastuti *et al.*, 2010). KJA activities as a contributor to the dominant Nitrogen and Phosphorus pollutants enter the WGM waters (Pujiastuti *et al.*, 2016). Nitrogen in water dissolves as ammonia, and undergoes biotransformation into nitrite and nitrate (Saha *et al.*, 2013). In KJA WGM, ammonia undergoes biotransformation naturally by *Nitrosomonas* sp. Into nitrite (N-NO₂), which is then transformed into nitrate by *Nitrobacter* sp. (Pujiastuti *et al.*, 2018). Nitrite compounds are toxic to fish, while nitrate compounds can stimulate the algae growth.

Chlorophyll-a is a green plant pigment, required for photosynthesis. Chlorophyll-a parameter indicates the level of algal biomass. estimated that the average weight is 1% of the biomass (Permen LH, 2009). Algae is green plant, so its presence can be measured by the chlorophyll-a parameter. Chlorophyll-a analysis can be carried out using Strickland & Parson

method (Parson *et al.*, 1984). Chlorophyll-a content has a positive correlation to nitrite (N-NO₂) and nitrate (N-NO₃) in water (Polan and Terbiyik, 2013). The high content of chlorophyll-a indicates the fertility of the waters, so that it can cause a reduction in dissolved oxygen in water (Wiryanto *et al.*, 2012). Reservoir water sediments at a depth of 25 m contain chlorophyll-a concentrations of 0.2-1.14 mg/m³ (Nuchsin, 2007).

The growth of green plants such as algae is an indicator of the eutrophication process in the water. In plain view, the occurrence of eutrophication in the reservoir is marked by the development of aquatic plants, such as water hyacinth, azola (Pujiastuti, 2003) and increasing chlorophyll-a content in water (Nuchsin, 2007; Sanjaya & Iriani, 2018). Reservoir water quality condition is classified based on eutrophication caused by increased levels of plant nutrients in the water, such as nitrogen and phosphorus. Nitrogen and phosphorus are limiting factors for eutrophication in water (PermenLH, 2009). As a limiting factor, phosphorus will limit the eutrophication process when the nitrogen content in water is more than 8 times the phosphorus level. Likewise, nitrogen is a limiting factor for eutrophication if the water content is less than eight times the phosphorus content (UNEP-IETC/ILEC, 2001). Eutrophication

is classified below four categories of trophic status.

Trophic status is defined as the status of reservoir water based on nutrient content and phytoplankton biomass content or productivity (Permen, L.H, 2009). The determination of the reservoir trophic status is based on water quality data and criteria for trophic status. There are four categories of trophic status, namely: 1) Oligotrophs, which are trophic status of reservoir water which contains low levels of nutrients (Permen, L.H, 2009; UNEP-IETC/ILEC, 2001), that is, if it contains an average total nitrogen content of $\leq 650 \mu\text{g/L}$, an average total phosphorus content is $<10 \mu\text{g/L}$ and an average chlorophyll-a content is $<2.0 \mu\text{g/L}$, and has a brightness of $\geq 10 \text{ m}$. The status of oligotrophs indicates that the water quality is still natural, has not been polluted yet from the source of nitrogen and phosphorus nutrients. 2) Mesotroph, is a trophic status of reservoir water which contains moderate levels of nutrients, namely if it contains an average total nitrogen content of $\leq 750 \mu\text{g/L}$, the average total phosphorus content is $<30 \mu\text{g/L}$ and the average chlorophyll-a is $<5.0 \mu\text{g/L}$, and has a brightness of $\geq 4 \text{ m}$. Mesotrophic status indicates an enhancement of nitrogen and phosphorus levels, but is still within tolerance limits because it has not shown any indication of water pollution. 3)

Eutrophs, is the trophic status of reservoir water containing high levels of nutrients, i.e. if it contains an average total nitrogen content of $\leq 1900 \mu\text{g/L}$, the average total phosphorus content is $<100 \mu\text{g/L}$ and an average chlorophyll-a content is $<15.0 \mu\text{g/L}$, and has a brightness of $\geq 2.5 \text{ m}$. Eutrophic status indicates that the reservoir water has been polluted by increased nitrogen and phosphorus levels. 4) Hypertrophic is the trophic status of reservoir water which contains very high levels of nutrients, that is, if it contains an average level of total nitrogen $> 1900 \mu\text{g/L}$, the average total phosphorus content is $\geq 100 \mu\text{g/L}$ and the average level chlorophyll-a is $\geq 200.0 \mu\text{g/L}$, and has a brightness of $<2.5 \text{ m}$. This Hypertrophic status indicates that the reservoir water has been heavily polluted by the enhancement of nitrogen and phosphorus. According to Laksitaningrum *et al.*, (2017), based on chlorophyll-a analysis, WGM water has five statuses including ultraligotrophic, oligotrophic, mesotrophic, mild eutrophic and moderate eutrophic. Meanwhile, based on the analysis of total phosphorus, WGM waters have three trophic status, namely moderate eutrophic, severe eutrophic and hypereutrophic.

The water has dynamic condition, so they need to be controlled periodically to maintain their sustainability. Fish farming activities in marine cage as the dominant

source of nitrogen and phosphorus pollutants need to be studied to determine the tropical status of the water.

MATERIALS AND METHODS

This study was conducted in the rainy season in 2019. The sampling location was in the floating net cage zone of Gajah Mungkur Wonogiri reservoir. Parameter analysis was carried out in the laboratory of water and waste water analysis at Setia Budi University, Surakarta.

The chemicals needed in this study include: 1) N-NH₃ analysis: ammonium-free distilled water, phosphate buffer, NaOH 1N, NaOH 6N, concentrated H₂SO₄, ZnSO₄, KNaC₄H₄O₆, H₃BO₄, Nessler reagent, ammonium standard, NaAsO₂. 2) N-NO₃ analysis: nitrate-free aquadest, stock nitrate 100 mg N-NO₃/L, water-free nitrate. 3) N-NO₂ analysis: nitrite-free aquadest, stock nitrite 100 mg N-NO₃/L, water-free nitrite. 4) P-PO₄ analysis: H₂SO₄ 5N solution, (K (SbO) C₄H₄O₆·½H₂O) potassium antimonyl tartrate solution, Ammonium molybdate ((NH₄) 6Mo₇O₂₄·4H₂O) solution, C₆H₈O₆ 0.1 M ascorbic acid solution, Potassium dihydrogen phosphate (KH₂PO₄). 5) Chlorophyll-a analysis: 4% formalin, aquadest, glycerin, O₂ and free CO₂ titration reagent, 0.45 µm filter paper, aluminum foil, MgCO₃, acetone. While the tools used include: water sampler,

Shimadzu UV-1800 UV spectrophotometer, a set of distillation tools and glassware. The sample of this study was WGM water, in the KJA fish cultivation zone and reservoir outlets. Water sample was taken in a representative manner from 11 points, namely 5 points in the KJA fish cultivation zone of PT Aquafarm Nusantara (P), 5 points for fish farmer KJA (T) and 1 point of WGM outlet (O). Data obtained from the laboratory were analyzed by comparing the content of phosphorus, nitrogen and chlorophyll-a to study the tropical status of WGM KJA water based on UNEP-IETC/ILEC, 2001 and PermenLH 2009.

This study procedure was based on the Indonesian National Standard (SNI). The research stages are: 1) representative water sampling based on SNI number 6989.57: 2008 (BSN, 2008). 2) field analysis: DO based on SNI Number 06-6989.14-2004, (BSN, 2004), pH based on SNI Number 06-6989.11-2004 (BSN, 2004), temperature based on SNI No. 06-6989.23: 2005 (BSN, 2005), 3) nitrogen analysis which includes parameters: nitrite (N-NO₂) according to SNI 6989.9-2004 (BSN, 2004), nitrate (N-NO₃) based on APHA 2012, Section 4500-NO₃, ammonia (N-NH₃) according to SNI 19-1655-1989 (BSN, 1989). 4) analysis of phosphorus (P-PO₄) according to SNI 06-6989-31: 2005 (BSN, 2005), 5) analysis of chlorophyll-a using the Strickland and Parson method.

Chlorophyll-a analysis procedure (Parson *et al.*, 1984): 1) Take 1-2 L of water, put it in a PE bottle (wrapped in aluminum foil or black plastic) and add 10 mL/L of 1% MgCO₃ solution sample. 2) Prepare 0.45 µm cellulose nitrate type membrane filter, 47 mm diameter, electric vacuum and Nalgen filtering apparatus. 3) Filter 100-2000 mL samples (depending on the level of plankton density). 4) Take the membrane filter on the surface carefully not to touch the surface, then folded into 2 equal parts, and folded again into 2 smaller parts, thus forming an angle of 45 °C. 5) Wrap the membrane filter with aluminum foil, then put it in a plastic clip, label it and store it in a closed ice box at 4 °C. 6) Then the filter paper containing chlorophyll-a was put into the tube and 10 mL of 90% acetone was added. The filter paper was crushed and kept in a cooler for 30 minutes, then centrifuged at 2000 rpm for 20 minutes. 7) Pour the clear liquid from the centrifuge into the cuvette, then the chlorophyll-a content was measured by a spectrophotometer at λ 662, 647, 630 and 750 nm.

RESULTS AND DISCUSSION

Laboratory test results for nitrogen pollutants measured as ammonia (N-NH₃), nitrite (N-NO₂), nitrate (N-NO₃) and phosphorus pollutants measured as phosphate (P-PO₄), at the sampling point KJA PT Aquafarm Nusantara (P), KJA Fish farmers (T), and WGM Outlet (O) presented in table 1. In the rainy season, the KJA zone of PT Aquafarm, fish farmers and the reservoir outlet contained ammonia nitrogen (N-NH₃) ranging from 0.0016-0.0148 mg/L. Ammonia pollutant in fish farmers' marine cage was greater than that of PT Aquafarm. The average ammonia content in fish farmers' marine cage was 0.0068 mg/L, while PT Aquafarm's marine cage was 0.037 mg/L. The ammonia pollutant content in the two sampling locations was still below the water quality standard for fisheries, a maximum of 0.02 mg/L. This condition was due to the ammonia in the water being transformed into nitrite naturally by the *Nitrosomonas* sp. which was found in the waters of KJA WGM.

Table 1. Distribution of Nitrogen and Phosphorus in KJA

Pollutants (mg / L)	Sampling Points										
	P1	P2	P3	P4	P5	T1	T2	T3	T4	T5	O
N-NH ₃	0.0025	0.0044	0.0023	0.0029	0.0064	0.0047	0.0098	0.0030	0.0148	0.0016	0.0055
N-NO ₂	0.0034	0.0023	0.0025	0.0046	0.0024	0.0025	0.0030	0.0030	0.0031	0.0023	0.0078
N-NO ₃	0.01	0.01	0.01	0.01	0.01	0.015	0.01	0.01	0.01	0.01	0.01
P-PO ₄	0.55	1.43	0.92	1.16	0.57	1.39	1.39	0.40	0.59	0.03	0.19

In table 1, the distribution of nitrite nitrogen (N-NO₂) pollutants at the sampling point of KJA PT Aquafarm (P) and fish farmers (T) ranges from 0.0023-0.0046 mg/L, increased at the reservoir outlet point by 0, 0078 mg/L. The average nitrite content of Aquafarm KJA is 0.0030 mg/L and fish farmer KJA is 0.0027 mg/L. The content of nitrite pollutants in KJA waters and reservoir outlets still met the water environmental quality standards in class 2 with a maximum nitrite content of 0.06 mg/L. This condition indicates that there was no nitrite contamination. It is since nitrite compounds in the water was transformed into nitrate compounds by the natural role of *Nitrobacter* sp. Based on the research of Pujiastuti, *et al.*, (2018), the positive zone of KJA WGM water contained *Nitrobacter* sp and *Nitrosomonas* sp. The distribution of nitrogen nitrate (N-NO₃) pollutant content is presented in table 1. At the sampling point, fish farmers' marine cage and aquafarm marine cage were 0.01 mg/L. The maximum quality standard for nitrate in water is 10 mg/L. Compared to these quality standards, the water quality at all KJA sampling points and reservoir outlets was below the quality standard, so it means that there has been no nitrate contamination. The distribution pattern of nitrogen pollutants, namely ammonia, nitrite and nitrate were almost evenly below the second class water quality

standard at all sampling points in Wonogiri WGM KJA. It indicates that the nitrogen content in KJA fish cultivation zone in the rainy season was still good, suitable for fish cultivation.

The distribution of phosphorus pollutants in KJA waters is presented in Table 1. Phosphorus pollutants at PT Aquafarm's KJA sampling points, fish farmers' marine cage and reservoir outlets were relatively high, ranging from 0.03 - 1.43 mg/L, with an average content of 0, 3760 mg/L. This distribution was not evenly distributed at all sampling points. The phosphorus pollutants in this marine cage come from fish feed that was not eaten by fish and fish feces which settle as waste. The phosphorus pollutant content at the PT Aquafarm Nusantara sampling point appears to be greater when compared to the KJA owned by fish farmers. It was due to different feeding patterns. At PT Aquafarm, it had a continuous feeding pattern to catch big fast fish targets, thus allowing more tilapia feed waste to settle as pollutants. The trend in the distribution of phosphorus pollutants had decreased until the reservoir outlet is 0.19 mg/L. It was since there had been mixing by the Keduang river water entering WGM. When compared with the second class water quality standard of a maximum of 0.02 mg/L, it can be seen that all sampling points have exceeded the quality standard.

There had been pollution from phosphorus pollutants in the fish culture zone in KJA at WGM in the rainy season. WGM KJA had a load of phosphorus and nitrogen pollutants from the remaining feed, feces and fish urine of 18,770.4 tons/year (Pujiastuti, *et al.* 2016).

The distribution of chlorophyll-a content in WGM KJA water is presented in table 2. Chlorophyll-a content at KJA WGM water, which sampled 3 times during the rainy season, containing chlorophyll-a which varies, ranging from 5.14 - 17.60 µg/L. The average chlorophyll-a at the

Aquafarm sampling point was 12.64 µg/L, fish farmers were 12.77 µg/L and outlets were 6.32 µg/L. The chlorophyll-a content at the sampling point of fish farmers was slightly higher than that of Aquafarm KJA. It was due to the higher content of phosphorus and nitrogen pollutants as plant nutrients, which triggers the development of green algae. Chlorophyll-a content decreased after arriving at the WGM outlet point. It was since the water leading to the outlet mixed with water from Keduang river, resulting in dilution.

Table 2. Distribution of chlorophyll-a KJA

Test (µg / L)	Sampling Points										
	P1	P2	P3	P4	P5	T1	T2	T3	T4	T5	O
I	13.75	8.77	13.51	15.59	15.52	11.29	13.44	11.81	10.41	13.06	5.60
II	12.17	13.26	14.44	5.14	15.49	15.47	16.6	12.24	17.6	16.59	5.99
III	11.51	12.37	11.90	11.60	14.57	9.84	11.55	11.74	5,57	14.33	7.38

Based on the chlorophyll-a content in table 2, the WGM KJA water at all sampling points and WGM outlet water have a mesotrophic status, because the chlorophyll-a content is greater than 5 µg / L. According to research Laksitaningrum *et al.* (2017), WGM water generally had

ultraligotropic, oligotropic, mesotrophic, mild eutropic and moderate eutropic status. Mesotrophic status in the water of KJA WGM showed an increase in nitrogen and phosphorus levels, but was still in tolerance limits, because it had not shown any indication of water pollution.

Figure 1. Nitrogen, Phosphorus and Chlorophyll-a

The effect of nitrogen and phosphorus pollutants on trophic status is presented in Figure 1. During the rainy season the average total nitrogen content at the sampling point of Aquafarm Nusantara (P) marine cage was 16.22 µg/L, smaller than that of fish farmers by 19.99 µg/L and the reservoir outlet is 23.25 µg/L. The three sampling locations had an oligotrophic trophic status, which indicated that the water quality was still natural and had not been contaminated with nitrogen. The trophical status of the oligotrophs in the WGM KJA water was due to natural biotransformation of nitrogen compounds derived from fish feed residue, feces and urine settling at the bottom of the reservoir as a source of pollutants. Biotransformation will occur naturally if the water contains nitrifying bacteria,

namely *Nitrosomonas* sp, which is able to remodel ammonia from the remaining feed into nitrite compounds. Meanwhile, the nitrite compounds in the water were immediately broken down into nitrate compounds naturally by the bacteria *Nitrobacter* sp (Saha *et al.*, 2013; Pujiastuti, *et al.*, 2018). Both of these bacteria were in WGM KJA water and develop naturally, because the quality of the water allows the bacteria to grow and to develop.

The nitrification process is a biological process, involving the role of aerobic bacteria, which is influenced by: 1) Dissolved Oxygen/DO (Podgorny & Leonov, 2013), Based on Titiresmi & Sopiah (2006), if DO < 2 mg / L can interfere with nitrification. WGM KJA water contained an average DO of 7.4 mg/L. 2) Water temperature, nitrifying bacteria

worked optimally at 35 °C. The water temperature of KJA WGM is 36 °C. 3) The optimum pH for the nitrification process was between 7.5-8.5, although nitrifying bacteria was sensitive to pH, these bacteria can adjust to pH values beyond the optimum range. The optimum pH of nitrification was 8.4, at pH 7 the efficiency can still be achieved at 80% and 90% of the maximum rate of the nitrification process at 7.8 - 8.9, while at pH outside 7.0 -9.8 the process rate which occurred in less than 50%. Nitrite is unstable at acidic pH (Shen *et al*, 2003). The rate of the nitrification process will decrease at pH 6.3 - 6.7 and at pH 5 - 5.5 the nitrification process will stop (Sykes *et al.*, 2006). WGM KJA water had a pH of 7.8 -7.9. It is the optimal pH of the nitrifying bacteria to carry out the biotransformation process. 4) Detention time, the length of time where the waste water contacts with decomposing microorganisms in water. The length of detention time used will affect the effectiveness of the nitrification process. 5) Ammonia nitrogen content (N-NH₃), can inhibit the growth of nitrifying bacteria if the concentration is greater than 750 mg/L, while the ammonia content above 1000 mg/L will be toxic to nitrifying bacteria (Titiresmi & Sopiah, 2006). Ammonia nitrogen KJA WGM ranged from

0.0016 - 0.0148 mg / L, did not inhibit the work of nitrifying bacteria. This is the optimal pH of the nitrifying bacteria to carry out the biotransformation process. 4) Detention time, the length of time the waste water contacts with decomposing microorganisms in water. The length of detention time used will affect the effectiveness of the nitrification process. 5) Ammonia nitrogen content (N-NH₃), can inhibit the growth of nitrifying bacteria if the concentration is greater than 750 mg/L, while the ammonia content above 1000 mg/L will be toxic to nitrifying bacteria (Titiresmi & Sopiah, 2006). ammonia nitrogen KJA WGM ranged from 0.0016 - 0.0148 mg/L, did not inhibit the work of nitrifying bacteria.

The total phosphorus to the tropical status of WGM water of the marine cage is shown in Figure 2. The quality of water for marine cage in the rainy season shows that phosphorus levels range from 273-1091µg/L in KJA PT Aquafarm Nusantara marine cage and range between 4-320 µg/L in fish farmer marine cage. Phosphorus as total P is a plant nutrient and is also one of the water quality parameters needed to determine the load capacity of reservoir water pollution to meet the specified tropical status.

Figure 2. Tropical Status of WGM KJA

In Figure 2, it can be seen that PT Aquafarm KJA water have a tropical hypereutrophic status, because they have a P-PO₄ number exceeding 100 ug / L. Samples from KJA water of fish farmers shows diversity, 2 locations shows hyperutrophic status, namely points T2 and T3. Sample T4 shows tropic status of eutrophs, Sample T5 shows tropic status of Mesotrophs, while sample T1 shows tropic status of oligotrophs.

The diversity of tropical status in the KJA zone was due to different feeding patterns, so that the amount of the rest feed that was not eaten by fish and settles on the bottom of the water was different. The more remaining feed that settles, it will cause increased phosphorus pollution.

According to Laksitaningrum, *et al.*, (2017), based on the analysis of total

phosphorus, WGM waters have three tropic statuses, namely moderate eutropic, severe eutropic and hypereutrophic. In the KJA fish cultivation zone, it had a moderate eutropic distribution.

Corophyll-a to the trophic status of WGM waters of KJA zone is presented in Figure 3. The presence of chlorophyll-a in water can also be used to determine the tropical status of the reservoir. The determination of the reservoir tropical status was based on water quality data and criteria for tropical status. Reservoir water with chlorophyll-a of <2 µg/L indicates oligotrophic tropic status, chlorophyll-a content of <5 µg/L indicates mesotrophic tropic status, whereas if containing chlorophyll-a <15 µg/L it indicates

eutrophic tropical status and if containing $\geq 100 \mu\text{g} / \text{L}$ it indicates hypereutrophic tropic status.

Figure 3. Chlorophyll-a and Tropical Status

The relationship between chlorophyll-a content and tropic status of WGM KJA is presented in Figure 3. Aquafarm Nusantara marine cage contains chlorophyll-a ranging from 5.14 - 15.59 $\mu\text{g} / \text{L}$, with an average chlorophyll-a of 12.64 $\mu\text{g} / \text{L}$, has a eutrophic status, meaning that it contains high levels of nutrients, This status indicates the water had been contaminated by increased levels of nitrogen and phosphorus, so that it can trigger the growth of green algae which was characterized by detection of chlorophyll-a in water. KJA water of fish farmer contained chlorophyll-a between 5.57-17.6 $\mu\text{g} / \text{L}$, with an average of 12.77 $\mu\text{g} / \text{L}$. This indicates eutrophic status, the KJA water Fish Farmers contained high plant nutrients, this status indicates that the

water had been polluted by increased levels of nitrogen and phosphorus. Chlorophyll-a content became smaller at the sampling point WGM outlet water compared to KJA water, which ranged from 5.60 - 7.38 $\mu\text{g} / \text{L}$, with an average content of 6.32 $\mu\text{g} / \text{L}$. The tropical status in WGM outlet waters was eutrophic, indicating that the water had been polluted by increased nitrogen and phosphorus levels. It is due to mixing of Keduang river water that entered the reservoir.

CONCLUSION

The WGM KJA water had eutrophic status during the rainy season. It shows that WGM KJA water had been polluted by the enhancement of nitrogen as 18,345 $\mu\text{g} / \text{L}$ and phosphorus as 420,65 $\mu\text{g} / \text{L}$.

These nitrogen and phosphorus pollutants increased the growth of chlorophyll-a in KJA water by 12.77 µg/L.

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