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The Most Efficient Seaweed Species as a Bioremediator of Intensive Pond Waste

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ABSTRACT

Seaweed species have a positive effect on the bioremediation of nutrient waste. However, waste absorption efficiency varies between species. This research aims to analyze the most efficient seaweed as bioremediation of intensive waste ponds. The method used in this research was three types of seaweed stocked based on treatment groups in each of three tanks measuring 100×100×100 cm made of bamboo, wood, and specially designed tarpaulin. This study was carried out on a laboratory scale for 42 days on three local seaweed species to evaluate the waste disposal efficiency of each seaweed species. This research was conducted at the Bone Marine and Fisheries Polytechnic Laboratory, Tulang Daerah, South Sulawesi Province, Indonesia, from September to October 2022. Measurement of ammonia (NH_3-N), nitrite (NO_2-N), nitrate (NO_3-N), and phosphate (PO_4-P) carried out at the Center for Brackish Water Aquaculture and Fisheries Extension in Maros, the concentrations of the four nutrient wastes were significantly different ($P<0.05$), indicating differences in nutrient removal for each seaweed species. The removal efficiency of NH_3-N, NO_2-N, NO_3-N, and PO -P by *G. verrucosa* (97.1, 99.7, 99.9, 99.7%) was significantly higher ($P<0.05$) compared with *E. spinosum* (90.5, 93.9, 96.4, 95.4%) and *K. alvarezii* (81.6%, 94.6%, 94.5%, and 95.4%, respectively). Meanwhile, *E. spinosum* was not significantly different ($P>0.05$) from *K. alvarezii* in removing NO_2-N, NO_3-N, and PO_4-P but was more efficient in reducing NH_3-N. Overall, *G. verrucosa* is the most efficient in intensive disposal of shrimp pond waste based on the results of this study.

Keywords: *Eucheuma spinosum*, *Gracillaria verrucosa*, intensive pond waste, *Kappaphycus alvarezii,* nutrient removal efficiency.

INTRODUCTION

Aquaculture has rapidly developed into the fastest-growing food-producing sector (Nederlof et al., 2021), with an average annual increase of 6.7% from 1990–2020 (FAO, 2022). It is predicted that the aquaculture sector in the coming decades will play an important role in supplying nutrients to the human population (Campanati et al., 2022) because the production of the fishing sector during the 2000–2020 period tends to stagnate, while the world population growth is increasing, which is expected to exceed 9 billion by 2050 (FAO, 2022). However, aquaculture development has caused environmental degradation, which can threaten the sustainability of aquaculture and food security (Amoussou et al., 2022; Boyd et al., 2020). The most documented environmental impact is the impact of waste from feed on organic enrichment in water and sediment (Papageorgiou et al., 2023). Several studies have quantified feed utilization in shrimp aquaculture activities. For example, Bouwman et al. (2013) reported that 20% nitrogen (N) and 72% phosphate (P) are wasted as solid waste (leftover feed and feces), and 45% N and 18% P are dissolved in water. It means that only about 25% N and 10% P in feed is retained by shrimp. Nederlof et al. (2021) recorded 39–63% N and 18–30% P in the feed released as waste. Sahu et al. (2013) reported that

70% N and 89% P were wasted in sediment and the shrimp pond water column. The quantification of waste from shrimp aquaculture activities is a threat because it negatively impacts aquatic ecosystems (Boyd et al., 2020). Since feed waste is inevitable in aquaculture activities (Heriansah et al., 2022), it is necessary to properly treat this waste through practical implementation to minimize its environmental impact.

In addition to its economic value for food and non-food (Chopin & Tacon, 2021), seaweed is known to have several important ecosystem services, including providing oxygen, excess nutrient bioremediation, reducing ocean acidification, CO_2 assimilation, and reducing greenhouse gas emissions (Nobre et al., 2010; Buschmann et al., 2017; Chung et al., 2017; Clements & Chopin, 2017; Kim et al., 2017). In particular, seaweed has been widely accepted as an environmentally friendly and cost-effective method that can reduce the excessive concentrations of nutrients in waste (Chopin et al., 2012; Nederlof et al., 2021). The advantage is that seaweed photosynthetically converts waste into nutrient packages so that its bioremediation is assimilative and increases the environment's assimilative capacity for nutrients (Neori et al., 2004).

Several studies have demonstrated the positive effects of bioremediation of seaweed cultivation in co-culture systems. For example, in polycultures with sea bream *(Sparus aurata)*, the seaweed *Ulva lactuca* assimilated 75% of the dissolved nitrogen released by the fish (Shpigel et al., 2018). Felaco et al. (2020) reported that *Solieria filiformis* seaweed, which was integrated with red drum fish, Sciaenops ocellatus, sea cucumber, and Isostichopus badionotus could absorb 50% and 43% of ammonium and orthophosphate, respectively. Kang et al. (2021) evaluated five species of seaweed *(Codium fragile, Ulva pertusa, Ecklonia stolonifera, Saccharina japonica,* and *Gracilariopsis chorda)* as nutrient biofilters for wastewater from aquaculture systems. These five species' N and P phosphate uptake efficiencies were 63–80% N and 30–43% N, respectively.

Regardless of the method used in each previous study, each seaweed species appears to have a different ability to absorb nutrients (Kang et al., 2021). This study evaluated the bioremediation capacity of three seaweed species *Kappaphycus spinosum*, there are many in Gorontalo, North Sulawesi. Pond waste usually includes large amounts of ammonia (NH_3-N), nitrite (NO_2-N), nitrate (NO_3-N), and orthophosphate (PO_4-P), which may depend on the type of cultivation practiced. Thus, we evaluated the uptake rates of these three types of nutrient waste to identify the most efficient species for remediating intensive shrimp pond effluents. The findings of this study will be useful for selecting local seaweed species to continue co-culturing sustainability, which has great practical value for farmers and strategic conceptual value for environmental policymakers.

MATERIALS AND METHODS

Experimental design

This research was conducted at the Marine and Fishery Polytechnic Laboratory of Bone, Bone Regency, South Sulawesi Province, Indonesia, from September to October 2022. Ammonia, nitrite, nitrate, and phosphate measurements were conducted at the Brackish Water Aquaculture Fisheries Center and Fisheries Extension in Maros, Indonesia. The experiment was randomly designed with three treatment groups (seaweed species) and three replicates (Table 1 and Fig. 1). Three species of seaweed (Fig. 2) were stocked based on the treatment groups in each of the three tanks measuring 100×100×100 cm made of bamboo, wood, and tarpaulin specifically designed for this experiment (Fig. 3).

Rearing management

Seaweed K. alvarezii lives in tidal areas with a water depth of around 1–5 meters at lowest tide, requires sunlight for photosynthesis, requires a growth pH range of 6–9 (optimal pH 7.5–8.0) and salinity of 28–34 ppt. The nutrients needed are obtained from water and grow well at a temperature range of 27–30°C, a brightness of 1.5 meters and a current speed of around 20–40 cm/s. This causes the biomass produced to be greater and the cultivation period to be shorter (35–45 days). This continues to increase by 3–5% every year. *G. gigas* seaweed is mostly cultivated in the sea,

Table 1. Treatment group

Treatments	Seaweed species
Sw1	*Kappaphycus alvarezii*
Sw2	*Gracillaria verrucosa*
Sw3	*Eucheuma spinosum*

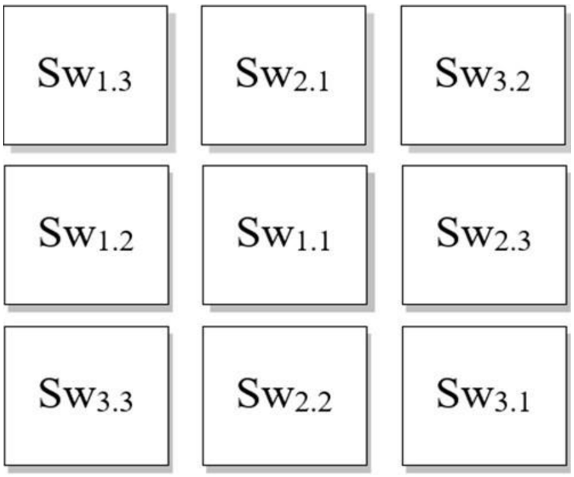

Fig. 1. Expriment unit layout

but cultivation in ponds is now starting to be carried out as an effort to increase production through area expansion. Meanwhile, *G. verrucosa* is more widely cultivated in ponds because can live in waters with a salinity of 15–30 ppt, and *E. spinosum*. The seaweed type *Eucheuma spinosum* can live in temperature conditions of 28–30°C with an average of 30°C. *E. spinosum* has a tolerance range for salinity ranging from 32–34 ppt with an average of an average of 33 ppt for these three types of grass from the outlet area of the intensive white shrimp pond managed by the Marine and Fisheries Polytechnic of Bone. Furthermore, seaweed was spread on five rope stretches for each trough, and each stretch consisted of eight double tie points at a distance of 10 cm (seaweed weight ± 10 g per tie point). Because the experiment was conducted indoors, light-emitting diodes (LED) (20 W) provided light. In addition, the tank was continuously aerated by the Resun LP60 air pump to maintain oxygen availability for 42 days of rearing. Water quality parameters, including dissolved oxygen, temperature, salinity, and pH, are monitored daily using a Lutron DO 5509 Dissolved Oxygen Meter, Hand refractometer RHS 10 ATC, and Lutron PH 201 pH meter.

Data collection procedures

In this study, nitrogen and phosphate were represented by the concentrations of ammonia, nitrite, nitrate, and phosphate. Concentrations were analyzed from 250 mL of water in the sample bottles for each experimental unit at the start and end of the study. The sampling, preservation, transportation, and detection of water samples were carried out carefully

Fig. 2. Experimental seaweed species

following the American Public Health Association (APHA, 2017). Nutrient removal efficiency (NRE) was quantified using the following equation (Pham & Bui, 2020):

$$NRE\ (\%) = (C_0 - C_f) / C_0 \times 100 \quad (1)$$

where: C_0 is the initial concentration and C_f is the final concentration of nutrients (NH_3-N, NO_2-N, NO_3-N, and PO_4-P).

Data analysis

The normal distribution and homogeneity of the variance of the data were first tested using the Shapiro-Wilk-test and Levene tests to meet the assumptions of parametric statistics. All data were normally distributed and homogeneous (P>0.05). Furthermore, one-way ANOVA was used to evaluate the effect of seaweed species on nutrient removal efficiency. Significant effects were compared using Tukey's honestly significant difference test. This statistical test was performed using IBM SPSS Statistics Version 25. For water quality data, it is analyzed descriptively by comparing the tolerance range of each type of seaweed based on valid references.

RESULTS

Concentrations of NH_3-N, NO_2-N, NO_3-N, and PO_4-P

A summary of the results of measuring the concentrations of ammonia (NH_3-N), nitrite (NO_2-N), nitrate (NO_3-N), and phosphate (PO_4-P) at the beginning and end of the experiment for each type of seaweed is shown in Figure 4. The concentrations of NH_3-N, NO_2-N, NO_3-N, and PO_4-P were relatively high at the start of the experiment before stocking the seaweed (blue color in Fig. 4), and the concentration values for each of these inorganic compounds were not significantly different (P>0.05) between the tanks of seaweed species. However, after 42 days of seaweed stocked, the concentrations of NH_3-N, NO_2-N, NO_3-N, and PO_4-P significantly decreased (P<0.05) (yellow color in Fig. 4). The final concentrations of NH_3-N, NO_2-N, NO_3-N, and PO_4-P significantly differed (P<0.05) for each species. Lower concentrations were recorded for *G. verrucosa* than

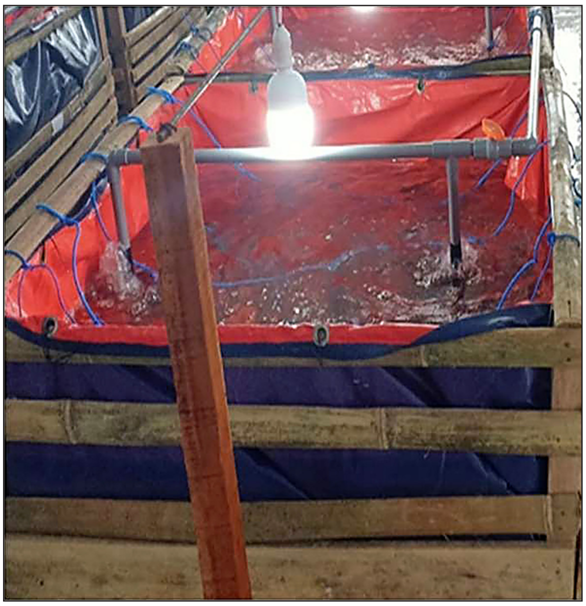

Fig. 3. Experimental tank

K. alvarezii and *E. spinosum* for all the organic compounds.

Nutrient removal efficiency

The NH_3-N, NO_2-N, NO_3-N, and PO_4-P reduction efficiencies for each type of seaweed are shown in Figure 5. The percentage of nutrient reduction by *G. verr*ucosa was significantly higher (P<0.05) than that of *E. spinosum* and *K. alvarezii* for all observed inorganic compounds. *E. spinosum* did not differ significantly (P>0.05) from *K. alvarezii* in reducing NO_2-N, NO_3-N, and PO_4-P, but *E. spinosum* was significantly higher than *K. alvarezii* in reducing NH_3-N. Overall, *G. verrucosa* was the most efficient at reducing NH_3-N, NO_2-N, NO_3-N, and PO_4-P.

General parameters of water quality

Table 2 lists the lowest and highest range values of the four general water quality parameters measured for each treatment. The average dissolved oxygen at the beginning of rearing was low, but increased at the end of the experiment in all types of seaweed treatment tanks. Salinity and temperature were relatively stable in all treatments. For pH, the concentration dynamics were lower at the beginning of the experiment than at the end. A higher pH was recorded in the *K. alvarezii* and *E. spinosum* than in the *G. verrucosa* tanks.

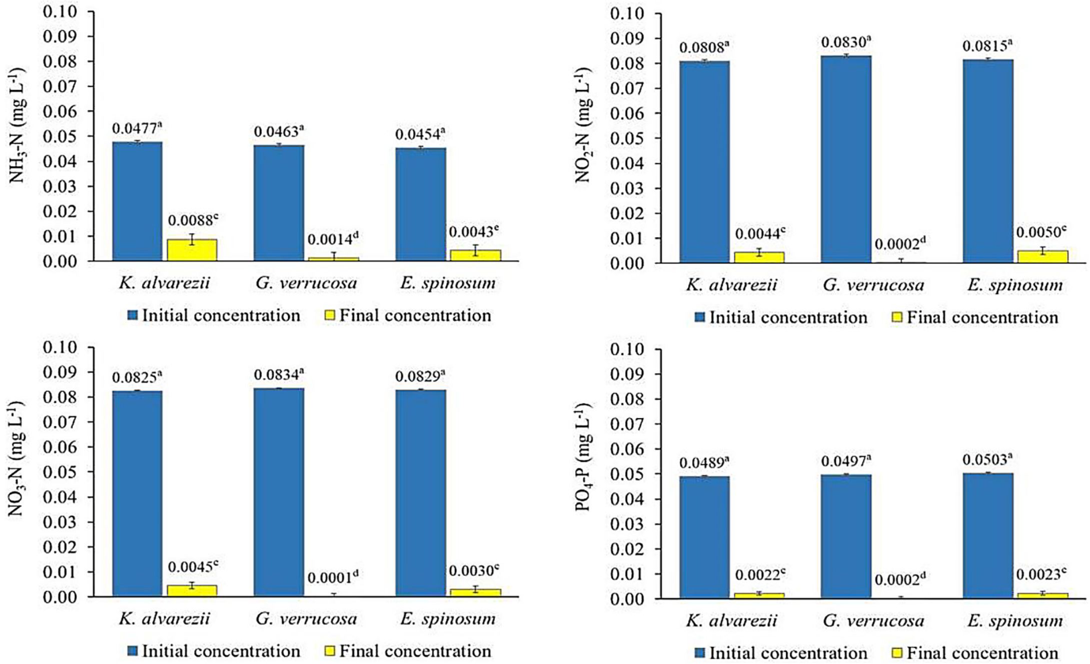

Fig. 4. Initial and final concentrations of NH3-N, NO2-N, NO3-N, and PO4-P

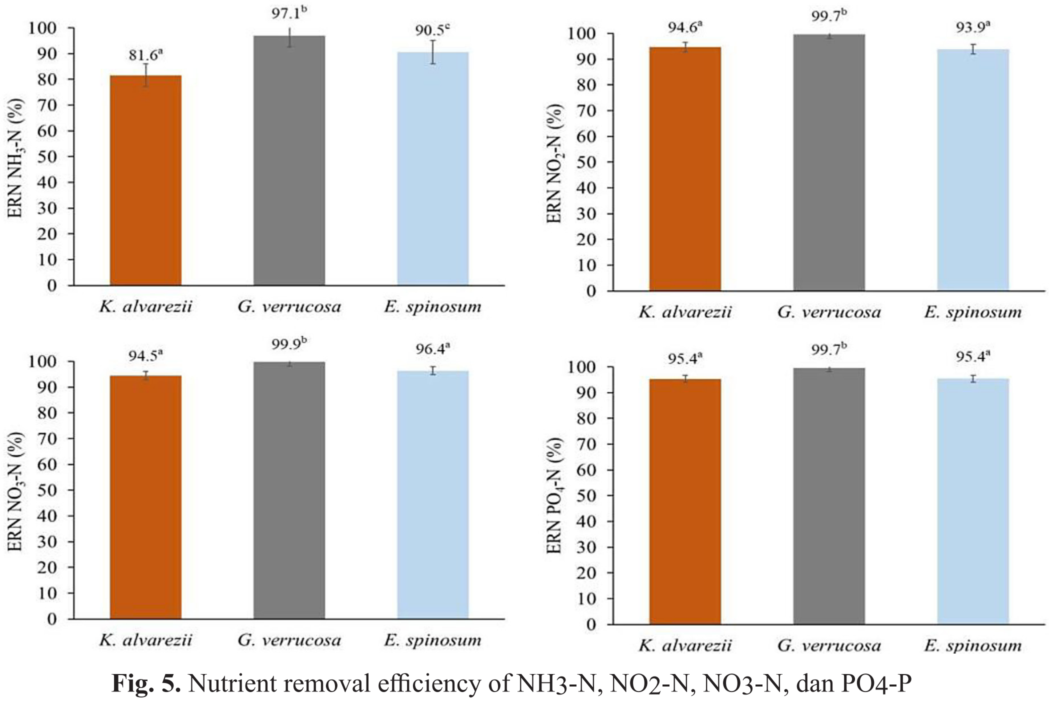

Fig. 5. Nutrient removal efficiency of NH3-N, NO2-N, NO3-N, dan PO4-P

DISCUSION

Concentrations of NH_3-N, NO_2-N, NO_3-N, and PO_4-P

In aquaculture practices, intensive aquaculture systems are used as an alternative to increasing aquaculture production, but this system also increases the disposal of inorganic waste owing to increased cultivation and feed inputs (Amoussou et al., 2022; Boyd et al., 2020). Nitrogen (N) and phosphorus (P) are the two most significant constituents of aquaculture wastewater originating from dissolved feed residues and feces (Campanati et al., 2022; Chiquito-Contreras et al., 2022). High concentrations of N and P can cause eutrophication with negative effects on aquatic ecosystems (Effendi et al., 2020). Nitrogen is

Table 2. Range of water quality parameters for 42 days of rearing

	Treatments	DO (mg L^{-1})	Salinity (ppt)	Temperature (°C)	pH
Sw1	*K. alvarezii*	3.66−8.03	28−30	27.3−28.7	6.58−8.08
Sw2	*G. verrucosa*	3.56−8.00	28−30	27.4−28.7	6.51−7.15
Sw3	*E. spinosum*	3.53−7.63	28−30	27.4−28.6	6.56−7.87

released into the water in the form of an ammonia compound (NH_3-N), which can further decompose into nitrites (NO_2-N) and nitrates (NO_3-N) (Dauda et al., 2019).

This study proved that the wastewater from the vaname shrimp-intensive pond contained NH_3-N, NO_2-N, NO_3-N, and PO_4-P. The concentration of inorganic compounds, 0.0454−0.0477 mg·L^{-1} NH_3-N, 0.0808−0.0830 mg·L^{-1} NO_2-N, 0.0825−0.0834 mg·L^{-1} NO_3-N, and 0.0489−0.0503 mg·L^{-1} PO_4-P, respectively. The concentrations of these organic compounds are lower than those in other vaname-shrimp-intensive ponds in several pond areas in Indonesia (Ariadi et al., 2019; Bosman et al., 2021; Mustafa et al., 2022; Pratiwi et al., 2020). The density and amount of feed are thought to be factors causing variations in the concentrations of organic compounds in this study (Chiquito-Contreras et al., 2022).

However, after 42 days of seaweed rearing, the observed concentrations of all inorganic compounds decreased significantly. Undoubtedly, we confirmed that this decrease was due to the work of seaweed as a waste bio-remediator (Chiquito-Contreras et al., 2022; Kang et al., 2021; Ramli et al., 2020). In the process of this research, the concentration values for each inorganic compound were not significantly different (P>0.05) between seaweed tanks. However, the concentrations of NH_3-N, NO_2-N, NO_3-N, and PO_4-P significantly decreased (P<0.05 Many studies and reviews in different environments have concluded that seaweeds effectively absorb inorganic nutrient waste (Arumugam et al., 2018). Seaweed contributes to the removal of dissolved nutrients (N and P) from higher trophic levels, such as fish, shrimp, and shellfish, and converts these compounds into biomass, reduces the effects of eutrophication, and stabilizes water quality (Nardelli et al., 2019). Seaweed is known to be able to absorb nitrogen at levels that exceed its growth and store nitrogen reserves to support further growth under conditions of nutrient deficiency (Wang et al., 2023). Owing to this adsorption ability, all seaweed species evaluated significantly reduced NH_3-N, NO_2-N, NO_3-N, and PO_4-P compounds. These results indicate that the three species of seaweed have the potential to act as bioremediators of intensive pond waste.

Nutrient removal efficiency (NRE)

The intensity of shrimp farming in ponds has contributed to the release of large amountsof inorganic nutrients in the form of N and P in water bodies, which leads to a decrease in water quality and aquaculture production. Meanwhile, the two most important nutrients are needed by seaweeds to maintain their physiological and metabolic functions (Arumugam et al., 2018; Kang et al., 2021). Therefore, plant uptake is one of the most effective ways to reduce nutrient waste in aquaculture systems (Irhayyim et al., 2020). Our results show that the reduction efficiencies of NH_3-N, NO_2-N, NO_3-N, and PO_4-P of the three observed seaweed species (*K. alvarezii, G. verrucosa,* and *E. spinosum*) were all more than 80%. Similar to the results in this study, several previous studies with various aquaculture methods and sites have reported that these three red seaweed species have demonstrated their great potential to remove nutrient waste from aquaculture effluents.

Azad et al. (2017) reported that *K. alvarezii* absorbed 59.5% and 61.6% of total N, and 5.5% and 3.4% of total phosphorus, respectively, in flow-through and recirculating system culture tanks in an integrated multi-trophic aquaculture (IMTA) system. *K. alvarezii* is grown in fish cage systems, and the removal of inorganic nutrients PO_4-P by approximately 30% (Kambey *et al*., 2020). Meanwhile, *K. alvarezii* absorbed 30% NH_3-N (Santos et al., 2022), 50.8% NO_2-N, 18.2% NO_3-N, and 26.8% PO_4-P (Hayashi et al., 2008) from fish farming waste. Likewise for the *G. verrucosa* species, 89.1% of the PO_4-P waste produced by mussels is eliminated (Ajjabi et al., 2018). The concentrations of NO_2-N, NO_3-N, and PO_4-P after the cultivation of *G. verrucosa* in the eutrophication area were reported by Huo et al. (2011), which decreased by 75.5%, 49.8%, and 49.0%, respectively. Widowati et al. (2021) reported that the capacity of polycultured *G. verrucosa* with mussels was confirmed by a total ammonia nitrogen (TAN) removal of 67%. For *E. spinosum* (trade name of *E. denticulatum*), no information was obtained regarding the efficiency of N and P reduction, but a recent study reported a significant linear relationship between the growth rate of *E. spinosum* and NO_3-N uptake (Narvarte et al., 2022.

Compared with the results in this study, the reduction of NH_3-N, NO_2-N, NO_3-N, and PO_4-P was higher than the results mentioned above. All the tested seaweed species removed significant amounts of N and P (over 80% each). It is also suspected that the variability of studymethods is a possible factor, so that nutrient reductions in this study were higher than those inprevious studies. In this study, all types of seaweed were cultivated in tanks supplied with intensive shrimp pond wastewater, which is rich in nutrients, particularly NO_2-N and NO_3-N. The study was conducted in relatively small tanks (100×100×100 cm) with controlled conditions (laboratory scale), relatively dense amounts of seaweed (40 double tie points per tank), and continuous aeration to create water movement.

The absorption rate of seaweed increases linearly with nutrient availability, even thoughit exceeds the need for its growth (Harrison & Hurd, 2001; Wang et al., 2023). Thus, the availability of nutrients in the area around the seaweed is one of the most important factors affecting its uptake (Narvarte et al., 2022). That is, the continuous movement of water throughaeration is thought to spread nutrients evenly across the distribution of seaweed in the tank. Increased water movement (stirring) is a fundamental driver of nutrient uptake, because it determines the rate and diffusion of nutrients across the thallus surface (Roleda & Hurd, 2019). This explanation may be related to the high ERN of NH_3-N, NO_2-N, NO_3-N, and PO_4-P in this study because the available nutrients were maximally absorbed by the seaweed.

The current study shows that *K. alvarezii, G. verrucosa*, and *E. spinosum*, all of them are capable of reducing inorganic nutrients (NH_3-N, NO_2-N, NO_3-N, and PO_4-P) nutrient waste from intensive shrimp ponds, hence the potential for waste bioremediation. However, significant differences were observed among the seaweeds with regard to their nutrient reduction efficiency. The most efficient type of seaweed in reducing all types of inorganic nutrients observed was *G. verrucosa*. These results provide valuable information regarding the selection of optimal seaweed species in a fish-seaweed integrated system. From several previous studies conducted separately on the three grass species as previously mentioned, it seems that *G. verrucosa* does tend to have a higher absorption efficiency than *K. alvarezii* and *E. spinosum*. In this study, the three species were evaluated simultaneously, the results obtained were the same trend, *G. verrucosa* species were the most efficient in absorbing nutrients.

The absorption rate of seaweed nutrients depends on several physical factors (temperature, salinity, light, and water movement), chemical factors (nutrient concentrations and limiting nutrient types, such as NO_3-N), and biological factors (nutritional history, life history, and morphology) (Harrison & Hurd, 2001; Narvarte et al., 2022). In this study, the temperature and salinity were relatively the same in each tank (Table 2), also the light source was the same (all tanks were indoors with LED lights), and each tank was supplied with the same aeration system. Thus, in this study, this physical factor was not considered a factor that caused differences in the level of nutrient absorption between species. Nutrient concentrationsand limiting nutrient types (NO_3-N) at the beginning of rearing were relatively the same (Fig.4); therefore, chemical factors were also not causal. For biological factors, nutrients for seaweed in this study were obtained from the same source (intensive shrimp pond waste); therefore, nutritional history is also not considered a factor affecting absorption variations.

Earlier life-history stages usually havehigher absorption rates (Harrison & Hurd, 2001). These life history factors (biological factors) may contribute to the nutrient uptake efficiency observed differently for each seaweedspecies, especially G. verrucosa (Narvarte et al., 2022). In addition, biological factors, in terms of morphology, among many seaweed species correlate with their properties, including the ability to absorb nutrients (Dudgeon et al., 1995). Visually, there are clear morphologicalvariations between *K. alvarezii, G. verrucosa*, and *E. spinosum*. *G. verrucosa* species are longer and denser than *K. alvarezii* and *E. spinosum*; therefore, more surface area per unit of biomass is available for light and nutrient uptake ((Dudgeon et al., 1995). Seaweeds with more surface membranes for absorption generally have higher nutrient uptake (Harrison & Hurd, 2001). This morphology results in a higher surface-area-to-volume ratio. Theoretically, seaweed witha high surface-area-to-volume ratio will have a faster nutrient uptake rate because nutrients areabsorbed across the surface of the seaweed thallus (Narvarte et al., 2022). These morphological differences may explain the higher efficiency of nutrient reduction in *G. verrucosa*.

General parameters of water quality

The results of measurements of dissolved oxygen, salinity, temperature, and water pH inall tanks during rearing (Table 2) are generally within the recommended range for *K. alvarezii, G. verrucosa,* and *E. spinosum.* For *K. alvarezii*, dissolved oxygen >4 mg L^{-1}, salinity 25–32 ppt, temperature 25–35°C, and pH 7–9 are the suitable ranges for growth (Aris & Labenua, 2020). Meanwhile, it is recommended to maintain *G. verrucosa* at a salinity range of 15–38 ppt, temperature of 18–33°C, and pH of 7–8 (Rejeki et al., 2018). The salinity, temperature, and pH of the water that are conducive to *E. spinosum* are 28–35 ppt, 25–30°C and 6.5−8.5, respectively (Munawan et al., 2021). Specifically for dissolved oxygen, seaweed contributes to oxygen production through the process of photosynthesis (Ajjabi et al., 2018), apart from the aeration process that was continuously carried out in this study.

The appropriate conditions for a number of water quality parameters in this study may support the maximum absorption of nutrients by each type of seaweed. Physical factors such as temperature and water movement affect the kinetics of seaweed nutrient uptake (Harrison & Hurd, 2001). This can be written that that salinity has an impact on macroalgae nutrient uptake (Choi et al., 2010). In relation to this study, for water quality with parameters that are conducive and relatively the same between tanks, it can be concluded that the nutrient absorption performance obtained is due to the performance of each type of seaweed.

CONCLUSIONS

Aquaculture nutrient waste has been highlighted in various discussions of sustainable aquaculture. The seaweed species *K. alvarezii, G. verrucosa*, and *E. spinosum* have the potential to bioremediate NH_3-N, NO_2-N, NO_3-N, and PO_4-P from intensive shrimp pond waste. In addition to being an important food and non-food ingredient, the results of the current study reinforce the potential of seaweed as the most profitable candidate for an integrated aquaculture system to support sustainable aquaculture projects. Of the three species observed, *G. verrucosa,* with a typical morphology, was the most efficient waste bioremediator. These results lead to the development of sustainable aquaculture in ponds. Further studies, particularly the nutrient uptake rates in seaweed tissue, may complement the current study results.

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