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# Phytoremediation by *Echinodorus palaefolius* to Reduce Nitrogen and Phosphate Waste of Intensive Culture *Anguilla bicolor* in Recirculation Aquaculture Systems

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*Abstract*— **Increase in aquaculture activities leads to a negative impact on the environment. Thus, phytoremediation through recirculation aquaculture system becomes one effort that can be applied. The study aimed to evaluate the administration of *Echinodorus palaefolius* (water jasmine plant) through increasing the capacity of plants as phytoremediators in reducing the waste of intensive culture eel. This study used a completely randomized design with three treatments of *E. palaefolius* density and repeated 3 times, as treatments were 1.04 g $L^{-1}$ (Ep1), 2.08 g $L^{-1}$ (Ep2), and 3.13 g $L^{-1}$ (Ep3). The initial average eel weight was 8.3 ± 0.13 g, with a stocking density of 4 g $L^{-1}$. The results showed a significant difference in the effect of *E. palaefolius* density on nutrient removal efficiency, as well as performance on eel and plant growth. The highest efficiency of nutrient removal in *E. palaefolius* with a density of 2.08 g L-1, nitrite (49.65 ± 4.52) %, nitrate (59.62 ± 1.89) %, phosphate (60.88 ± 1. 03) %, and TAN (46.03 ± 0.63) %. At *E. palaefolius* density 2.08 g L-1 produced eel specific growth rates (0.99 ± 0.02) % lowest feed conversion (1.97 ± 0.03), and highest increase of *E. palaefolius* biomass (262, 33 ± 2.60 g with daily growth 4.37 ± 0.43 g day-1). Thus, it can be concluded that the density of *E. palaefolius* 2.08 g L-1 produces the best efficiency in removing nutrients, the growth performance of eels and plants**.

*Keywords*— **Eel culture; nutrient removal efficiency; RAS; water jasmine plant; wastewater.**

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## I. INTRODUCTION

The development of the aquaculture industry experiences a massive escalation in fulfilling animal protein market supply. The statistical data of The Ministry of Fisheries and Marine Affairs noted that in 2011, 2012, 2013, and 2014, the total volume of aquaculture production increased each year as many of 7,928,962 tons, 9,675,553 tons, 13,300,126 tons, and 14,332,733 tons, respectively, or 18% of improving production each year [1]. Eel is one of the most consumed species in various countries, especially East Asian (Japan, Taiwan, Korea, China, and Hongkong), Europe (Italy, German, France, and Netherland), and The United States of America.

The worldwide production of eel in 2014, 2015, and 2016 were approximately 259,445 tons, 282,282 tons, and 293,293 tons, respectively [2]. The global market demand reaches about 350,000 tons/year, with market price ranges relatively 12.5 US$/kg – 16 US$/kg. Japan, as an example, the most leading export destination of eel in the world with the total consumption of eel approaches 130,000 tons/year, is only able to accomplish 29.4% of its market demand (38,228 tons). The production capacity of eel in Indonesia is projected approaching 120,000 tons, yet the reality is only 10,000 tons or 8.33% of the target production [3]. The market demand for eel is a prospective market opportunity and the possibility to be fulfilled by conducting improvement production.

Intensive aquaculture of eel will impact extra feed application. In intensive aquaculture, fish are reared in high density, and almost every source of the nutrition is obtained from the high protein feed. The feed retention of fish was only 20-30% [4]. Meanwhile, the rest of it was excreted to the environment in ammonia and organic protein form. Generally, 60-90% of the nitrogen excretion was thrown in soluble form, while phosphorus was excreted in metabolism waste form [5].

The organic materials are accumulated in the rearing media. In the long term of rearing, it potentially causes anaerobic decomposition, which produces toxic gas, i.e., ammonia. The major source of ammonia in rearing media is protein feed, both uneaten feed and metabolism waste. Ammonia exists in a rearing media in the form of total ammonia nitrogen (TAN), the ionized ammonia ($NH_4^+$), and unionized ammonia ($NH_3$). Decreasing the accumulation of waste and maintain the water quality, especially in limited supply, it is urgently required a comprehensive system to overcome the problem. Along with developing aquaculture technology, a recirculation system is one of the applicable alternatives in breaking down the aquaculture waste.

The recirculated phytoremediation system is potentially able to optimize water usage and water quality. The wastewater aquaculture contains organic materials that are needed by a plant as a nutrient source to grow out [6]. The recirculation principle is the reuse of rearing media, which is already emitted from the aquaculture system. The beneficial factors of the recirculation system are minimizing water usage, maintaining pH level, and reducing toxic compounds, i.e., ammonia and nitrite [7], [8]. The excellence of phytoremediation compared to the other waste management technique are natural and low-cost technology. It could decrease organic materials to create a synergy between plants, fish, and the environment, and it does not require high technique [9].

The application of *L. perpusilla* in wastewater management effectively decreased nitrogen waste as many of 4.48±0.04 g and 75% of $NH_3$-N (0.87 to 0.31 mg $L^{-1}$) [10]. The mustard green was able to cut the concentration of TAN, $NO_3^-$, and $PO_4$ as many of 69%, 56.67%, and 66.79%, respectively [11], *Pistia stratiotes* could reduce TAN, $NO_3^-$, and $PO_4$ approximately 81.90%, 48.62 %, 54.46%, respectively, and vetiver grass managed to diminish 48.36% of TAN and 19.94% of $PO_4$ [12]. This study applied water jasmine plant *Echinodorus palaefolius* as a phytoremediator. [13] explained that the Mexican sword plant was able to lower Pb concentration in the waste reactor until below 0.0764 mg $L^{-1}$. An absorbent metal plant from a waste reactor absorbed 4.87 mg $kg^{-1}$ with 81.72% of removal efficiency, while an absorbent metal plant from a control reactor absorbed 6.38 mg $kg^{-1}$ with 86.05% of removal efficiency. *E. palaefolius* and natural zeolite to remove mercury from contaminated water in a laboratory-scale apparatus. Hg removal was taken place by a combined mechanism of both adsorption and phytoremediation. The removal of 91.84% was achieved at the end of the experiment showing a promising application of SSF-CW to overcome mercury contamination in water, especially in the ex-artisanal gold mining sites in Indonesia [14]. Treatment using aquatic plant *E.amazonicus* and *E.palaefolius* as phytoremediator highly affected the declining concentration of TAN, $NO_2$, $NO_3$, and $PO_4$ in fish culture wastewater. The ability of *E.palaefolius* to utilize the nutrient of fish culture waste was found to be better than that of *E. amazonicus* [15].

The water jasmine plant can remediate the waste. Hence, a further study about the capacity of water jasmine plants as phytoremediators in an intensive eel culture is required. Besides, the water jasmine plant has an aesthetics aspect so that it can be used in an aquascape, both indoor and outdoor. This study aimed to evaluate the capacity of the water jasmine plant as a phytoremediator in eliminating intensive culture waste of eel so that it is expected that the intensive eel production will be elevated.

## II. Materials and Methods

This study used a complete randomized design with three treatments and three replications. The treatment was a different density of water jasmine plants. The treatments consisted of 1.04 g $L^{-1}$ (Ep1), 2.08 g $L^{-1}$ (Ep2), and 3.13 g $L^{-1}$ (Ep3). The stock density of eel was 4 g $L^{-1}$. The feed used in this study was paste with 45% of protein content.

### A. Experiment Preparation

Two kinds of containers used in this study were eel rearing containers and aquatic plant rearing containers. The rearing eel was a round tarp basin, the diameter was 50 cm, and the height was 55 cm, equipped with nine recirculation systems. The water jasmine plant was reared in an aquarium sized in 60 cm × 40 cm × 40 cm. The bottom of the aquarium was set to double the bottom filter system consisted of pipe gutter as a buffer, soft gauze, and Dacron as filter and sand as the substrate for the plant. The average weight of the tested eel was 8.3±0.13 g/individual, with stocking density 4 g $L^{-1,}$ and it was randomly placed in the experiment unit.

The eel was taken from a farmer in Tulungangung, East Java. Before the study began, the eel was adapted for two weeks in freshwater and fed using grouper feed with 45.25% of protein content. During the adaptation, the health status and vitality of glass eel were observed to ensure the glass eel was proper to be an experimental subject. A 24 hour before treatment, the tested glass eel was fasted to eliminate the previous feed impact. Then weight measurement was conducted. The aquatic plant, Mexican sword plant, was obtained from an ornamental plant farmer, then adapted in a fiber tank five days before treatments.

### B. Data Collection and Experiment Parameters

The raring period was 60 days. The tested eel was fed (based on the first step of the study) 4% of biomass with feeding frequency three times (08.00, 13.00, and 18.00). The eel biomass was measured every 30 days and started on day 0 to calculate the growth and feeding method. The growth performance was observed through body weight. The protein and lipid content were measured at the beginning and the end of the study. The overall parameters were survival rate, daily growth rate, feed conversion, feeding efficiency, protein, lipid, and energy retention. The observation of blood glucose was conducted as a secondary stress indicator as a result of applying a recirculation and phytoremediation system. The blood profile was also analyzed, and the parameters were erythrocyte, leucocyte, hemoglobin, and hematocrit.

The aquatic plant was weighted the initial weight, and the final weight was measured at the end of the study (day 60) to calculate the final biomass. The nitrogen and phosphorus measurement in the plant biomass was done at the beginning and at the end of the study. The water quality parameters which daily analyzed were temperature, pH level, dissolved oxygen, while $NO_3^-$, $NO_2^-$, $PO_4$-P, TAN, and TSS was observed once in two weeks [16]. The calculation of nutrient removal efficiency, TAN, nitrite, nitrate, and phosphate was also done once in two weeks. The absorption rate of N and P by the water jasmine plant was calculated using the formulation by Kitadai and Kadowaki [17].

## III. Results and Discussions

### A. Results

The water quality parameters consisted of temperature, pH level, dissolved oxygen, alkalinity, $CO_2$, total suspended solids (TSS), total ammonia nitrogen (TAN), ammonia ($NH_3$), ammonium ($NH_4$), nitrite ($NO_2^-$), nitrate ($NO_3^-$), and orthophosphate ($PO_4$). The results of the measurement are presented in Table 1. The stock density of water jasmine plant in Table 1 below has some terms, such as Ep1 = 1.04 g $L^{-1}$; Ep2 = 2.08 g $L^{-1}$ ; Ep3 = 3.13 g $L^{-1}$

TABLE I
WATER QUALITY PARAMETERS DURING A 60-DAY OF EEL REARING

| Parameter | Ep1 | Ep2 | Ep3 |
|---|---|---|---|
| Temperature ( °C) | 29.0 - 30.7 | 29.1 - 30.5 | 29.1 - 30.7 |
| DO (mg $L^{-1}$) | 4.2 - 6.7 | 4.4 - 6.8 | 4.3 - 6.8 |
| pH | 7.1 - 7.3 | 7.1 - 7.5 | 7.1 - 7.5 |
| Alkalinity (mg $L^{-1}$) | 59 - 124 | 59 - 120 | 59 -132 |
| $CO_2$ (mg $L^{-1}$) | 2.68 - 3.88 | 2.68 - 3.84 | 2.68 - 3.92 |
| TSS (mg $L^{-1}$) | 1.68 - 6.64 | 1.68 – 4.47 | 1.68 – 6.08 |
| TAN (mg $L^{-1}$) | 0.15 - 0.35 | 0.15 - 0.23 | 0.15 - 0.33 |
| $NO_2$ (mg $L^{-1}$) | 0.01 - 0.25 | 0.01 - 0.22 | 0.01 - 0.31 |
| $NO_3$ (mg $L^{-1}$) | 13.35 - 49.34 | 13.35 - 35.60 | 13.35 - 48.28 |
| $PO_4$ (mg $L^{-1}$) | 0.6 - 7.96 | 0.6 - 5.85 | 0.6 - 8.78 |

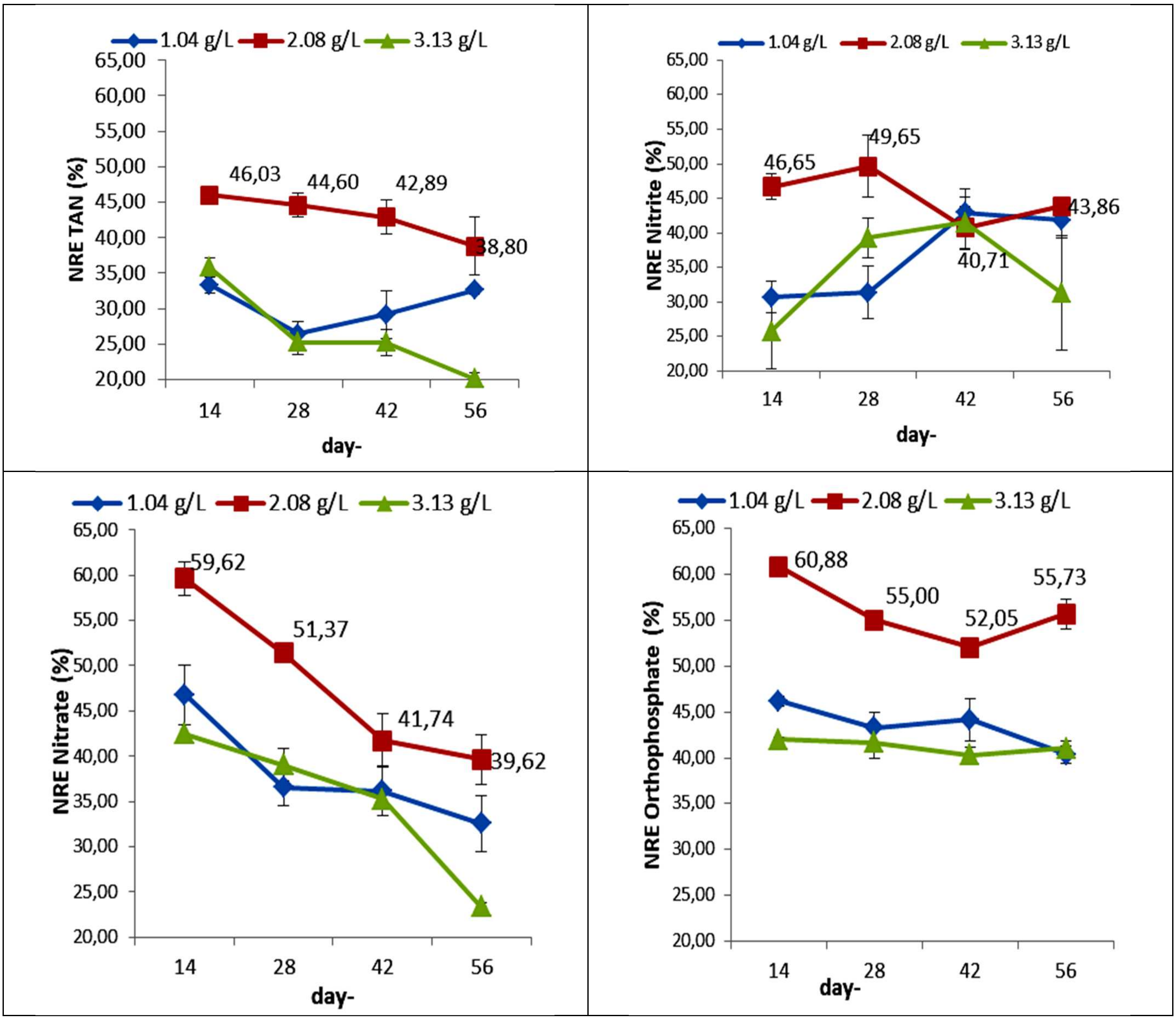


Fig. 1 Nutrient removal efficiency of (%) TAN (a), Nitrite (b), Nitrate (c), and Phosphate (d)

Figure 1 showed nutrient removal efficiency (NRE) every two weeks in each treatment. The NRE of nitrate ($NO_3$) and orthophosphate ($PO_4$) in each treatment tended to decrease every two weeks, except the Ep2, increased at the 8$^{th}$ week. The NRE of TAN declined in the Ep2 and Ep3 treatment, while the Ep1 only declined at 4$^{th}$ week and increased at 6$^{th}$ and 8$^{th}$ week. The NRE of nitrite fluctuated almost in all treatments.

The growth performance parameters of eel (survival rate, Δbiomass, specific growth rate, feeding efficiency, feed conversion, protein and lipid retention, and coefficient of variance) is shown below in Table 2.

TABLE II

GROWTH PERFORMANCE OF EEL DURING THE REARING PERIOD

| Parameter | Ep1 | Ep2 | Ep3 |
|---|---|---|---|
| Survival rate (%) | 100.00±0.00 | 100.00±0.00 | 98.85±1.99 |
| Δ biomass (g) | 114.5±3.48[a] | 152.17±2.42[b] | 105.70±2.91[a] |
| Specific growth rate (%) | 0.79±0.01[a] | 0.99±0.02[b] | 0.74±0.02[a] |
| Feeding efficiency (%) | 38.17±2.01[a] | 50.72±1.40[b] | 35.23±1.68[a] |
| Feed conversion | 2.62±0.08[b] | 1.97±0.03[a] | 2.84±0.14[b] |
| Protein retention (%) | 26.32±1.38[a] | 32.97±2.65[b] | 22.18±0.80[a] |
| Lipid retention (%) | 43.99±1.71[ab] | 55.46±2.85[b] | 39.13±1.31[a] |
| Coefficient of variance (%) | 20.67±4.46 | 24.24±1.37 | 23.68±2.17 |

Different superscript in the same row indicated a significant difference in 5% of a significant level (Duncan multiple range tests)

The result of statistical analysis described a significant difference ($P<0.05$) in all growth parameter, except survival rate and coefficient of variance. The Ep2 treatment (*E. palaefolius* 2.08 g/L) presented the highest value of Δbiomass, specific growth rate, feeding efficiency, feed conversion, and protein & lipid retention. The physiological responses in all treatments are explained by the blood profile parameters, blood glucose, and total serum protein. Those parameters are shown below in Table 3.

TABLE III

BLOOD PROFILE, GLUCOSE, AND TOTAL SERUM PROTEIN OF TESTED EEL AT THE END OF THE REARING PERIOD

| Parameter | Ep1 | Ep2 | Ep3 |
|---|---|---|---|
| Hematocrit (%) | 26.21±0.17 | 27.14±0.10 | 27.15±0.45 |
| Erythrocyte ($10^6$ cell $mL^{-1}$) | 7.72±0.14[a] | 8.07±0.23[b] | 7.68±0.82[a] |
| Hb (g/%) | 6.67±0.13[a] | 7.13±0.12[b] | 7.23±0.10[b] |
| Leucocyte ($10^4$ cell $mL^{-1}$) | 6.33±0.02[b] | 6.23±0.02[b] | 5.53±0.10[a] |
| Glucose (mg $dL^{-1}$) | 34.23±0.48 | 26.77±2.15 | 27.10±3.12 |
| Cholesterol | 77.37±0.49[b] | 74.23±1.69 | 70.70±0.38[a] |
| Total serum protein (mg $L^{-1}$) | 32.40±0.55[b] | 24.47±0.68[a] | 22.77±0.41[a] |

Different superscript in the same row indicated a significant difference in 5% of a significant level (Duncan multiple range tests)

The erythrocyte, leucocyte, cholesterol, and total serum protein were different significantly between treatments ($P<0.05$). On the contrary, there was no significant difference in hematocrit and glucose content ($P>0.05$).

During the 60-day of rearing, the water jasmine plant grew by utilizing nutrient from the intensive eel culture. The growth performance of the plant was observed by measuring the additional biomass (Δ biomass), daily growth, nitrogen retention, and phosphorus retention in the plant tissues. The result is presented below in Table 4.

TABLE IV

THE GROWTH PERFORMANCE OF MEXICAN SWORD PLANT *E. PALAEFOLIUS*

| Parameter | Ep1 | Ep2 | Ep3 |
|---|---|---|---|
| Δ biomass (g) | 124.33±4.25[a] | 262.33±2.60[c] | 210.00±2.89[b] |
| Daily growth (g/day) | 2.07±0.07[a] | 4.37±0.43[b] | 3.50±0.78[b] |
| Nitrogen retention (g) | 99.87±4.39[a] | 170.84±0.54[c] | 143.11±6.53[b] |
| Phosphorus retention (g) | 68.23±3.62[a] | 149.43±0.39[c] | 127.12±1.64[b] |

Different superscript in the same row indicated a significant difference in 5% of a significant level (Duncan multiple range tests)

The statistical analysis showed a significant difference between treatments ($P<0.05$) in all growth parameters of the water jasmine plant. The Ep2 had the highest value of Δ biomass, daily growth, and nitrogen & phosphorus retention. The absorption rate of nitrogen and phosphorus also presented a significant difference ($P<0.05$). The highest value of nitrogen and phosphorus absorption rate was obtained in the Ep2, which is 0.12 mg $g^{-1}$ $day^{-1}$ and 0.11 mg $g^{-1}$ $day^{-1}$. The graph of nitrogen and phosphorus absorption rate was shown in Figure 2.

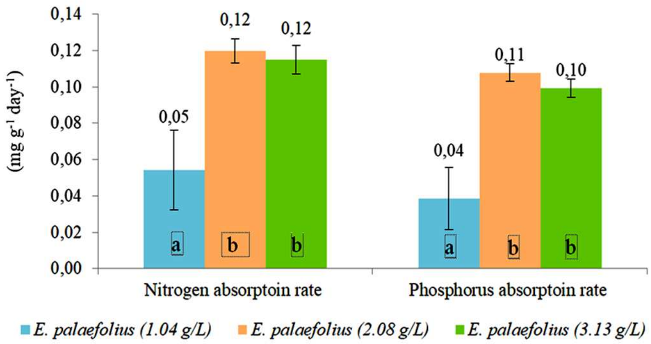


Fig. 2 The nitrogen and phosphorus absorption rate of water jasmine plant

## B. *Discussions*

The eel rearing using phytoremediation technology is closely affected by several factors, such as temperature, pH level, and dissolved oxygen. One of the crucial physical environmental factors is temperature. The temperature change will directly affect the metabolism rate of the aquatic organism [18]. The higher the temperature gets, the metabolism rate will get higher as well. A high metabolism rate will induce feed consumption and produce a high level of waste at the same time. [19] stated that the tolerable range of temperature for eel rearing is 27–31°C and the temperature during the treatment was on the tolerable range for eel rearing (29.0–30.7°C). The eel could live in the temperature range from 28°C to 33°C [19]. A rapid temperature fluctuation possibly causes stress which is characterized by a high level of blood glucose, and it potentially interferes growth [21].

The pH level during the rearing period ranged from 7.1 to 7.5. It is considered normal and relatively optimum for eel rearing (6.0–8.0) [22] and (7.0–8.0) [18]. The dissolved oxygen during the treatment ranged from 4.2–6.8 mg.$L^{-1}$. The result was relatively normal (>4 mg. $L^{-1}$). The dissolved oxygen utilizes to transform organic material [19] s into a soluble inorganic nutrient so that the plant can absorb it.

The alkalinity during the rearing period was believed still in an optimum range of 59–132 mg.$L^{-1}$. The result of the alkalinity level was in line with [19], which stated that the tolerable alkalinity was 58–123 mg.$L^{-1}$. TSS is total solids in the aquaculture system, but it does not completely soluble. Thus, the rearing media becomes turbid and muddy [23]. The TSS level during the study ranged from 1.68–6.64 mg.$L^{-1}$. It got higher because of the waste culture of the eel during the study.

The carbon dioxide ($CO_2$) was utilized by the aquatic plant in photosynthesis. The result of $CO_2$ during the overall study period was 2.68–3.88 mg.$L^{-1}$. It was considered normal, compared to the previous study [24] who stated that a rainbow trout could survive and tolerated $CO_2$ until 16 mg.$L^{-1}$.

An aquaculture activity and all its related system undoubtedly produce a high level of organic waste and nutrients. Generally, most of the nitrogen waste (60–90%) exists on soluble molecules (ammonia), while phosphorus is around 25-85% [5]. The ammonia concentration in this study was stated in TAN value. TAN, $NH_3$, and $NH_4^+$ value was 0.15–0.23 mg $L^{-1}$, 0.002–0.003 mg $L^{-1}$; 0.15–0.23 mg $L^{-1}$, respectively (Table 2). It was lower than the result by [25] who stated that the TAN, $NH_3$, and $NH_4$ value in a remediator system using *Eichhornia crassipes* and *Pistia stratiotes* were 1.34 mg $L^{-1}$; 1,79 mg $L^{-1}$; 0,14 mg $L^{-1}$ and 0,2 mg $L^{-1}$; 0.13 mg $L^{-1}$; 0.22 mg $L^{-1}$, respectively.

The water quality in a recirculation system and phytoremediation-based technology was considered as optimum. It was generated by the waste utilization of the fish/eel by the aquatic plant. A similar result was reported that water spinach could diminish 84.6% of TAN level, 34.8% of nitrate concentration, and 44% of phosphate [6] and identical outcome has been also found by another previous study [12]. It was described that vetiver grass reduced 48.36% of TAN level and 19.94% of $PO_4$. The most advanced result in this study was obtained in the Ep2 treatment in eliminating TAN, nitrite, nitrate, and phosphate concentration (density of the plant was 2.08 g/L). The highest removal percentage of TAN, nitrate, and phosphate was in the 2nd week (46.03%, 59.64%, and 60.88%, respectively), while the highest removal percentage of nitrite, nitrate, and phosphate resulted in the 4th week (49.65%). The lowest nutrient removal efficiency resulted in Ep3 treatment (Figure 1). Plant as a phytoremediator agent utilizes inorganic nutrients through its root [11]. To support fish growth, ammonium is not a toxic compound. In an adequately oxidated environment, ammonia will be transformed into an intermediate product (nitrite).

Nitrite is a relatively unstable compound because, on sufficient oxygen, it will easily be oxidated by *Nitrobacter* to nitrate. The overall nitrite concentration in this study was tolerable by the eel. The nitrite changes in the environment were still in control because of the adequate level of oxygen. In a circulating system, nitrite concentration should be lower than 1 mg $L^{-1}$ [23]. The nitrite concentration must lower than 0.1 mg $L^{-1}$[22], [26]. The *Nitrobacter* oxidates nitrite into nitrate. Nitrate is the final product of the nitrogen cycle in aerobic conditions. It is completely harmless for the aquatic organism and acts as a nutrient source for aquatic plant beside $NH_4$. The nitrate concentration in this study still supports the eel growth and survival rate because it ranged from 13.35–49.34 mg $L^{-1}$. The $NO_3^-$ in a recirculation system was approximately 40.8 mg $L^{-1}$ [7]. Another previous study recommended the $NO_3$ should be lower than 50 mg $L^{-1}$ for aquaculture activity [27].

According to the growth performance of the eel (Table 2), the density of the water jasmine plant as a phytoremediator in a recirculation system of eel culture was significantly influenced towards eel growth ($P<0.05$). In the Ep2 treatment (2.08 g $L^{-1}$) resulted in the highest growth performance ($P<0.05$), consisting of 0.99% of the specific growth rate, 50.72% of feeding efficiency, 1.97 of feed conversion, 32.97% of protein retention, and 55.46% of lipid retention. It was possibly caused by its ability to utilize the nutrient from aquaculture waste. It was also the conceivable factor that caused an ideal level of water quality in the Ep2 treatment. The application of *Lemna gibba* in a recirculation system decreased the feed conversion, increased protein efficiency ratio, and growth performance of Nile tilapia [28], [29]. Feed conversion in aquaculture industry profoundly needs certain consideration because it affects production cost directly. Feed cost can reach up to 70% of total production cost [30]. Thereby, feed conversion cutback will precisely affect the profitability because of the lower feed cost. The blood glucose, total serum protein, and blood profile parameters indicated that the tested eel had no stress indicator.

The calculation of the Complete Blood Cell Count is an important and powerful diagnostic tool of the minimum database, which can be used to monitor the health status response of fish to several changes such as nutrition, water quality, and disease [31]. There is an increased interest in the study of haematological parameters and the description of fish blood cell structure considered important for nursery purposes [32]. It has been reported in another previous study that salmon (*Arius leptaspis*) and tarpon (*Megalops cyprinoides*) that tarpon can take oxygen directly from the air lead to have a higher number of red blood cells than salmon [33]. Indications of low red blood cells and hematocrit in fish cause anaemia when fish stop eating due to illness or stress. The reduction in the number of red blood cells was probably caused by an increase in stocking density [32], [34].

Stress condition will increase the level of fish blood glucose [35] that increase maintain of their homeostasis as a result of decreased insulin [36] and then chromaffin cells will release the hormone catecholamines, adrenaline, noradrenaline into the blood. Eventually, the stress hormone will combine with the cortisol hormone leading to an increase in blood glucose through the process of glucogenesis and glycogenolysis. The plasma glucose levels increase by an increase in stocking density, possibly caused an increase in catecholamine and cortisol in controlling carbohydrate metabolism [37]. In this study, differences in cork fish stocking density caused differences in hematological parameter conditions. Although the results were very varied and not always conclusive, many authors also report the high stocking density effect on several hematological parameters [32], [37]–[39],

The present study was to see the physiological response of eel to the treatment and analyze other blood chemical parameters, including cholesterol and total serum protein. A decrease in total protein levels in the blood indicates that fish are traumatized by a bacterial infection, which reduces the appetite of the fish or the fish stops eating, effected a decrease in protein levels of the blood plasma [40]. Cholesterol, blood glucose, and total protein levels are secondary stress responses in fish [41]. An increase in cholesterol and blood glucose levels indicated that the fish was experiencing stress over the treatment. Likewise, a decrease in total protein levels in fish blood also indicated that the fish has begun to decrease their appetite. Blood glucose, hematocrit, and leucocyte, normally ranges from 29–43 mg $dL^{-1}$ [42], 24–36% [43], and $3.60–7.58\times10^4$ cell $mL^{-1}$, respectively.

The growth performance of the Mexican sword plant indicated a positive response. It was characterized by biomass improvement in all treatments, nitrogen retention, and phosphorus (Table 4). The Mexican sword plant utilized the aquaculture waste to support its growth. It was proved by the increase in nutrient removal efficiency every two weeks (Figure 2). Generally, a floating aquatic plant greatly grows using $NH_4$ as a major nitrogen source. It was because the energy needed for assimilation and energy production from $NH_4$ was lower than using $NO_3$ as major nitrogen source [44], [45]. However, the water jasmine plant was known as absorbed higher $NO_3$ than $NH_4$ (Figure 2). The water jasmine plant *E. palaefolius* is an emerged aquatic plant. The root grows at the bottom of the substrate, and the stem appears to the water surface. Therefore, most part of the plant could absorb the nutrient.

The nitrate transport in the root plasma membrane and nitrate reductase activity (NRA) is commonly induced by $NO_3$ supply in the environment. Most of floating aquatic plant has a high level of NRA in the root, while the emerged and half-submerged in the water held the high level of NRA in the leaf and rod. The decrease of $NO_3$ and leaf assimilation has an advantage because the ATP needed for $NO_3$ absorption occurs in the photophosphorylation located in the chloroplast [27]. Different level of NRE in a variable density of water jasmine plant was caused by the lower nutrient absorption rate of the Ep1 treatment (1.04 g/L), compared to the Ep2 treatment. The Ep1 treatment was not able to reduce aquaculture waste as efficiently as the Ep2 treatment because of the lower density of water jasmine plants. It also led to the returning point of the culture waste back to the system. The water quality got worse, and it inhibited the eel from growing.

The Ep2 showed better water quality parameter, and physiologically, the tested eel tended to utilize nutrients in the feed greater and more efficient. Undoubtedly, the growth performance will be more advanced. The NRE level in the Ep3 treatment was considered lower than the Ep2 treatment. It was assumed that the Ep3 had a more rooting capacity. Unfortunately, it is inversely related to the substrate capacity [46], [47]. The young root is the main site of nutrient absorption because the lignification process has not occurred yet. The nutrient absorption process by the old roots is slower due to the thick epidermis and hypodermis tissues caused by the lignification process [27]. Even though the nutrient absorption site of the water jasmine plant can be done by the leaves and rods, roots tissues held an essential role as well. It was proved by the highest nitrogen, and phosphorus absorption rate was obtained in the Ep2 treatment. It was shown by the nitrogen and phosphorus retention in the plant tissues. The growth performance of the water jasmine plant in the Ep2 was also higher, compared to the Ep1 and Ep3 treatment. The better absorption of aquaculture waste resulted in a better environment for the eels to survive and grow (Table 2). Thereby, feeding efficiency was improved. It was confirmed by the high level of nutrition retention and growth performance of the eels.

The total amount of feed during 60-day maintenance was 350 g. The N content in each feed was 7.24%, so the consumed N in the feed was 2528.4 g. Eel Production Ep1: 389.26 g; Ep 2: 426.93 g; and Ep3: 380.46 g. The total N content in feed was calculated as 100%. In the total nitrogen balance in the system, the N was retained in the eel fish body by 39.64% (Ep1), 56.17% (Ep2), and 39.54% (Ep3), while those were accumulated in sediment and gaseous by 43.64% (Ep1), 20.47% (Ep2), and 34.61% (Ep3). Nitrogen was measured in plants, 15.57% (Ep1), 21.78% (Ep2), and 23.99% (Ep3). The highest yield of N retained in the body of eel in phytoremediation systems using water jasmine plants with a density of 2.08 g $L^{-1}$ was 56.17%, so that it could support the production of eel by 426.93 g, while the highest accumulation of N in sediment in the recirculation system with a density of water jasmine was 1.04 g $L^{-1}$. The nitrogen mass balance in this system was be illustrated in Figure 3.

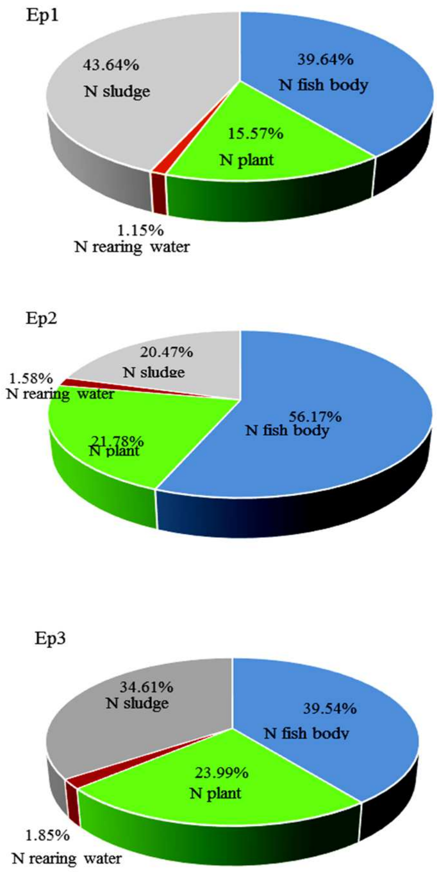


Fig. 3 Mass balance of Nitrogen in the Ep1, Ep2, and Ep3

A total of nitrogen balance in aquaculture systems is nitrogen assimilation in fish bodies varies from 25% to 35% of total nitrogen input without regard to differences in fish species [7], [8]. The results of this study indicated that N retention in recirculation systems with a density of water jasmine was 2.08 g $L^{-1}$ higher because the utilization of feed was more efficient.

The results of this study also showed a better achievement than the results of previous studies [29], namely N retention of tilapia reared in the recirculation system using *L. gibba* plants only reached 14 - 20%. The more nitrogen waste has used the plant as a nutrient, the concentration of nitrogen in water will decrease so that the water quality is to be better. Under optimum water quality conditions, the ability of fish to retain nitrogen will increase and eventually their growth will increase. The results of this study showed the water jasmine plants (2.08 g $L^{-1}$ density) had the highest ability to utilize eel waste as nutrients for growth. In addition, the environment that was formed did not interfere with the life of eel characterized by their physiological responses such as the form of blood and blood glucose levels in normal circumstances. Furthermore, these conditions could increase the efficiency of feed utilization and growth performance of eel.

## IV. Conclusion

According to the result, it can be concluded that the Water jasmine plant in density 2.08 g $L^{-1}$ resulted in higher nutrient removal efficiency compared to the other treatments (TAN 46.03%, nitrite 49.65%, nitrate 59.64%, and orthophosphate 60.88%). The highest growth performance was obtained in the 2.08 g $L^{-1}$ of water jasmine plant, equal with 400 g $m^{-2}$


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