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A Kinetic Approach for Employing Two Duckweed Species, *Lemna minor*, and *Spirodela polyrhiza*, in the Sustainable Aquaculture Wastewater Treatment and Fish Feed Production

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ABSTRACT

Duckweed, a widely used natural fish feed, has also become more popular as the phytoremediation agent for wastewater, including one sourced from aquaculture. These two features indicate that duckweed can be utilized in a sustainable aquaculture system by treating and reclaiming nutrients from wastewater and then harvesting them for fish feed production. Hence, this study attempted to assess the approach of the two most known duckweed species, i.e., *Lemna minor* and *Spirodela polyrhiza*, in depleting NH_4 and PO_4 from synthetic controlled aquaculture wastewater as well as to understand their yield based on the N:P ratio. Cultivation in synthetic aquaculture wastewater media was carried out, followed by nutrient uptake and growth analysis. According to statistical analysis, both *L. minor* and *S. polyrhiza* could remove NH_4 and PO_4 with a relatively equal rate (p-val > 0.050). Nonetheless, both duckweed species absorb nitrogen more easily than phosphorous (p-val < 0.050). Considering the yield based on nutrient uptake, NH_4 drove a more efficient yield for *L. minor* to *S. polyrhiza* at 16.70 g dry biomass/g NH_4 and 14.14 g dry biomass/g NH_4, respectively. Meanwhile, a higher yield was observed on *S. polyrhiza* than on *L. minor* regarding PO_4 concentration, at 19.31 g dry biomass/g PO_4 and 9.10 g dry biomass/g PO_4, respectively. Therefore, a strategy to remove nutrients and produce biomass for fish feed can be formulated based on the N:P concentration ratio, where *L. minor* tends to produce biomass more rapidly in a higher N:P ratio, whereas *S. polyrhiza* works in the opposite.

1. Introduction

Fisheries are one of the most critical sectors in Indonesia, where up to 54% of nationwide protein consumption comes from this activity's product (Tom *et al.* 2021). Indonesia is the world's third largest fish producer country, with 4.4 million tonnes of fish produced in 2018 (CEA 2018). This advancement in fishery eventually affected the hydrosphere's natural condition if untreated fishery wastewater is channeled to river bodies, initiating eutrophication and decreasing water quality (Phillips *et al.* 2015). A study even reported that 81.25% of the fishery industries, or aquaculture, in a region of Indonesia discharged wastewater into streams without prior treatment (Ariyunita & Listyawati 2020).

It is indeed that traditional wastewater treatment processes, e.g., a combination of aerobic and anaerobic maturation ponds, have been used in aquaculture up to a certain level in Indonesia. Nevertheless, this system produces greenhouse gases like CO_2 and CH_4 (Han *et al.* 2019). Another technology, constructed wetlands, is known for removing nitrogen and phosphorous but requires a 2.7 times larger area than the fish ponds (Sindilariu *et al.* 2007; Martins *et al.* 2010). In addition, these traditional treatments have not enabled nutrient recovery from the wastewater (Han *et al.* 2019). Nutrient recovery itself can be addressed by the

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Recirculating Aquaculture System (RAS), but the complexity of its process and the high operational costs make the system not feasible in a developing country (Martins *et al.* 2010; Ahmed & Turchini 2021).

Recently, duckweed (Lemnaceae), a type of water plant, has become more popular as an agent for remediating wastewater by uptaking nitrogen and phosphorous from aquaculture wastewater (Paolacci *et al.* 2022). This macrophyte could lower organic matter at 94.8% and 96.7% for COD and BOD, respectively (Mohedano *et al.* 2014). In addition, duckweed decreased total suspended solids and electrical conductivity by 81.11% and 2.60%, respectively, while improving the survival rate of fish fingerlings in an aquaculture system (Sarkheil & Safari 2020). Of a dozen duckweed species, *Lemna minor* and *Spirodela polyrhiza* (both colloquially referred to as "common duckweed") have been widely studied for their ability in phytoremediation. A study showed that *L. minor* removed 96 % NH_3 and 96% $PO4^{3-}$ from wastewater for agriculture and aquaculture (Selvarani *et al.* 2015). Also, $NH4^+$ concentration rapidly decreased following treatment using *L. minor* at the optimum surface density of 80% (Paolacci *et al.* 2021). Meanwhile, *S. polyrhiza* was studied for its ability to remove $NH4^+$ and $PO4^{3-}$ from fish wastewater to an undetected level (Ng *et al.* 2017). This species was also observed to remove NO_3^- and NO_2^- ultimately, given the density reached 11.67 g fresh biomass/L wastewater (Ng & Chan 2021).

Along with its ability to uptake nutrients, duckweed is famous for its potential as a principal constituent of alternative organic fish feed (Chakrabarti *et al.* 2018). Nutrients such as N and P drive the kinetic of duckweed growth, followed by the assimilation to macrophyte body mass (Cheng & Stomp 2009). A study on *Lemna* showed that high carbohydrate and protein biomass was yielded between 132.62 and 200.95 g/m^2.d during wastewater treatment (Verma & Suthar 2014). *L. minor*-based feed was also known to significantly increase feed utilization efficiency, relative growth rate, and survival rate of tilapia (*Oreochromis niloticus*) (Herawati *et al.* 2020). On the other hand, *S. polyrhiza* was observed to possess a 45.8% starch content according to its dry weight and, hence, suitable for animal feed (Cheng & Stomp 2009). There is no doubt that *L. minor* and *S. polyrhiza* are the two most commonly used ingredients of organic animal feed, including fish (Minich & Michael 2024). These findings allowed the wastewater treatment system to become more sustainable since nutrients from water are recovered and incorporated into fish. After all, the application of duckweed addresses sanitation and food supply (Roman & Brennan 2019).

Kinetic features such as nutrient uptake and biomass production rates are needed to implement duckweed in an engineered system. The formulation of nutrient uptake rate (dS/dt) and biomass production rate (dX/dt) results in the yield of duckweed over the available nutrients (dX/dS) (Mihelcic & Zimmerman 2021). In excess nutrients, duckweed grows in the logarithmic phase, where the maximum uptake and biomass production rates can be obtained (Zhang *et al.* 2014). These maximum rates can be further utilized to design the hydraulics parameters such as hydraulic retention time and flow rates in which duckweed is used as the main phytoremediation agent of wastewater (Papadopoulos & Tsihrintzis 2010).

Despite the promising application in wastewater treatment and feed formulation, the use of both *L. minor* and *S. polyrhiza* in Indonesia has yet to be studied thoroughly. This is primarily related to applying native varieties against local aquaculture wastewater. It is important to keep the regional ecosystem balanced and avoid invasiveness. In addition, the strategy of choosing one species above the other has yet to be studied inclusively. Therefore, this study attempted to characterize the potential of local varieties of *L. minor* and *S. polyrhiza* preference in using either NH_4 or PO_4 for their growth. It is expected from this study that we can understand more about which duckweed species are more suitable for aquaculture wastewater with a specific N:P ratio, as well as their aim for treating wastewater or producing biomass.

2. Materials and Methods

2.1. Duckweed Collection and Cultivation

Local varieties of *L. minor* and *S. polyrhiza* were obtained from plant breeders in Malang, East Java, Indonesia. Duckweeds were cultivated in Hoagland's media in a container with a volume of 3 L covering a surface area of 600 cm^2 (Paterson *et al.* 2020). Each duckweed species was cultivated in triplicates and acclimatized to the above synthetic media for around a week before the experiment. Figure 1 shows the cultivation settings.

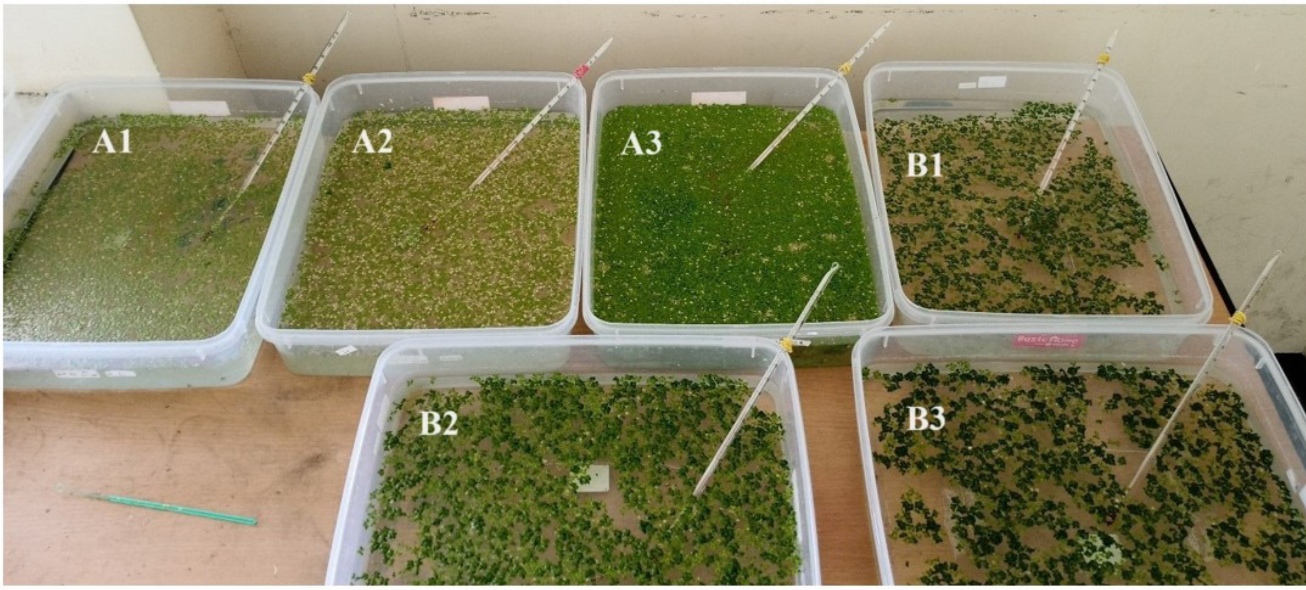

Figure 1. Laboratory settings of duckweed culture of *L. minor* (A1-A3) and *S. polyrhiza* (B1-B3)

2.2. Nutrient Removal Analysis

After acclimatization, both duckweed species went through a semi-continuous cultivation cycle. Every three days, 50 % m/m of total duckweed was harvested from the media, allowing the plant to grow continuously. In each harvest, media was sampled and analyzed for NH_4 and PO_4 using the spectrophotometry standard method (American Public Health Association *et al.* 2017). In addition, temperature, pH, dissolved oxygen, and conductivity were measured accordingly using direct measuring devices. The obtained values were compared to the actual nutrient of fresh Hoagland's media to generate the nutrient removal rate in mg-nutrient/L.d. After harvesting, each container was filled again with fresh Hoagland's media until reaching the design volume. The cultivation cycle lasted for 30 days.

2.3. Growth Analysis

Harvested duckweed from each cycle was weighed to obtain the wet biomass weight and then dehydrated in the oven for two days at 90°C. The dried duckweed was weighed to obtain dry biomass weight and moisture content number. The dry biomass weight was analyzed with the nutrient removal for both NH_4 and PO_4. The analysis generated the growth rate as yield in g dry duckweed/mg nutrients as formulated in Equation 1.

$$\frac{ds}{dt} = -\frac{1}{y}\left(\frac{dx}{dt}\right)$$

Where,
S: NH_4 or PO_4 concentration (g)
X: duckweed (g dry biomass)
Y: yield (g dry biomass/g)

Both nutrient uptake and yield between *L. minor* and *S. polyrhiza* were compared and analyzed statistically.

3. Results

3.1. NH_4 and PO_4 Removal Pattern

Both *L. minor* and *S. polyrhiza* showed the ability to reduce NH_4 and PO_4 from the media. As can be seen in Figure 2, NH_4 removal was observed more rapidly than PO_4 removal. This happened for the two species. During the 3-day cultivation, both *L. minor* and *S. polyrhiza* reduced NH_4 concentration in the media by roughly threefold what was observed for PO_4. In addition, the inconsistency of nutrient removal was perceived in PO_4 more often than in NH_4. The steadiness of nutrient removal was attained on NH_4 better than PO_4.

The initial NH_4 concentration of Hoagland's media was measured as 6.09 mg/L. *L. minor* and *S. polyrhiza* reduced this nutrient to 2.17 mg/L (SD = 1.11) and 1.64 mg/L (SD = 0.59), or 64.4% and 73.1%, respectively, for a detention time of three days. On the other hand, both duckweed species were able to lower PO_4

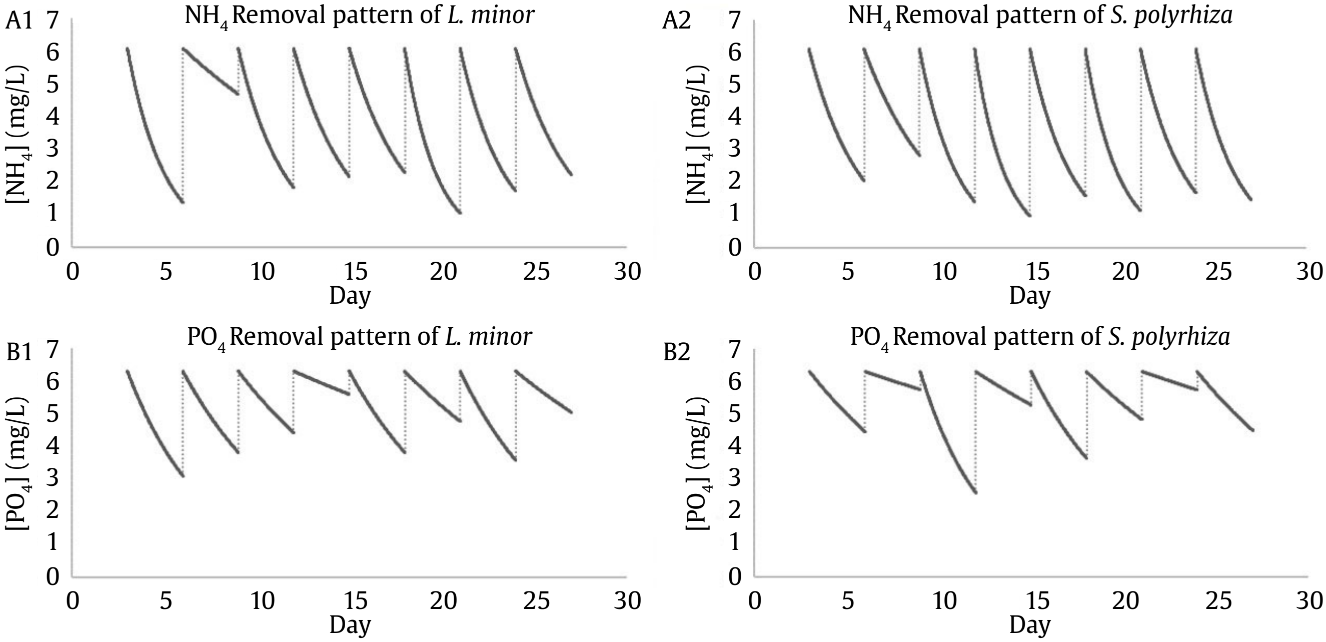

Figure 2. The pattern of NH_4 (A1, A2) and PO_4 (B1, B2) removal by *L. minor* and *S. polyrhiza* during a series of 3-day cultivation

concentration to 3.61 mg/L (SD = 0.75) and 3.84 mg/L (SD = 1.04), for *L. minor* and *S. polyrhiza*, respectively, from its initial concentration of 6.31 mg/L. These values are within the range of NH_4 and PO_4 duckweed removal in the first 36-hour aquaculture wastewater treatment, as reported by other previous studies.

3.2. Nutrient Uptake Rates by *L. minor* and *S. polyrhiza*

Figure 3 shows the comparison of uptake rate between *L. minor* and *S. polyrhiza* for both NH_4 and PO_4. In line with the temporal removal pattern, PO_4 removal rates were observed to be lower than the NH_4 removal rate. At $\alpha = 0.050$, NH_4 uptake rates were significantly higher than PO_4 uptake rates. The nutrient uptake rates for *L. minor* were 1.31 mg/L.d and 0.90 mg/L/d (p-val = 0.011) for NH_4 and PO_4, respectively. On the other hand, the nutrient uptake rates for *S. polyrhiza* were 1.48 mg/L.d and 0.82 mg/L.d (p-val<0.001) for NH_4 and PO_4, respectively. This led to the idea that N must have a comparably higher concentration to ensure P is removed up to a certain level.

There was no significant difference in the nutrient removal ability between *L. minor* and *S. polyrhiza* in NH_4 (p-val = 0.248) and PO_4 uptake rates (p-val = 0.617).

3.3. *L. minor* and *S. polyrhiza* Yield based on N and P

To better understand each duckweed species' potential for producing biomass from wastewater recovery, yield was considered. Yield in the unit of g biomass dry weight per mg of removed nutrients was measured to obtain the efficiency of biomass accumulation in line with the prospective usage of duckweed as alternative fish feed. Figure 4 shows the yield of each duckweed species according to the nutrient they uptake, either NH_4 or PO_4.

The growth of *L. minor* was observed to depend more on nitrogen rather than phosphorus content in the media. On the other hand, phosphorous drove the growth of *S. polyrhiza* more prevalently. These can be seen from the yield value against either NH_4 or PO_4 content decrease. For *L. minor*, the yield was 16.70 g dry biomass/mg NH_4 (SD = 20.15) and 9.10 g dry biomass/mg PO_4 (SD = 5.10). For *S. polyrhiza*, the yield was 14.14 g dry biomass/mg NH_4 (SD = 12.58) and 19.31 g dry biomass/mg PO_4 (SD = 15.19). Hence, at this point, it can be assumed that the N:P ratio is a critical parameter in determining the biomass for harvesting that is in line with the duckweed species selection.

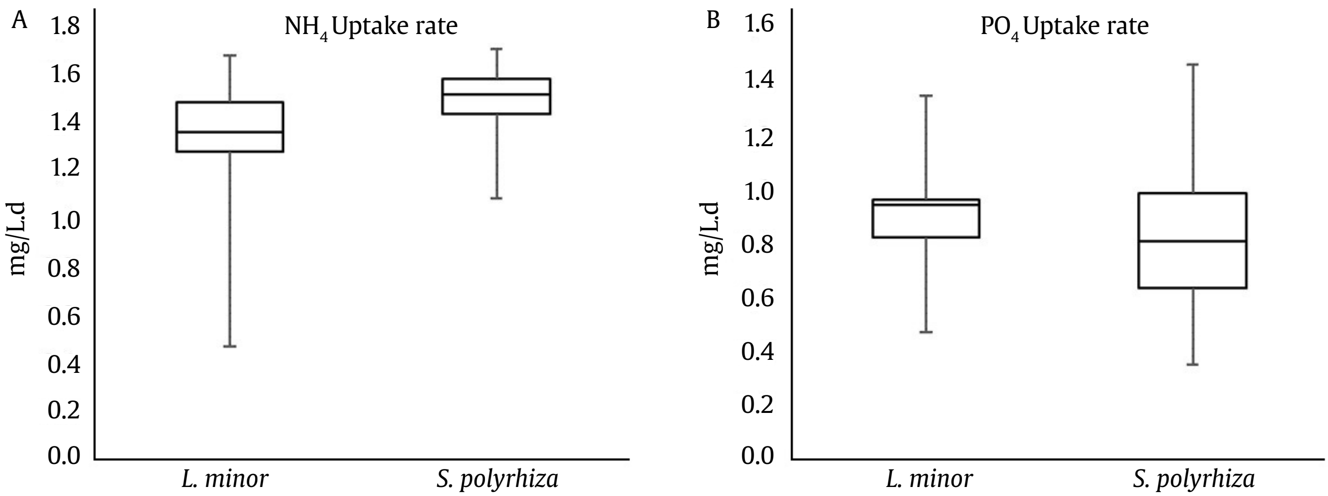

Figure 3. (A) NH_4 and (B) PO_4 uptake rates for *L. minor* and *S. polyrhiza*

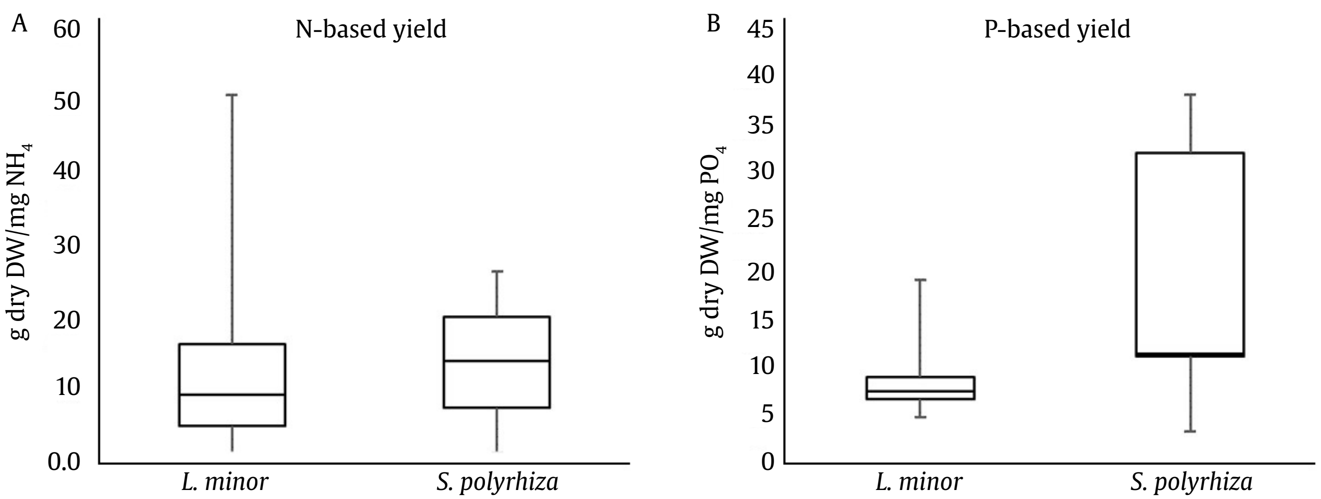

Figure 4. Yield of *L. minor* and *S. polyrhiza* based on (A) NH_4 (A) and (B) PO_4 removal

4. Discussion

Nitrogen is fixed to the duckweed biomass by the aid of N fixing cyanobacteria and microalgae, although mainly, duckweed is capable of assimilating nitrogen via its root due to the expression of significant nitrogen assimilation genes (Körner & Vermaat 1998; Zhou *et al.* 2021). Ammonium itself serves as the most preferred nitrogen form in the water for duckweed growth, even though at high concentrations, toxicity may be induced (Cedergreen & Madsen 2002; Wang *et al.* 2014). Even though duckweed also needs phosphorous to promote its growth and biomass accumulation, the amount of phosphorous plants need is relatively smaller than nitrogen (Al-Nozaily 2001). In typical duckweed biomass, N comprises up to 7.8% of total mass, whereas P comprises only up to 2.8% (Iqbal *et al.* 2019). Therefore, the lower and slower PO_4 removal rate is expected in a removal cycle covering the same detention time as if it is done for NH_4.

In addition, it was suggested that P removal using duckweed needs more time than N removal. The P removal and additional detention time are increasing (Liu *et al.* 2017). It must be noted that this condition was noticed with the usage of synthetic media rather than real wastewater. Lower nutrient removal on natural wastewater may be expected since the removal does not depend solely on duckweed but on other factors, e.g., natural decomposition and microorganism activities (Iqbal *et al.* 2019). These preceding findings elucidated the growth patterns of both *L. minor* and *S. polyrhiza*, as seen in Figure 2.

Another study showed that the % removal efficiency of *Lemna* and *Spirodela* fell to 61% and 72%, respectively. *Spirodela* reduced NH_4 by 75% within 120 hours of treatment and became the most prominent duckweed in treating ammonium (Galaviz-Villa *et al.* 2016). This feature could be due to this species adhering to consuming ammoniacal nitrogen as the primary nitrogen source (Petersen *et al.* 2021).

From Figure 3, it can be seen that the removal rate of PO_4 was inferior to that of NH_4. This led to the idea that N must have a comparably higher concentration to ensure P is removed up to a certain level. This idea was supported by the fact that a high N:P ratio is needed to make wastewater treatment work efficiently (Xu & Shen 2011). Generally, the ideal N:P ratio was at least 7.0 (Muradov *et al.* 2014).

Since there was no evidence to suggest that there is a significant difference in nutrient uptake ability between the two species, we can conclude that both species have equal potential in removing nitrogen and phosphorous from wastewater. The comparison between *L. minor* and *S. polyrhiza* in removing nutrients has been carried out in previous studies. Both species had an equal removal efficiency of nitrogen at 98.5-98.8% and phosphorous at 86.2-90.2% during the 10-day treatment of eutrophic wastewater (Chen *et al.* 2018). Aquaculture wastewater was also treated with both *L. minor* and *S. polyrhiza* and showed a similar removal efficiency for NH_3-N and total phosphorous content at 74-75% and 63-73%, respectively (Galaviz-Villa *et al.* 2016).

From the yield analysis, as seen in Figure 4, we understood that N:P ratio plays a significant role in determining the ability of each species to produce biomass while removing nutrients. The higher N:P ratio was known to add to the survivability of *Lemna* species in high-nutrient media (Fulton *et al.* 2009). On the other hand, phosphorus is vital to *Spirodela* growth. In a media with limited phosphorous, the plant could not survive due to photosynthesis alteration, while nitrogen metabolism was barely affected (Reid & Bieleski 1970). The fact that *S. polyrhiza* grew less competitively than *L. minor* may also be due to the NH_4 concentration in the media itself. The growth rate of *S. polyrhiza* depends on the NH_4 concentration, in which relatively high ammonium ion inhibits the anion transport to the cell membrane and causes the toxicity effect (Caicedo 2000).

The N:P ratio is critical in determining the biomass for harvesting in line with the duckweed species selection. For some time, *Lemna* was recognized as a more proper option for fish feed to *Spirodela* due to its growth rate (Hassan & Edwards 1992). *L. minor* possesses a high nutrient content, including 19.02% nitrogen-free extract, 29.92% crude fiber, 23.47% crude protein, and 3.99% crude fat (Herawati *et al.* 2020). On the other hand, *S. polyrhiza* also has a comparable nutrient content, including 57.89% nitrogen-free extract, 14.47% crude fiber, 13.10% crude protein, and 1.74% fat (Said *et al.* 2022). Hence, should *Spirodela* be chosen, lowering the N:P ratio will increase yield and provide more biomass than *Lemna* could attain.

L. minor and *S. polyrhiza* are the two most common duckweeds used for treating aquaculture wastewater and harvested as alternative fish feed. Each species has a different approach to attaining optimum nutrient recovery. It is known from this study that local varieties of *L. minor* and *S. polyrhiza* have a similar capability to remove nitrogen in the form of NH_4 and phosphorous in the form of PO_4 from wastewater. Nonetheless, to produce biomass, *L. minor* requires a relatively higher N:P ratio; meanwhile, *S. polyrhiza* is fitter to a relatively lower N:P ratio. Therefore, a strategy to recover nutrients from aquaculture wastewater employing duckweed and utilizing respective duckweed for fish feed efficiently can be formulated based on the N:P ratio and species used. Is it suggested that the detention time during wastewater treatment be increased to ensure the nutrient uptake and biomass accumulation have reached their optimum point. Therefore, a study to understand the specific growth rate μ_{max} must be performed before applying the duckweed in a sustainable aquaculture wastewater treatment system.

In conclusion, in this study, we investigated the efficiency of *L. minor* and *S. polyrhiza* in removing nitrogen (NH_4) and phosphorus (PO_4) from wastewater, as well as their biomass production potential under varying nutrient conditions. Our findings reveal several key insights into the nutrient removal capabilities and biomass yield of these two common duckweed species. Firstly, ammonium emerges as the preferred nitrogen form for duckweed growth, although high concentrations can induce toxicity. In contrast, phosphorus, while essential for growth, is required in smaller quantities compared to nitrogen. This discrepancy is reflected in the lower phosphorus removal rates observed in our study, necessitating longer detention times for effective phosphorus removal.

The comparison between *L. minor* and *S. polyrhiza* revealed comparable efficiency in nitrogen and phosphorus removal from wastewater. While both species exhibit similar nutrient uptake abilities, their optimal biomass production conditions differ. *L. minor* thrives in higher nitrogen-to-phosphorus ratios, whereas *S. polyrhiza* performs better in lower ratios. This distinction is crucial for formulating strategies to maximize biomass production and nutrient recovery from aquaculture wastewater. Furthermore, nutrient content analysis underscores the potential of both species as alternative fish feed sources. Depending on the desired nutrient balance, either *L. minor* or *S. polyrhiza* can be selected to optimize biomass yield. However, the selection should align with the specific nitrogen-to-phosphorus ratio requirements of each species to ensure efficient growth and nutrient uptake.

Our study highlights the importance of considering nitrogen-to-phosphorus ratios and species-specific growth characteristics when utilizing duckweed for wastewater treatment and biomass production. Future research should focus on elucidating species-specific growth rates and optimizing detention times to maximize nutrient uptake and biomass accumulation in sustainable aquaculture wastewater treatment systems.

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