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Waste Water Reduction Using Four Densities of Seaweed to Improve Water Quality for White Leg Shrimp (*Litopenaeus white leg*) Culture: Laboratory Scale

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ABSTRACT

The low environmental quality of pond water is caused, among other things, by the large amount of organic waste and nutrients (feed components) in pond water outflow. The remaining feed and feces (feces) decompose in pond water before being dumped into the nearby rivers, resulting in the discharge of high levels of nutrients and organic waste. One method for enhancing the effluent quality from aquaculture is a biofilter. In order to reduce and absorb dissolved organic contaminants, biofilters are used. It is possible to employ Gracilaria sp. as a biofilter, which is an efficient technique to increase sanitation. The study' goals were to determine how well Gracilaria sp. developed and how well seaweed absorbed N and P elements from vaname shrimp culture waste, specifically ammonia (NH_3), nitrite (NO_2), and nitrate (NO_3). Three different treatments for the weights of the seaweed were employed in the study: 50, 75, and 100 grams. The analysis showed that the 100 g of Gracilaria sp. was very effective in absorbing N and P waste. It was discovered that N and P component absorption was, respectively, 75.10% and 75.15% effective.

Introduction

Aquaculture activities generate significant quantities of nutrient-rich effluents containing organic and inorganic compounds such as nitrogen, phosphorus, and carbon (Samadan *et al.,* 2023a), which may cause the eutrophication of surrounding water bodies. Moreover, these effluents can increase the occurrence of pathogenic microorganisms and the introduction of invasive species (Crab *et al.,* 2012). Nutrient enrichment is a big issue in fish farming, and it's linked to waste excretion. In an aquaculture system, 50–80% of the feed nitrogen and 35–85% of the feed phosphorus are retained (Schneider, 2005; Boyd and Queiroz, 2001). The principal nitrogen excretory product of fish is inorganic ammonia, which affects their growth even at low concentrations (Benli and Koksal, 2005). Due to their complexity and cost of operation, advanced water treatments such as membrane filtration, anammox process (Lu *et al.,* 2020) and heterogeneous photocatalysis (Zinatloo-Ajabshir and Salavati-Niasari, 2019) are limited. Indonesia is a country that has not paid much attention to the processing of shrimp farming waste (ASC, 2019). Shrimp farming waste comes from an uneaten feed, shrimp feces, shells resulting from the molting of shrimp, and other micro-organisms in the aquaculture water, such as plankton and microorganisms (ASC, 2019; Samadan *et al.*, 2023b). Approximately 24-37% N and 13-20% P from the feed can be absorbed by the shrimp and become the tissue of the shrimp body, and the rest becomes waste (Avnimelech, 2006; Rustadi *et al.,* 2022). Apart from that, shrimp farming waste also contains high nitrogen and phosphorus, 7-32% in the form of nitrogen (N) and 30-84% in the form of phosphorus (P) (ASC, 2019; Samadan *et al.,* 2023a). It was further reported that the retention of N and P feed in white leg shrimp culture was 22.27% and 9.79% respectively so that nutrients wasted in pond waters reached 77.73%

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nitrogen and 90.21% phosphorus (Fahrur et al., 2016). In addition, Djunaedi et al., (2016) stated that organic matter derived from feces, metabolic results, and leftover feed that is not eaten by shrimp can trigger an increase in the concentration of toxic compounds such as ammonia (NH3) in the culture medium. Meanwhile, Thakur and Lin (2003) accumulation of organic matter can decrease if the dissolved oxygen (DO) value is high. Dissolved oxygen is used by aerobic bacteria, namely Nitrosomonas and Nitrobacter to decompose organic matter.

The elements N and P are important in waste treatment, if the concentrations of these two elements are successfully suppressed, the risk of eutrophication in free waters is reduced (Funge *et al.,* 1998). In overcoming this, it is necessary to make filters that can manage shrimp farming organic waste so that the quality of the water used is maintained. The use of biofilter plants can speed up the decomposition process or reduce the concentration of N and P which are contained in a lot of cultivation waste (Jones *et al.,* 2003). Biofilter plants can reduce the concentration of waste by 20% -45% (ASC, 2019). One of the plants that is often used as a biofilter is seaweed (Msuya and Neori, 2002; Jones *et al.,* 2003; Komarawidjaja, 2005; and ASC, 2019).

Seaweed as a biofilter is one method that is quite interesting and easy to apply, seaweed can react (able to store and accumulate organic matter in its cells) (Msuya and Neori, 2002). Growth of Gracilaria sp. seaweed. highly dependent on the availability of nutrients such as nitrogen (N), phosphorus (P), carbon (C), and others. Nutrients are needed by plants as a source of energy used to construct various cell components during the process of growth and development (Budiyani *et al.,* 2012). Izzati (2011), reported that *Gracilaria* sp is the most efficient biofilter for use in ecological engineering applications that function to improve water quality in rearing media. In addition, this type of seaweed grows abundantly on the coast. Therefore, this effort is also to utilize natural resources in overcoming shrimp farming waste. Thus, the purpose of this study was to determine the effectiveness of *Gracilaria* sp. as a biofilter in absorbing white leg shrimp farming waste in controlled containers.

Materials and Methods

This research will be carried out with several series of works starting in April - June 2022. The vanamei shrimp farming experiment was carried out at the Indonesian Institute of Sciences (LIPI) Field Station, Sasa Village, Ternate City. Meanwhile, water and waste samples were analyzed at the Laboratory of the Faculty of Fisheries and Marine Sciences, University of Hasanuddin Makassar.

Materials

Some of the equipment used in the research include; a water checker, refractometer, thermometer, and test kit to measure water quality such as salinity, temperature, pH, NH3, NO3, and NO2. While the materials used for shrimp and seaweed maintenance include aquariums, plastic pans, aeration hoses, aeration stones, plastic buckets, white leg shrimp fry (PL15), seaweed (*Gracilaria* sp.), bran, molasses, yeast, and feed.

Reasearch Prosedure

The stages of work follow the following steps:

Collection of Seeds

Gracillaria sp seeds were taken naturally from the coastal waters of Kalumata, South Ternate District, Ternate City. The seeds obtained were then put into a sample bag that had been prepared. The seeds that had been collected were then taken to the laboratory to be weighed.

Seed weighing

The seeds that have been collected are then cleaned and weighed for the initial weight of the thallus to be used, namely 25, 50, 75, and 100 gr which is then used for the planting process. The thallus was weighed using a digital balance (accuracy 0.1 gr).

Planting seeds and maintenance

The thallus that has been weighed is then released into a container that has been prepared using coral reefs as a substrate for its attachment. The process of maintaining *Gracillaria* sp. carried out for 45 days.

Waste input

After the thallus has been acclimatized for a week, then the shrimp culture wastewater is added to each different seaweed treatment. Waste is given as much as 500 ml in each treatment. In treatment A (control), waste was given 1x during the study, for treatments B, C, and D the waste was administered every 2 weeks during the study.

Data Collection

Observations of seaweed growth performance and water quality measurements are carried out every week. To obtain data during the study, the parameters observed were the effectiveness of waste absorption by seaweed, absolute weight growth, specific growth, and water quality such as salinity, temperature, pH, dissolved oxygen, TSS, nitrate, nitrite, and total N and P.

Research Design

This study was designed in the form of an experiment using a completely randomized design (CRD). The experiment included differences in the weight of seaweed, each consisting of four treatments, and each treatment was repeated three times so that the number of experimental units was 12 units each. The treatment scheme that was tried was as follows. Treatment of the difference in weight of seaweed Treatment A: seaweed (25 gr); Treatment B: seaweed (50 gr); Treatment C: seaweed (75 gr); and Treatment D: seaweed (100 g). The placement of the treatments in the experimental units was done randomly.

Measured Variables

Waste absorption effectiveness

Seaweed weight measurements are carried out every week, namely the specific growth rate and absolute growth (Amin *et al.,* 2005). Measuring the effectiveness of seaweed as a biofilter is done by taking 50 grams of seaweed samples before and after treatments, then the samples are analyzed in the laboratory to determine the nitrogen and phosphorus content. (APHA, 2005). The efficiency of filtering waste N and P in the water that enters container and exits the container (Drennan *et al.* 2006; Eding *et al.* 2006):

$$Pollutant\ removal = \frac{Pollutant_{in}\_Pollutant_{out}}{Pollutant_{in}} \times 100$$

Note: Polutanin, is a water pollutant that enters the tub; Polutanout is, water pollutant that come out of the tub. Optimum standards for environmental sanitation according to the WWTP technical guidelines by the Ministry of Health of the Republic of Indonesia in 2011 refer to Metcalf and Eddy (1991).

Absolute Weight Gain

To calculate absolute weight growth, the following formula is used according to Amin *et al.* (2005):

$$W = Wt - W_0$$

Note: W is absolute weight growth (gr), Wt is the final weight (gr), W_0 is the initial weight (gr)

Specific Growth

The specific growth rate was calculated at the end of the treatment using the Dawes *et al.* (1994) formula as follows:

$$SGR(\%) = \ln Wt - \frac{\ln W_0}{t} x\ 100$$

SGR is the specific growth rate (%), W0 is the wet weight of seaweed at the beginning of the study (gr), Wt is the wet weight of seaweed at the end of the study (gr) and t is the rearing time (days)

Water quality

The water quality parameters measured included temperature, pH, TDS, and dissolved oxygen (DO) were measured every week, from the beginning of the study to the end of the study.

Data Analysis

Before carrying out the analysis of variance, the data were tested for normality and homogeneity. Data on the effectiveness of total N and P filtration in each biofilter treatment were analyzed using ANOVA with F test at a 95% confidence level to determine the effect of seaweed weight (Zar, 2010). Fisher's test (LSD) is carried out if the treatment has a significant effect on the variables measured. Data processing was performed using StatCal software version 1.0. While another water quality is analyzed descriptively and displayed in tabular form.

Result

Effectiveness of N and P Absorption

The results of the analysis of the effectiveness of N and P absorption of *Gracilaria* sp. in each treatment that was tried are shown in Tables 1 and 2 below. It appears that the four treatments tested had differences in the percentage of absorption of N and P. The treatment with a weight of 100 grams of seaweed appeared to be more effective in absorbing white leg shrimp N waste compared to the other treatments, which was 75.1% (Treatment D). Whereas in absorbing P it was very effective in the treatment with a weight of 75 grams of seaweed which was 78.73% (Treatment C). The effectiveness of N and P absorption of *Gracilaria* sp. is shown in Figures 1 and 2.

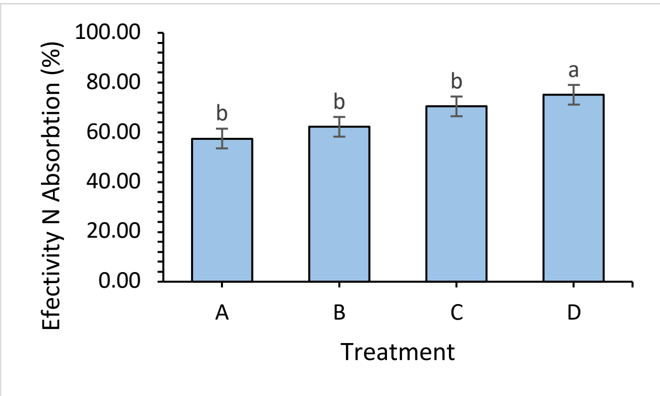

Figure 1. Effectiveness of N absorption of seaweed (*Gracilaria* sp) in each treatment

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The results of analysis of variance (ANOVA) on the effectiveness of absorption of N and P waste in the four treatments are presented in Tables 1 and 2 below.

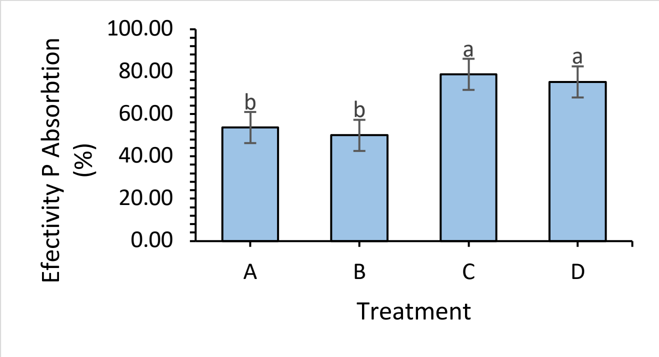

Figure 2. Effectiveness of P absorption of seaweed (*Gracilaria* sp) in each treatment

The four treatments tested affected the absorption of waste N and P. Effective absorption of waste N was found in treatment D, while absorption of waste P was very effective in treatment D.

Ammonia (NH_3), Nitrite (NO_2) and Nitrate (NO_3) Concentrations

The results of measuring the concentration of ammonia (NH_3) in the four treatments during the study showed different concentrations between the treatments. Treatment D was the highest concentration compared to other treatments. The average concentration of ammonia during the study is shown in Figure 3.

Table 1. Analysis of variance of the effectiveness of N uptake by *Gracilaria* sp.

Varians	db	SQ	QM	F-Stat	Sig.	F.05	F.01
Treatment (P)	3	564.59	188.19	918.77**	0.00	4.07	7.59
Error	8	1.63	0.20				
Total	11	566.24					

Ket** significant

Table 2. Analysis of variance parameter of the effectiveness of P uptake by *Gracilaria* sp.

Varians	db	SQ	QM	F-Stat	Sig.	F.05	F.01
Perlakuan (P)	3	1941.21	647.07	2334.43**	0.00	4.07	7.59
Galat	8	2.22	0.28				
Total	11	1943.43					

**: significant

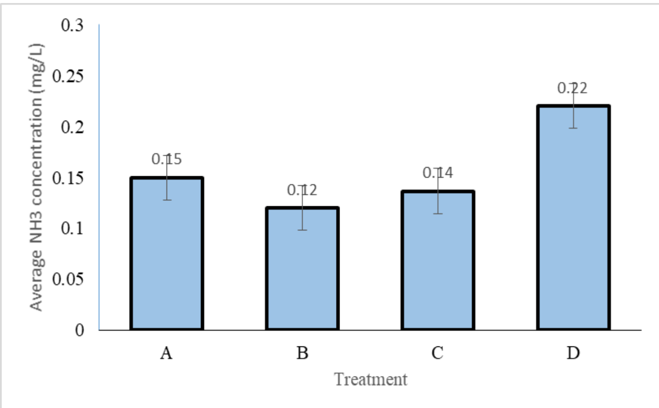

Figure 3. The average concentration of NH_3 in each treatment

The results of measuring the concentration of nitrite (NO_2) during the study in the four treatments showed fluctuations. The concentration of nitrite in treatment A was relatively the same as in treatment C, while the concentration of nitrate in treatments B and D was also relatively the same. The average concentration of nitrite (NO_2) in each treatment is shown in Figure 4.

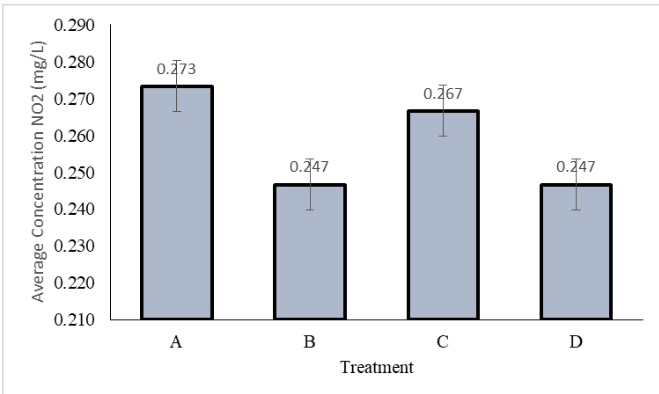

Figure 4. The average concentration of NO_2 in each treatment

The results of measurements of nitrate (NO_3) concentrations during the study in the four treatments appeared to be different from those of ammonia and nitrite. The highest nitrate concentration was found in treatment A, while the other treatments were relatively the same. The average concentration of nitrate (NO_3) for each treatment is presented in Figure 5 below.

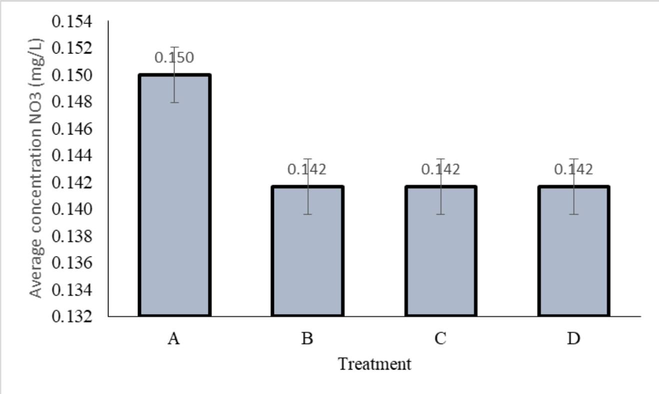

Figure 5. The average concentration of NO_3 in each treatment

The results of the analysis of variance (ANOVA) on the average concentrations of ammonia (NH_3), nitrite (NO_2), and nitrate (NO_3) for the four treatments showed that there was no significant difference in the weight of seaweed on the concentrations of ammonia, nitrite, and nitrate. Analysis of variance in ammonia, nitrite, and nitrate concentrations during the study is shown in Tables 3, 4, and 5.

Growth of *Gracilaria* sp.

The results of observing the absolute growth of Gracilaria sp. during the study on the four treatments showed that there were differences in every 7 days of measurement. It appears that treatment D has the highest growth value compared to other treatments. The average growth of *Gracilaria* sp. during the study is shown in Figure 6.

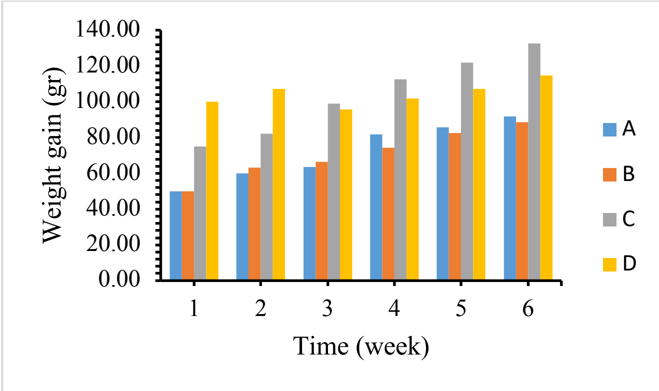

Figure 6. The average growth of *Gracilaria* sp.

The calculations of the four treatments' individual growth rates reveal that treatment D grows at a slower rate than treatments A, B, and C. Figure 7 displays the study's four treatments' average specific growth rates on a per-treatment basis.

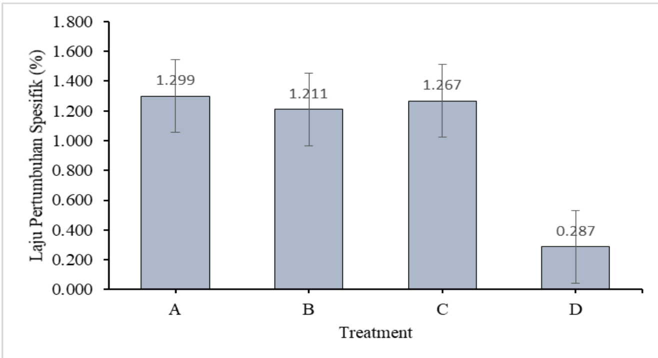

Figure 7. The average specific growth rate (%) of *Gracilaria* sp.

The results of the analysis of variance (ANOVA) on the absolute growth of *Gracilaria* sp. showed that there was no significant effect on the four treatments on seaweed growth. Likewise, the results of the specific growth rate analysis also showed the same thing, where there was no significant effect on the four treatments tried. The results of the analysis of the two parameters are presented in Tables 6 and 7.

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Table 3. Analysis of variance of ammonia (NH_3) concentration

Varians	db	SQ	QM	F-stat	Sig.	F.05	F.01
Treatment (T)	3	0.0044	0.0015	0.56^{ns}	0.66	4.07	7.59
Error	8	0.0208	0.0026				
Total	11	0.0252					

ns: not significant

Table 4. Analysis of variance of nitrite (NO_2) concentration parameters

Varians	db	SQ	MQ	F-stat	Sig.	F.05	F.01
Treatment (T)	3	0.0036	0.0012	1.98^{ns}	0.19	4.07	7.59
Error	8	0.0048	0.0006				
Total	11	0.0084					

ns: not significant

Table 5. Analysis of variance of nitrite (NO_3) concentration parameters

Varians	db	SQ	MQ	F-stat	Sig.	F.05	F.01
Treatment (T)	3	9.8958	3.2986	0.91^{ns}	0.48	4.07	7.59
Error	8	29.1667	3.6458				
Total	11	39.0625					

ns: not significant

Table 6. Analysis of variance of absolute growth parameters of *Gracilaria* sp.

Varians	db	SQ	MQ	F-stat	Sig.	F.05	F.01
Treatment (T)	3	2835.00	945.00	2.54^{ns}	0.13	4.07	7.59
Error	8	2976.67	372.08				
Total	11	5811.67					

ns: not significant

Table 7. Analysis of variance parameter specific growth rate (SGR) of *Gracilaria* sp.

Varians	db	SQ	MQ	F-stat	Sig.	F.05	F.01
Treatment (T)	3	2.14	0.71	3.29^{ns}	0.08	4.07	7.59
Error	8	1.73	0.22				
Total	11	3.87					

ns: not significant

Table 8. Parameters of water quality during the study

Treatment	Water Quality				
	Temp (^{0}C)	Salinities ($^0/_{00}$)	pH	DO (mg/L)	TDS (mg/L)
A	28.90-29.72	32.58-34.36	8.3-8.34	21.27-29.31	17.15-22.96
B	29,60-30,13	33,32-35,76	8,22-8,34	17,05-21,46	21,37-23,15
C	27,69-29,13	27,45-34,32	8,30-8,34	19,28-22,89	20,46-21,84
D	26,67-29,75	33,56-33,56	8,22-8,25	19,13-23,11	19,81-21,99

Water quality

The results of measuring water quality parameters during the study such as temperature, salinity, pH, DO and TDS showed no extreme fluctuations. All parameters are still optimal for seaweed growth. The results of measuring water quality during the study of the four treatments are presented in Table 8.

Discussion

The analysis's findings demonstrated that 75 grams of *Gracilaria* sp. (Treatment D) were particularly successful at removing N and P from white leg shrimp waste. Elements N and P have an absorption efficiency of 75.10% and 75.15% respectively. It seems that the marine algae *Gracilaria* sp. are highly good at absorbing nitrogen in the form of nitrate and phosphate. Red algae belonging to the genus Gracilaria have the capacity to bioremediate, or take up nutrients from the water.

According to research done by Wang *et al.* (2018), *Gracilaria* sp. is capable of efficiently absorbing nitrogen and phosphate from saltwater. *Gracilaria* sp. samples from Chinese coastal waters were used in the investigation, which was conducted in a laboratory. According to the findings, *Gracilaria* sp. was able to lower the levels of phosphate and nitrogen in saltwater by 80% and 90%, respectively. Additionally, studies by Zheng *et al.* (2020) in Korea have produced comparable findings. The findings of the experiment demonstrated that *Gracilaria* sp. was capable of reducing the quantities of nitrogen and phosphate by 70% and 60%, respectively, after 10 days.

The algae *Gracilaria* sp., one of the components that can lower shrimp productivity, can collect and use nitrogen (in the form of nitrate) and phosphorus (in the form of phosphate), according to Komarawidjaja (2005). Other plants, like water spinach, are also capable of doing the same when it comes to receiving nutrients from the water (Samadan *et al.*, 2023a). The amounts of ammonia (NH3), nitrite (NO^2), and nitrate (NO^3) were unaffected by the treatment of seaweed weight. The integration of *Gracilaria* sp. in vaname shrimp ponds can act as shading for white leg shrimp, according to further explanation that *Gracilaria* sp. generally grows best at high sunlight intensity (Komarawidjaja, 2005). White leg shrimp productivity will decrease if the water temperature is too high and exposed to sunshine for an extended period of time (Wyban *et al.*, 1995). High sunlight exposure will increase water temperature. Depending on the kind, the ideal temperature for *Gracilaria* sp. ranges from 20 to 30^{0}C. White leg shrimp prefer temperatures between 23 and 30^{0}C (Wyban *et al.*, 1995; Boyd, 1998). The environmental requirements for seaweed development and shrimp life are essentially identical in terms of environmental quality. The contents of ammonia (NH_3), nitrite (NO_2), and nitrate (NO_3) were unaffected by the treatment of seaweed weight, according to Avnimelech (1999). The growth performance does not influence absolute growth parameters and particular growth rates, which is the same thing. According to Msuya and Neori (2022), *Gracilaria* sp. may absorb nitrogen and phosphorus up to 0.4 gr. N/m2/day in water that has been contaminated with organic debris. The seaweed *Gracilaria* sp. is swiftly capable of lowering the concentration of dissolved nutrients in aquaculture pond wastewater, according to Jones *et al.* (2003). *Gracilaria* sp., a fresh seaweed weighing 500 grams, absorbed nitrogen, with the average concentration of N-total nutrients falling from 1.2 mg/L to 0.4 mg/L. According to Pantjara *et al.* (2010), 60–80% of the organic matter in wastewater can be reduced in water in a short period of time. The findings of this study show that seaweed can effectively absorb N and P waste from white leg shrimp aquaculture with an initial weight of 100 grams (Pandjaitan *et al.,* 2014). According to de Schryver and Verstraete (2009), this fact demonstrates that even while the weight of the grass is lower than in some earlier tests, it is still effective at absorbing the waste from white leg shrimp culture. In addition, this study demonstrates that the outcomes rely on the kind of algae employed, the environmental factors, and other factors.

Gracilaria is a type of seaweed that is widely found in Indonesia and has an extraordinary ability to absorb nitrogen (N) and phosphorus (P) waste in vaname shrimp farming. This ability makes it an ideal marine algae biofilter to maintain pond water quality and improve environmental sustainability Tanaka *et al.* (2020). According to Mawi *et al.* (2019) that this ability is closely related to Gracilaria in photosynthesizing. Besides that, the broad surface of this algae, where the greater the weight of the thallus allows more thallus to receive sunlight actively Izzati (2011); Mawi *et al.* (2019). In addition, *Gracilaria* has the ability of phytoextraction (Komarawidjaya, 2005), which is the process of absorption and storage of heavy metals and other pollutants from the environment. This mechanism involves the cell wall and specialized cell organelles that bind N and P, then store them in the cell vacuole Afriansyah *et al.* (2016).

Conclusions

Based on the findings, it was determined that the weights of 75 and 100 gr of *Gracilaria* sp seaweed were very good at absorbing N and P from white leg shrimp farming waste and promoting the absolute development of *Gracilaria* sp seaweed. the best, weighing 100 grams.

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