Use seaweeds on nutrient reduction in super-intensive vaname pond wastewater

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Abstract. This research was carried out at the Waste Water Treatment Plant (IPAL) in super intensive vaname shrimp cultivation. Wastewater discharged into the sea through wastewater treatment has high N and P contents. Grass is known to have effective absorption of N and P. Therefore, this study aims to determine the absorption capacity of the seaweed *Gracillaria gigas*, *Gracillaria verrucosa*, *and Eucheuma spinosum* on N and P in wastewater from super intensive pond wastewater treatment plants. The experiment utilized aquariums measuring 40 x 30 x 25 cm, with a volume of 30 liters each. The seaweed types were tested at a density of 2.8 g/L, while the method employed was a complete randomized design (CRD) with three treatments and three replications. Furthermore, the water for the experiment was taken from the pump of the equalization pond in the super-intensive wastewater treatment plant. The seaweed parameters measured were the final average weight, absolute growth, and specific growth rate. The absorption rate of total ammonia nitrogen (TAN), nitrite, nitrate, and phosphate was assessed with time intervals of 6, 12, 24, 48, 72, 96, 120, and 144 hours. The results showed that the growth of the three seaweed types was significantly different ($p<0.05$). The highest average reduction rate of total N was found in *Eucheuma spinosum* and *Gracillaria verrucosa* plants which were in the "very effective" (VE) category in absorbing total N and PO_4.

1 Introduction

Studies on wastewater treatment plants (WWTP) in super-intensive shrimp farming were needed to minimize and prevent the emergence of environmental problems in aquaculture. The deterioration of water quality in fish and shrimp farming primarily results from uneaten feed. This was evidenced in feces and urine which accumulate in the maintenance media and are discharged to the environment during water changes. The higher the density applied, the greater the waste generated.

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According to a study on stocking densities of 500 and 600 shrimp/m^2, the N retention was 30.47% and 33.34%, while P retention was 16.59% and 18.05%, respectively [1]. The N and P waste load generated can reach 406.57 kg N and 100.33 kg P (500 shrimp/m^2), as well as 532.30 kg N and 119.50 kg P (600 shrimp/m^2) [2, 3]. This directly affects the characteristics of wastewater in ponds, which is then discharged through the central drain outlet. The wastewater contains concentrations of total suspended solids (TSS), TAN, N-Total, PO_4, and total organic matter (TOM) which are significantly above the allowable threshold [4]. Super-intensive pond wastewater management in WWTP consisting of sedimentation, oxygenation, bioconversion, and storage plots can improve water quality. This is evidenced in parameters including total suspended solids, biochemical oxygen demand (BOD), pH, and dissolved oxygen (DO). However, the concentrations of PO_4 and Total N remain above the allowable threshold [5]. When discharged directly into public waters, these components potentially cause eutrophication [6]. Therefore, biological management using seaweed as a biofilter needs to be implemented.

Seaweed is used as a biological indicator in ponds and public waters [7, 8]. It has the ability to absorb nutrients N and P [9, 10], leading to the application in bioremediation of shrimp ponds [11]. Therefore, this study aims to determine the absorption capacity of the seaweed *Gracillaria gigas*, *Gracillaria verrucosa*, and *Eucheuma spinosum* on N and P in wastewater from super intensive pond wastewater treatment plants.

2 Method

This study was conducted at the Experimental Pond Installation (EPI) of the Maros Brackish Water Aquaculture Research and Fisheries Extension Center located in Punaga Village, Manggara Bombang sub-district, Takalar Regency.

The container used was an aquarium measuring 40 x 30 x 30 cm, with a water volume of 30 liters/container. A total of nine aquariums were set up, each equipped with a blower installation. The complete randomized design was employed with three treatments and three replications. The seaweed species tested included A) *Gracilaria gigas* B) *Gracillaria verrucosa*, and C) *Eucheuma spinosum*. Observations were carried out on the absolute and specific growth rate using the formula:

2.1 Observed variables

The waste water used is waste water originating from the super intensive vanname shrimp cultivation waste water treatment plant. Meanwhile, seaweed comes from the waters and ponds of Takalar district. The seaweed is selected and its health is ensured, such as no wounds, white spots (*ice-ice*) on the surface of the seaweed, bright color and freshness. Seaweed is weighed before being placed in the rearing container as initial data and maintained for 6 days. Sampling of seaweed growth was carried out at the beginning and end of the study.

Observations were carried out on the absolute and specific growth rate using the formula:

2.1.1 Absolute growth

Absolute growth was calculated based on initial and final weight [12]. The formula used is as follows:

$$PM \quad = Wt - Wo \qquad (1)$$

Where : PM = Absolud growth (g), Wt = Final weight (g), W_0 = Initial weight (g)

2.1.2 Specific Growth Rate (SGR)

The specific growth rate (SGR) of seaweed was measured for six days, from the beginning to the end of the study. According to Dawes et. al. [13], SGR can be calculated using the formula:

$$SGR= Ln\ Wt - Ln\ Wo/t\ X\ 100 \qquad (2)$$

Where: SGR = Specific Growth Rate (%), Wt= Seaweed Weight at Final Time (g), Wo= Seaweed Weight at Initial Time (g), t = Observation Period (days)

2.2 Water quality

Water sampling was evaluated based on SNI 6989.57: 2008 concerning water and wastewater section 57 regarding surface water sampling methods. Based on these methods, sampling used a 500 mL polyethylene bottle. Water quality variables were observed every 0, 6, 12, 24, 48, 72, 96, 120, and 144 hours. The variables observed included Total Ammonia Nitrogen (TAN), Nitrite (NO_2), Nitrate (NO_3), Total N, and Phosphate (PO_4) (Table 1). Total N is the sum of soluble inorganic nitrogen NO_3, NO_2, and NO_3 [14, 15].

Table 1. Water quality variables Total N, TAN, $NO^{3-}N$, NO_2, PO_4 and analysis methods used

Water quality variables	Method or tool	References	Name of container
Total N		Effendi, 2003	
TAN	Phenat (spectroquan)	Anon, 1991	Polyetline Bottle
NO_3	Cadmium reduction (spectroquan)	Anon, 1991	Polyetline Bottle
NO_2	Colorimetry(Spectroquan)	Anon, 2004	Polyetline Bottle
PO_4	Ascorbic acid (spectroquan)	Anon, 1991	Polyetline Bottle

2.3 Nutrient Decrease Rate (NDR)

The nutrient-reducing ability of seaweed was calculated based on the formula [16]. Where the initial concentration is the data before treatment of seaweed, and the final concentration is the result of analysis after treatment. Therefore, the nutrient decrease rate was calculated using the formula:

$$\text{Nutrien decrease rate} = \frac{T1\ Nutrient\ concentration - T2\ Nutrient\ concentration}{T3} \qquad (3)$$

Where: T1 = Initial Concentration, T2 = Final Concentration, T3 = Time (Days)

2.4 Efficiency of N and P

The nutrient decrease rate data was then used to calculate the seaweed absorption efficiency of N and P during the study with the formula:

$$Seaweed\ efficiency\ (\%) = (A-B)/A \times 100 \quad (4)$$

The calculation results of seaweed efficiency were categorized based on [17] as follows: Very efficient x > 80%; efficient: 60% < x = 80%; moderately efficient: 40% < x = 60%; less efficient: 20% < x = 40% and inefficient: x = < 20%.

2.5 Data processing

The data obtained were first tested using the Shapiro-Wilk test to determine the normal distribution. Moreover, Analysis of Variance (ANOVA) was employed to analyze the differences between data variants. Further tests using Post hoc test (Tukey) were carried out to determine the smallest significant difference between each treatment. The Shapiro-Wilk and ANOVA tests were conducted at the 95% confidence level using SPSS software version 25.

3 Results

The ANOVA results on the absorption of N and P wastewater by seaweed showed a significant impact on growth (Table 2). The Post hoc test (Tukey) indicated that the final weight of *G. gigas* (A) was not significantly different ($p>0.05$) from *G. verrucosa* (B). However, both treatments exhibited significant differences compared to *E. spinosum* (C). The final weight growth data was then used to calculate absolute growth.

Absolute growth refers to the weight gain of seaweed during six days of maintenance. In this study, absolute growth exhibited significant differences ($p<0.05$) between treatments. However, further test Post hoc tests showed that treatment C significantly differed from A, while treatment A was not significantly different from B. The specific growth rate was calculated to determine the daily growth of seaweed.

Analysis of variance results showed significant differences ($p<0.05$) between treatments. Further tests demonstrated that treatments A and B were not significantly different ($p>0.05$) but both differed significantly from C.

Table 2. Growth seaweed initial Weight, Final Weight, Absolute Growth (AG), Specific Growth Rate (SGR) during the research

Variable	A	B	C
	***Gracillaria gigas* sp.**	***Gracillaria verrucosa***	***Eucheuma spinosum* sp.**
	Average	**Average**	**Average**
Initial weight (g)	84.00±0.00	84.00±0.00	84.00±0.00
Final weight (g)	102.03±3.21^{a}	100.97±4.85^{a}	83.40±8.26^{b}
Absolute growth (g)	18.83±3.21^{a}	17.77±4.85^{a}	-0.20±8.26^{b}
SGR (%)	3.31±0.52^{a}	3.12±0.80^{a}	-0.11±1.69^{b}

Information: Values with the same superscript in the column indicate no significant difference ($p>0.05$).

Seaweed growth was affected by the availability of N and P in the rearing water media. A decrease in these nutrients indicated efficient absorption by seaweed, specifically for NO_3 and PO_4. However, the process of Total Ammonia Nitrogen reduction was monitored to observe the extent of the decrease that occurred during the study. The results of water quality observations for Total Ammonia Nitrogen in controlled containers are shown in Table 3. The highest rate of decrease was observed in *Eecheuma spinosum* sp. at 0.4843±0.91 ppm/day, followed by *Gracillaria verrucosa* and *Gracillaria gigas* sp. at 0.2484±0.41 and 0.2970±0.70 ppm/day, respectively.

Table 3. Rate of Total Ammonia Nitrogen decrease in *Gracillaria gigas* sp., *Gracillaria verrucosa*, and *Eucheuma spinosum* sp. treatments

Observation	A			B			C		
	***Gracillaria gigas* sp.**			***Gracillaria verrucosa***			***Eucheuma spinosum* sp.**		
	Beginning	**After**	**NDR ppm/day**	**Beginning**	**After**	**NDR ppm/day**	**Beginning**	**After**	**NDR ppm/day**
1	0.8977	0.1500	1.4953	0.9567	0.4400	1.0333	1.1130	0.1183	1.9893
2	0.1500	0.5083	-0.3583	0.4400	0.3917	0.0483	0.1183	0.4283	-0.3100
3	0.5083	0.4483	0.6980	0.3917	0.3033	0.4867	0.4283	0.3100	1.1196
4	0.4483	0.2332	-0.2958	0.3033	0.1900	-0.0708	0.3100	0.2833	-0.2967
5	0.2332	0.1761	0.2088	0.1900	0.0442	0.1770	0.2833	0.2149	0.3618
6	0.1761	0.0735	0.0342	0.0442	0.0471	-0.0010	0.2149	0.0901	0.0416
Average	0.4023	0.2677	0.2970	0.3876	0.2360	0.2484	0.4113	0.2408	0.4843
stdv	0.28	0.17	0.70	0.31	0.17	0.41	0.36	0.13	0.91

Description: NDR = TAN (Total Ammonia Nitrogen) Rate of Decrease

Nitrite results from the breakdown of ammonia into NO_3 during the nitrification process carried out by Nitrosomonas bacteria. Due to its temporary nature, the concentration of NO_2 fluctuates. However, the reduction rate needs to be observed to understand the overall process. The highest rate of reduction was found in seaweed species *G. verrucosa* at 0.0446±0.06 ppm/day, followed by *Eucheuma spinosum* sp. and *G. gigas* at 0.0438±0.06 ppm/day and 0.0387±0.06 ppm/day respectively.

Table 4. Nitrite (NO_2) decrease rate in the treatment of *Gracillaria gigas* sp., *Gracillaria verrucosa*, and *Eucheuma spinosum* sp.

Observation	A			B			C		
	***Gracillaria gigas* sp.**			***Gracillaria verrucosa***			***Eucheuma spinosum* sp.**		
	Before	**After**	**NDR ppm/day**	**Before**	**After**	**NDR ppm/day**	**Before**	**After**	**NDR ppm/day**
1	0.0753	0.0103	0.1300	0.0747	0.0008	0.1477	0.0747	0.0008	0.1477
2	0.0103	0.0097	0.0007	0.0008	0.0050	-0.0042	0.0008	0.0067	-0.0059
3	0.0097	0.0113	0.0791	0.0050	0.0093	0.0923	0.0067	0.0093	0.0923
4	0.0113	0.0047	-0.0020	0.0093	0.0037	-0.0039	0.0093	0.0077	-0.0068
5	0.0047	0.0030	0.0304	0.0037	0.0010	0.0365	0.0077	0.0111	0.0325
6	0.0030	0.0219	-0.0063	0.0010	0.0025	-0.0005	0.0111	0.0030	0.0027
Average	0.0191	0.0102	0.0387	0.0157	0.0037	0.0446	0.0184	0.0064	0.0438
stdv	0.03	0.01	0.06	0.03	0.00	0.06	0.03	0.00	0.06

Description: NDR = Nitrite Decrease Rate

Nitrate is directly absorbed by seaweed, and undergoes continuous fluctuations in its concentration, leading to a gradual decrease each day. This nutrient was needed by plants, and in this study, the highest rate of decrease was observed in *Eucheuma spinosum* sp. at 0.0617±0.21 ppm/day, followed by *Gracillaria gigas* sp. and

Gracillaria verrucosa at 0.0430±0.20 ppm/day and 0.0449±0.43 ppm/day respectively.

Table 5. Nitrate (NO_3) decrease rate in the treatment of *Gracillaria gigas* sp., *Gracillaria verrucosa*, and *Eucheuma spinosum* sp.

Observation	A			B			C		
	***Gracillaria gigas* sp.**			***Gracillaria verrucosa***			***Eucheuma spinosum sp.***		
	Before	**After**	**NDR ppm/day**	**Before**	**After**	**NDR ppm/day**	**Before**	**After**	**NDR ppm/day**
1	0.2167	0.0277	0.3780	0.2251	0.0344	0.9953	0.2406	0.0306	0.4200
2	0.0277	0.1343	-0.1067	0.0344	0.1641	-0.1298	0.0306	0.1411	-0.1105
3	0.1343	0.1517	0.1509	0.1641	0.1846	0.1312	0.1411	0.1469	0.1821
4	0.1517	0.1988	-0.1528	0.1846	0.1248	-0.1273	0.1469	0.1519	-0.1312
5	0.1988	0.3036	-0.0611	0.1248	0.1009	0.0121	0.1519	0.1214	0.0243
6	0.3036	0.1552	0.0495	0.1009	0.1368	-0.0120	0.1214	0.1647	-0.0144
Average	0.1721	0.1619	0.0430	0.1390	0.1243	0.0449	0.1388	0.1261	0.0617
stdv	0.09	0.09	0.20	0.07	0.05	0.43	0.07	0.05	0.21

Description: NDR= Nitrite Decrease Rate

In this study, the highest decrease in Phosphate (PO_4) occurred in *Gracillaria verrucosa* by 0.2542±0.45 ppm/day, followed by *Eucheuma spinosum* and *Gracillaria gigas* sp at 0.1614±0.37 ppm/day and 0.0819±0.07 ppm/day.

Table 6. Phosphate (PO_4) decrease rate in *Gracillaria gigas* sp., *Gracillaria verrucosa*, and *Eucheuma spinosum* sp. treatments

Observation	A			B			C		
	***Gracillaria gigas* sp.**			***Gracillaria verrucosa***			***Eucheuma spinosum sp.***		
	Before	**After**	**PDR ppm/day**	**Before**	**After**	**PDR ppm/day**	**Before**	**After**	**PDR ppm/day**
1	0.4217	0.1320	0.5793	0.5450	0.0473	0.9953	0.4343	0.0600	0.7487
2	0.1320	0.2747	-0.1427	0.0473	0.1987	-0.1513	0.0600	0.2000	-0.1400
3	0.2747	0.1737	0.2704	0.1987	0.1547	0.5604	0.2000	0.0873	0.4409
4	0.1737	0.2230	-0.1828	0.1547	0.0317	-0.0915	0.0873	0.0790	-0.1095
5	0.2230	0.0460	0.0898	0.0317	0.0289	0.2126	0.0790	0.0657	0.1501
6	0.0460	0.522	-0.0021	0.0289	0.0303	-0.0004	0.0657	0.4316	-0.1220
Average	0.2118	0.1503	0.1020	0.1677	0.0819	0.2542	0.1544	0.1539	0.1614
stdv	0.13	0.09	0.29	0.20	0.07	0.45	0.15	0.15	0.37

Description: PDR= Phosphate Decrease Rate

The rate of decrease in total nitrogen (TN) for each treatment is shown in Table 7. The highest average rate of decrease was found in *Eucheuma spinosum* at 0.4583±0.87 ppm/day, and *Gracillaria gigas* sp. at 0.2858±0.68 ppm/day. Meanwhile, the lowest was observed in *Gracillaria verrucosa* at 0.2722±0.43 ppm/day.

Table 7. Rate of Total N Decrease in *Gracillaria gigas* sp., *Gracillaria verrucosa*, and *Eucheuma spinosum* sp. treatments

Observation	A			B			C		
	***Gracillaria ggas* sp.**			***Gracillaria verrucosa***			***Eucheuma spinosum* sp.**		
	Before	**After**	**TNDR ppm/day**	**Before**	**After**	**TNDR ppm/day**	**Before**	**After**	**TNDR ppm/day**
1	0.8714	0.1430	1.4568	0.9256	0.3997	1.0517	1.0683	0.1126	1.9114

Observation	A			B			C		
	***Gracillaria ggas* sp.**			***Gracillaria verrucosa***			***Eucheuma spinosum* sp.**		
	Before	**After**	**TNDR ppm/day**	**Before**	**After**	**TNDR ppm/day**	**Before**	**After**	**TNDR ppm/day**
2	0.1430	0.4862	-0.3433	0.3997	0.3878	0.0119	0.1126	0.4157	-0.3031
3	0.4862	0.4373	0.6797	0.3878	0.3152	0.4910	0.4157	0.3125	1.0660
4	0.4373	0.2547	-0.2990	0.3152	0.1893	-0.0887	0.3125	0.2894	-0.2962
5	0.2547	0.2275	0.1809	0.1893	0.0628	0.1713	0.2894	0.2226	0.3374
6	0.2275	0.1077	0.0399	0.0628	0.0741	-0.0037	0.2226	0.1194	0.0344
Average	0.4033	0.2760	0.2858	0.3801	0.2382	0.2722	0.4035	0.2453	0.4583
stdv	0.26	0.15	0.68	0.30	0.15	0.43	0.34	0.12	0.87

Description: TNDR= Total Nitrogen Decrease Rate

The effectiveness of seaweed in absorbing nitrate (NO_3) from super-intensive wastewater ponds was found to be the highest in *Gracillaria gigas* sp. by 60.30%, falling in the efficient category (E). *Eucheuma spinosum* sp. with an absorption rate of 46.35% was classified as moderately efficient (ME), while the lowest was found in *G. verrucosa* by 38.96%, falling in the less efficient category (ME). On the other hand, the highest total N values were recorded for *Gracillaria verrucosa*, *Gracillaria gigas* sp., and *Eucheuma spinosum* sp., with 92.06%, 89.83%, and 88.09% respectively, in the very efficient category (VE). The highest phosphate (PO_4) was found in *Gracillaria gigas* sp. and *Gracillaria verrucosa* treatment, while the lowest was detected in *Eucheuma spinosum* sp. with values of 94.26% (VE), 87.51% (VE) and 43.84% (ME) respectively.

Table 8. Effectiveness of *Gracillaria gigas* sp., *Gracillaria verrucosa*, and *Eucheuma spinosum* sp. seaweeds in absorbing N and P nutrients in super-intensive pond wastewater.

Variable	A		B		C	
	***Gracillaria gigas* sp. (%)**	**Effectiveness**	***Gracillaria verrucosa* (%)**	**Effectiveness**	***Eucheuma spinosum* sp. (%)**	**Effectiveness**
TAN (mg/L)	92.18±3.77	VE	95.28±1.10a	VE	90.22±8.66	VE
NO_2 (mg/L)	70.96±24.27	E	96.69±1.61	SE	92.77±3.03	VE
NO_3 (mg/L)	60.30±20.92	E	38.96±6.07	ME	46.35±16.02	ME
Total N (mg/L)	89.83±4.47	VE	92.06±0.48	VE	88.09±7.81	VE
PO_4 (mg/L)	87.51±6.10	VE	94.26±1.94	VE	43.84±45.27	ME

Description: VE (very efficient); E (efficient), ME (moderately efficient); LE (less efficient)

4 Discussion

Vaname shrimp farming waste taken from super-intensive pond WWTP was found to contain total ammonia nitrogen, NO_3, and PO_4 that exceeded the threshold allowed in the government regulation of marine and fisheries Number 75 of 2016 (Table 9). The content will be discharged into public waters due to wastewater treatment in conventional WWTP. Therefore, biological management such as the application of seaweed can absorb N and P.

Table 9. Wastewater N and P concentrations of super-intensive vaname shrimp ponds.

Variable	Concentration	Effluent Standard Permen KP. No. 75/2016
TAN (mg/L)	0.9891	≤ 0,05
NO_2 (mg/L)	0.0749	≤ 1
NO_3 (mg/L)	0.2274	0,5
PO_4 (mg/L)	0.4670	0,01

The high growth of *Gracillaria verrucosa* and *Gracillaria gigas* sp is an indicator of the ability to absorb total N and PO_4 optimally. NO_3 and PO_4 availability greatly affects seaweed growth [11]. At lower concentrations of N and P, seaweed growth was reduced [18]. Total ammonia nitrogen consists of ammonia (NH_{3-}) and ammonium (NH_{4+}), while inorganic nitrogen is composed of ammonia (NH_{3-}), ammonium (NH_{4+}), NO_2, NO_3, and N_2 [19]. The decomposition of NH_{4+} into NO_2 and NO_3 is called nitrification. This process occurs optimally under aerobic conditions or sufficient oxygen and in the presence of decomposing bacteria. The super-intensive vaname shrimp uses probiotics during the rearing period, hence abundant decomposing bacteria are available. The process of NO_3 utilization by seaweed is a key step in nitrogen removal during nitrification. The rate of decrease in the concentration of total ammonia nitrogen, NO_2, and NO_3 demonstrates the biological process of seaweed in absorbing available nutrients.

Seaweed absorbs N and P by diffusion through the entire surface of the talus. The cell membrane, located outside the cell wall, acts as a protector of the cellular contents in the body and regulates the passage of nutrients in and out. The semi-permeable nature selectively allows certain substances to enter the cell. The amount of nutrients that diffuse internally depends on the concentration inside and outside. When the nutrient outside is higher than the amount inside, diffusion occurs freely into the cell according to its needs. As the nutrients gradually enter the cells, the metabolic activity increases and contributes to the development of the vacuoles, which serve as vital defense mechanisms [20]. Therefore, the process affects the effective performance of seaweed.

Gracillaria verrucosa as a biological biofilter was highly efficient in removing total N and PO_4 from super-intensive shrimp farming wastewater. These positive results indicate the importance of developing the application of seaweed in WWTP across super-intensive, intensive, and estuarine areas affected by aquaculture wastewater. Waste management using WWTP manually was effective in reducing the concentration of total suspended solids, but N and P remained high [1]. After the application of *Gracillaria verrucosa* and *Gracillaria gigas* sp., the performance of WWTP improved, falling in the very effective category [21, 22]. *Eucheuma spinosum* in this study had the lowest growth and effectiveness because it experienced death starting on the fifth day. This was due to its sensitivity to biofouling attachment caused by high total suspended solids in media water [23, 24]. *Eucheuma spinosum* sp. thrives in low total suspended solid concentrations, making it more suitable for applications in estuarine areas directly affected by shrimp farming waste discharges.

5 Conclusion

Final weight, absolute growth and daily growth rate between *Gracillaria gigas* sp., dan *Garcillaria verrucosa* were not significantly different P>0.05, while *Eucheuma spinosum* was significanly different p<0.05, to *Gracillaria gigas* and *Gracillaria verrucosa*. *Gracilaria verrucosa*, *Gracilaria gigas* sp. and *Eucheuma spinosum* sp. are very effective in absorbing Total N, as well as phosphates except *Eucheuma spinosum* which has a very low category.

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