



## Nitrogen assimilation potential of seaweed (*Gracilaria verrucosa*) in polyculture with Pacific white shrimp (*Penaeus vannamei*)

<sup>1</sup>Yudi N. Ihsan, <sup>1</sup>Subiyanto, <sup>2</sup>Tri D. K. Pribadi, <sup>3</sup>Carsten Schulz

<sup>1</sup> Faculty of Fisheries and Marine Sciences, Universitas Padjadjaran, Bandung, Indonesia;

<sup>2</sup> Faculty of Mathematics and Natural Sciences, Universitas Padjadjaran, Bandung, Indonesia; <sup>3</sup> Gesellschaft für Marine Aquakultur mbH, Kiel University Hafentörn 3, Büsum, Germany. Corresponding author: Y. N. Ihsan, yudi.ihsan@unpad.ac.id

**Abstract.** In order to evaluate the nutrient absorption efficiency of combined shrimp and seaweed production, nitrogen fluxes in polycultures were compared with shrimp monoculture systems. Therefore, triplicate concrete tanks, with a volume of 3 m<sup>3</sup>, were stocked with shrimp *Penaeus vannamei* (6-7 g, 5 ind/100 litres) and seaweed (*Gracilaria verrucosa*) in densities of 0, 3.125, 6.250, and 9.375 g L<sup>-1</sup>. The culture period lasted four weeks and water samples were taken every week to measure nutrient fluxes. The use of seaweed at a density of 3.125 g L<sup>-1</sup> in shrimp polyculture showed the highest ability for nitrogen assimilation originating from shrimp waste. This treatment increased shrimp survival rate from 63% (without seaweed) to 83% and the growth performance of shrimp from 247.78 g (without seaweed) to 350.20 g. Remaining nitrogen excreted by shrimp amounted to 15.36 g, which was mainly (14.62 g) utilized by seaweed to form a biomass of 16.90 kg. Therefore, polyculture systems using seaweed seem to act more efficiently with regard to nutrient accumulation.

**Key words:** aquaculture, biofiltration, eutrophication, nutrient.

**Introduction.** Shrimp aquaculture has developed quickly since the 1980s in Southeast Asian countries including Indonesia. However, the rapid industrial growth of aquaculture has raised environmental concerns about eutrophication and depletion of natural habitats (Naylor et al 2000; Zhang 2003; Zhou et al 2003; Mao et al 2006; Cosme et al 2017; Ménesguen & Lacroix 2018). Animal mariculture and other anthropogenic activities generate large quantities of organic and inorganic waste. April to October, 1997 comparative studies on the nitrogen budgets of closed shrimp polyculture systems showed that, in all the studied polyculture systems, nitrogen from feeds and fertilizers were the main input items, which comprised 70.7-83.9% of the total input nitrogen, 3.2-7.4% of which was provided by nitrogen fixation (Qi et al 2001). Released nutrients increase eutrophication processes and the accumulation of acute toxic substances for aquatic animals (Radhakrishnan 2001).

Integrated multi-trophic aquaculture techniques are good candidates to overcome these sustainability problems (Hishamunda & Ridler 2004). These systems have been proposed as a tool for developing environmentally sounded aquaculture practices and resource management within a balanced coastal ecosystem approach (Troell et al 2003; Neori et al 2004). One of the integration that can be done is integration of algae with fish or shrimp in the aquaculture system has been suggested as an effective measure to reuse the dissolved nutrients from remnant feed, faeces and excretory products of aquatic animals, to biologically remedy the water quality and control diseases (Du et al 2013).

Seaweed can absorb significant amounts of waste nutrients, controlling eutrophication, and consequently, improving the health and stability of marine ecosystems (Buschmann et al 2001; Chopin et al 2001; Troell et al 2003; Fei 2004; Neori et al 2004; Ma et al 2018). The physiological mechanisms of seaweed biofiltration have

been studied with the scallop *Chlamys farreri* in an integrated multi-trophic aquaculture (IMTA) system (Mao et al 2009), fish cage farms (Hayashi et al 2008), shrimp culture ponds (Jones et al 2001; Nelson et al 2001), and polyculture ponds containing shrimp *Penaeus vannamei* and seaweed *Gracilaria verrucosa*.

In an aquaculture system most of the nitrogenous and phosphorus dissolved inorganic waste products are excreted in the form of ammonium ( $\text{NH}_4^+$ ) and phosphate ( $\text{PO}_4^{3-}$ ) (Huang et al 2017; Lepine et al 2018). Both compounds are potentially toxic to aquatic organisms and increase eutrophication potential. The mechanical and chemical treatment and other processes to remove the excess of ammonium and phosphate from waste water and the culture ponds are very expensive and may also affect the environment (Troell et al 2003). Seaweed has been studied in recent years for nutrient removal strategies. This treatment technique is considered to be the most inexpensive and environmentally sounded clarification way (Radhakrishnan 2001; Neori et al 2004).

The subject of the present study is the parameterization of the rate of nitrogen uptake by the seaweed from shrimp wastewater of polyculture systems in order to reduce the nutrient releases into the environment.

**Material and Method.** The experiment was conducted in Sungai Buntu, West Java over July to August 2012 in an outdoor laboratory facility (Figure 1). Shrimp (*Penaeus vannamei*) and seaweed (*G. verrucosa*) were used in this study are available from Faculty of Fisheries and Marine Sciences Universitas Padjadjaran. This study used a completely randomized block design conducted in two phases. Phase I aimed to determine optimal stocking densities and ammonia excretion rates of shrimps, whereas the second phase quantified the nitrogen assimilation of seaweed at four seaweed stocking densities.

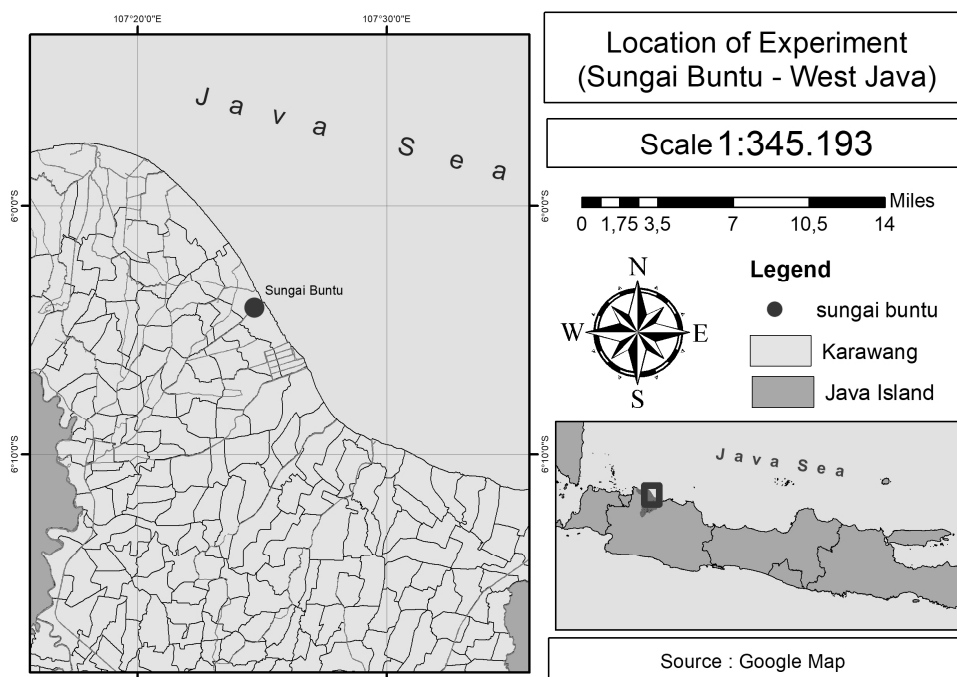


Figure 1. Map of Research Institute for Brackishwater Fisheries, West Java, Indonesia.

The experiment in phase I was divided into 3 treatments using glass aquaria with shrimp stocking densities of 5, 10, and 15 ind/100 liters of water. For each treatment two replications were conducted. Phase I was carried out for 1 week. The aquarium was filled with 100 liters of aerated sea water, and the environment was controlled with temperatures in the range of 27-30°C and salinity ranging from 25 to 28 ppt. Shrimp were fasted for one day then weighed; afterwards they were fed for one week and weighed again at the end of the experiment. On the last day, shrimp were transferred into two containers (10 liters), which had been filled with aerated sea water and exposed for 8 hours to ultraviolet (UV) light for disinfection of other nitrogen-consuming

organisms. Stocking density in the containers was one shrimp per 5 liters. Two containers without shrimp served as a control. Sampling was carried out 6 times at 1 h intervals from 0-5 h. Afterwards ammonium nitrogen was measured in accordance to APHA standard methods (2005).

In phase 2, nitrogen assimilation of seaweed at four stocking densities of seaweed was investigated with 3 replications using concrete tanks (3 m<sup>3</sup> volume, 1 m \* 3 m \* 1 m). Seaweed stocking densities were 0 g L<sup>-1</sup> (treatment A), 3.125 g L<sup>-1</sup> (treatment B), 6.250 g L<sup>-1</sup> (treatment C), and 9.375 g L<sup>-1</sup> of seaweed (treatment D). Determination of seaweed density was modified from Radhakrishnan (2001). Shrimp stocking density used in experimental phase 2 amounted 5 shrimp/100 liters with an initial weight of 6-7 g, based on the outcome of growth and survival results of phase 1 experiments. Individual and total weight of the replicate tank loading groups was not significantly different ( $p > 0.05$ ). The shrimp were fed a commercial diet with a protein content of 40% four times a day, i.e. at 07:00, 12:00, 17:00 and 22:00. The feed quantity was dynamically assigned to shrimp biomass increase estimated from control sampling. The daily feed ratio provided 7% of the weight of shrimp at sampling, modified in accordance with Baliao & Tookwinas (2002). Subsamples of shrimps (number of individuals) and algae from each tank were weighed every week.

**Sampling.** In both phases of the experiment, the following water quality parameters were measured: temperature, salinity, dissolved oxygen (DO), pH, ammonium, nitrate, nitrite, and total nitrogen (TN). Additionally, the number and weight of shrimp and survival rate, growth rate and feed conversion ratio (FCR) were assessed.

In phase 2, every week, ammonium, nitrate, nitrite, and TN were analysed. The samples were taken over a 24-hour period at 3 hour intervals. Water samples were taken from 20 cm below the water surface. Everyday at 08:00 and 16:00, the water temperature, salinity, and DO were monitored in situ with a portable water-quality analyser (TOA model WQC-20A Electronics Ltd., Japan). Water samples were collected using plastic bottles attached to the end of a stick.

They were immediately filtered through Whatman GF/F filters 0.7 µm millipore for soluble nutrients analyses (NO<sub>3</sub><sup>-</sup>, NO<sub>2</sub><sup>-</sup>, NH<sub>4</sub><sup>+</sup>). The ammonium concentration in the filtrate was measured immediately following filtration. TN, nitrate, and nitrite concentrations were measured using the APHA (2005) standard method. The standard methods for the determination of ammonium, nitrite and nitrate were based on moderate alkaline solution with hypochlorite, diazotization, and cadmium reduction followed by diazotization, respectively. The spectrophotometer wave lengths for ammonium, nitrite, and nitrate were 630, 542, and 542 nm, respectively. The nutrient analyses were performed using a spectrophotometer Shimadzu UV-2400.

The nitrogen content in the feed, shrimp, and seaweed was measured using the Kjeldahl method. Subsamples of shrimp (quantity) and algae (quantity) were analyzed at the beginning and the end of the experiment to assess changes in nitrogenous composition.

**Calculation.** Survival rate (SR) was calculated as a ratio of the shrimp quantity at stocking and sampling time using the following formula:

$$SR = (N_t / N_0) \times 100$$

where: SR = survival rate;

N<sub>0</sub> = number of shrimp on day 0 (individuals);

N<sub>t</sub> = number of shrimp on day t (individuals).

Specific growth rate was calculated with the formula (Busacker et al 1990):

$$SGR = ((\ln W_t - \ln W_0) / t) \times 100$$

where: SGR = specific growth rate;

W<sub>0</sub> = weight on day-0 (g individual<sup>-1</sup>);

W<sub>t</sub> = weight on day-t (g individual<sup>-1</sup>);

t = time of experiment (day).

The feed conversion ratio (FCR) was calculated as the ratio between the amount of feed given to shrimp biomass increment at a certain period (NRC 2011) using the formula:

$$FCR = F / \Delta B$$

where: FCR = feed conversion ratio;

F = amount of feed given during experiment (kg);

$\Delta B$  = addition of shrimp biomass during experiment (kg).

Nitrogen retention was calculated based on the following equation:

$$NR = \Sigma TN_t - \Sigma TN_0$$

where: NR = nitrogen retention (g);

$\Sigma TN_t$  = amount of total nitrogen on day-t (g);

$\Sigma TN_0$  = amount of total nitrogen on day-0 (g).

Ammonium excretion was calculated based on the following equation:

$$AE = ((N_t - N_0) / (W \times T_{t-0}))$$

where: AE = ammonium excretion ( $\text{mg-N g}^{-1} \text{h}^{-1}$ );

$N_t$  = ammonium concentration on time-t ( $\text{mg L}^{-1}$ );

$N_0$  = ammonium concentration on time-0 ( $\text{mg L}^{-1}$ );

W = weight of shrimp (g);

$T_{t-0}$  = sampling interval.

**Statistics.** Growth rate, survival rate, nitrogen retention and nutrient fluxes from experiments were analyzed statistically. All data were checked for normality (Kolmogorov-Smirnov test) and homogeneity of variances (HOV, Brown Forsythe test). Differences of means of triplicates in phase 2 were evaluated for significance by the range tests of Tukey HSD ( $p < 0.05$ ) for homogeneous variance and for inhomogeneous variances Dunnett-T3 test was used. Calculations were performed with the SPSS software package (SPSS 9.0.1, 1999).

## Results

**Phase I.** In phase 1, the highest survival rate (SR) and daily weight gain occurred at densities of 5 ind/100 liters (Table 1). The survival rate (SR) in this density was 100% and average daily growth (ADG) of shrimp was  $0.20 \text{ g day}^{-1}$ . The survival rate at densities of 10 and 15 ind/100 liters was 80% and 86.7%, respectively. Based on this data a density of 5 ind/100 liters was proposed for the experimental set up in phase II.

Table 1  
Growth of shrimp in phase 1

Amount of shrimp	Unit	Weight (T0)		Weight (Tt)		Average daily gain ( $\text{g day}^{-1}$ )	Survival rate (%)
		Total	average	Total	Average		
5	ind/100 L	34.1	6.8	41.1	8.2	0.20	100.0
10	ind/100 L	75.2	7.5	68.6	8.6	0.15	80.0
15	ind/100 L	107.1	7.1	103.0	7.9	0.11	86.7

During the short-term (5h) ammonium excretion trials, ammonia concentrations in the containers increased until the 4<sup>th</sup> hour from  $0.45$  to  $0.67 \text{ mg L}^{-1}$ , before starting to decline during the 5<sup>th</sup> hour (Table 2). The highest rate of ammonium excretion was found during the 4<sup>th</sup> hour ( $0.67 \text{ mg L}^{-1}$ ). The average (over 5 h) value of ammonium excretion was  $0.004 \text{ mg g}^{-1} \text{h}^{-1}$ . The results of the first phase ammonia excretion trial are summarized in Table 2.

Table 2

## Ammonium excretion

Containers	Time observation (h)						Weight of shrimp(g)	Ammonium excretion (mg g <sup>-1</sup> h <sup>-1</sup> )
	0	1	2	3	4	5		
Replicate 1	0.356	0.438	0.535	0.603	0.671	0.620	7.89	0.005
Replicate 2	0.544	0.586	0.540	0.580	0.660	0.643	8.21	0.003
Average	0.450	0.512	0.537	0.591	0.665	0.631	8.05	0.004

**Phase II**

*Growth of shrimp.* The total weight of shrimp at the end of the experiment was significantly different between treatments ( $p < 0.05$ ). The lowest weight of shrimp was 243.78 g in treatment A (without seaweed), whereas the weight of the other treatment tanks ranged from 314.71 g to 350.20 g (Table 3). The daily growth rates of shrimp were not significantly different ( $p > 0.05$ ) among the treatment without seaweed and the treatment with stocking of seaweed 3.125 g L<sup>-1</sup> (B), 6.250 g L<sup>-1</sup> (C), and 9.375 g L<sup>-1</sup> (D) (Figure 2).

Table 3

## Weight, retention, and FCR

	Treatment			
	A	B	C	D
Total N-Feed (g)	16.44	18.09	16.9	18.41
	<i>Start</i>			
Weight shrimp (g)	266.41	265.95	269.74	266.81
N shrimp (%)	7.44	7.43	7.53	7.45
Weight seaweed (g)		1562.6	3125.26	4688.29
N Seaweed (%)		8.06	16.13	24.19
	<i>End</i>			
Weight shrimp (g)	243.78 <sup>a</sup>	350.2 <sup>b</sup>	327.96 <sup>c</sup>	314.71 <sup>d</sup>
N shrimp (%)	1.78 <sup>a</sup>	10.16 <sup>b</sup>	9.13 <sup>b</sup>	9.22 <sup>b</sup>
Weight seaweed (g)		3255.12 <sup>a</sup>	5963.78 <sup>b</sup>	6563.24 <sup>b</sup>
N Seaweed (%)		22.68 <sup>a</sup>	24.67 <sup>a</sup>	36.65 <sup>b</sup>
	<i>N-retention</i>			
Shrimp (g)	0.59 <sup>a</sup>	2.73 <sup>b</sup>	1.6 <sup>c</sup>	1.78 <sup>c</sup>
Seaweed (g)		14.62 <sup>a</sup>	8.54 <sup>b</sup>	12.46 <sup>c</sup>
N production of shrimp	15.85 <sup>a</sup>	15.36 <sup>a</sup>	15.3 <sup>a</sup>	16.63 <sup>a</sup>
N in water (g)	15.85 <sup>a</sup>	0.74 <sup>b</sup>	6.76 <sup>c</sup>	4.18 <sup>c</sup>
FCR	2.69 <sup>a</sup>	1.99 <sup>a</sup>	2.02 <sup>a</sup>	2.24 <sup>a</sup>
Daily growth rate of seaweed (%)		2.62 <sup>a</sup>	2.31 <sup>a</sup>	1.20 <sup>b</sup>

Values with the same superscript letter do not differ significantly ( $p > 0.05$ ).

The survival rate (SR) of shrimp in phase II, showed that from the first week until the end of the study there was a significant difference ( $p < 0.05$ ) in each week between treatments using seaweed and without seaweed. The SR of shrimp at the end of experiment in treatment with the seaweed *G. verrucosa* (treatment B, C, and D) were 82.7%, 78.67%, and 76.00%, respectively, while in the treatment without seaweed (treatment A) was 62.67% (Figure 3). The absolute survival rate at the end of the experiment was significantly higher in all seaweed tanks than in the control tanks (without seaweed).

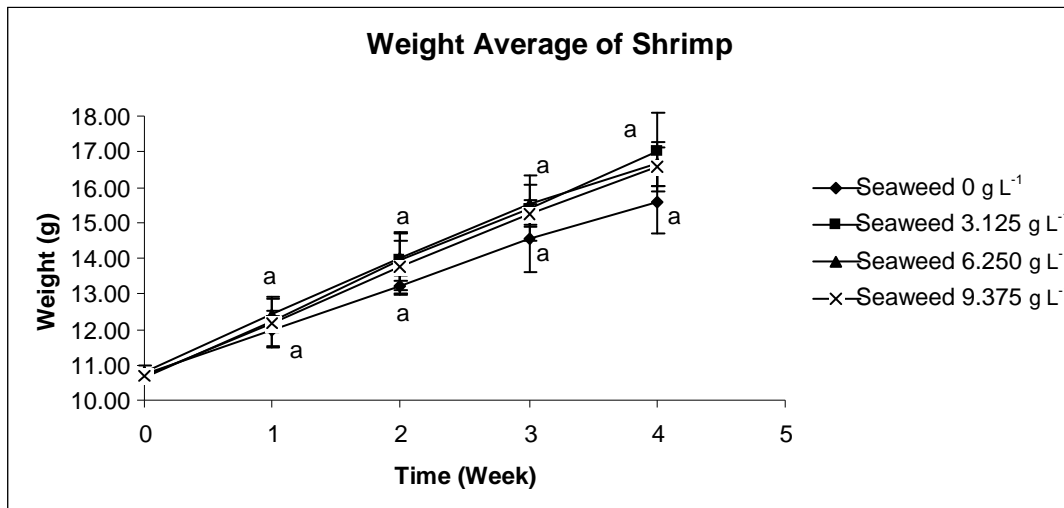


Figure 2. Individual weight of shrimp.

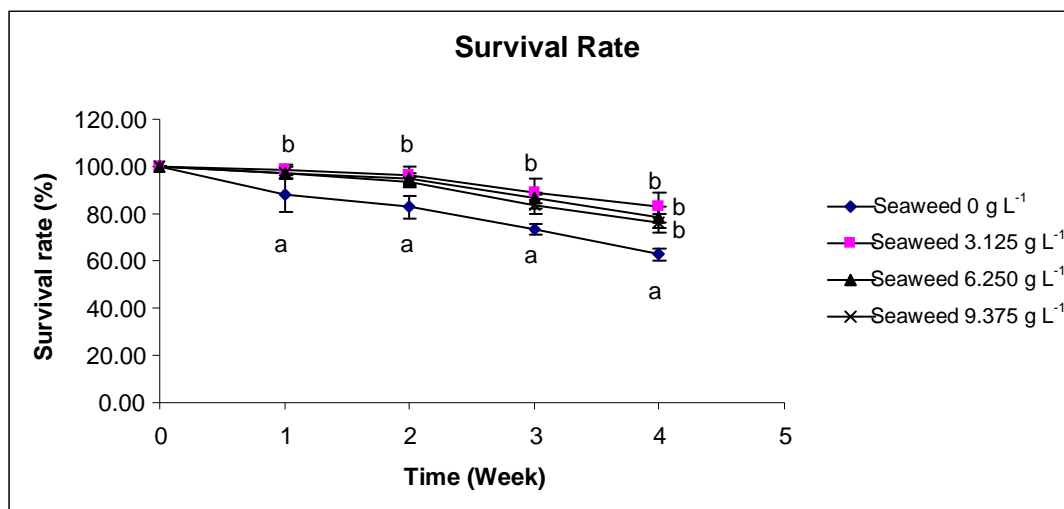


Figure 3. Survival rate of shrimp.

**Growth of seaweed.** The daily growth rate of seaweed was significantly different ( $p < 0.05$ ) between treatments, with the highest average growth rate ( $2.62 \pm 0.06$ ) in the tanks with lowest seaweed stocking density  $3.125 \text{ g L}^{-1}$  (treatment B) (Table 3). The seaweed growth rate in the  $6.250 \text{ g L}^{-1}$  seaweed stocking group (treatment C) was significantly lower (2.31%) than the aforementioned, and the lowest growth rate (1.20%) was found in the density of  $9.375 \text{ g L}^{-1}$  (treatment D).

**Feed conversion ratio (FCR) and nitrogen retention.** The FCR of shrimp was not significantly different between treatments ( $p > 0.05$ ). The lowest FCR value occurred in the treatment with stocking density of seaweed  $3.125 \text{ g L}^{-1}$  (treatment B) i.e. 1.99 and the highest occurred in the treatment without seaweed (treatment A) i.e. 2.69.

Nitrogen retention of shrimp in each treatment was significantly different ( $p < 0.05$ ) between treatments. Shrimps that were held in tanks with a low stocking density of seaweed ( $3.125 \text{ g L}^{-1}$ , treatment B) had the highest N-retention (2.73) whereas in treatment C (stocking seaweed  $6.250 \text{ g L}^{-1}$ ) and treatment D (stocking seaweed  $9.375 \text{ g L}^{-1}$ ) were 1.6 and 1.78. The lowest N-retention occurred in treatment A (without seaweed). Nitrogen retention of seaweed *G. verrucosa* was significantly different ( $p < 0.05$ ) between treatment  $3.125 \text{ g L}^{-1}$  (treatment B) and the others. Nitrogen retention of

seaweed *G. verrucosa* at treatment (B) was 14.62, while in treatment (C) it was 8.54 and (D) 12.46.

**Nutrient flux.** In this study, the total nitrogen (TN) concentration increased gradually. Figure 4 shows that the concentration of total nitrogen (TN) increased in all treatments. TN in treatment (A) increased from 0.82 to 2.73 mg L<sup>-1</sup>, while in treatment (B) TN ranged from 0.65 to 1.67 mg L<sup>-1</sup> and treatment (C) from 0.65 to 1.58 mg L<sup>-1</sup>. The lowest increase occurred in treatment (D) ranging from 0.55 to 1.46 mg L<sup>-1</sup>. This means the highest increase of TN occurred in treatment (A), which is significantly different to other treatments ( $p < 0.05$ ).

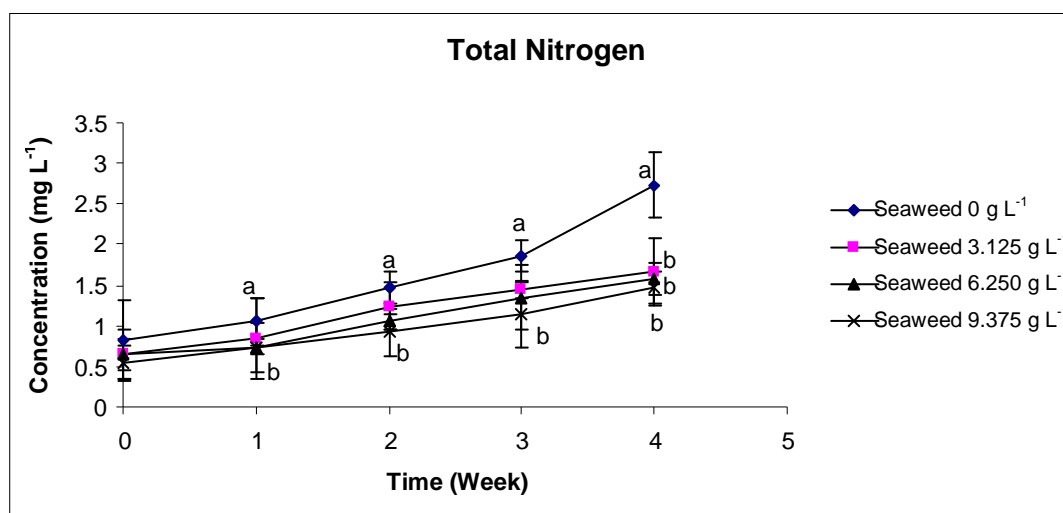


Figure 4. Concentration of total nitrogen (TN).

Even though fresh water was supplied to the ponds, the ammonium-nitrogen concentrations occurred in different amounts in all treatments. The highest concentration of ammonium occurred in treatment A (without seaweed) at week 2, i.e. 0.94 mg L<sup>-1</sup> (Figure 5).

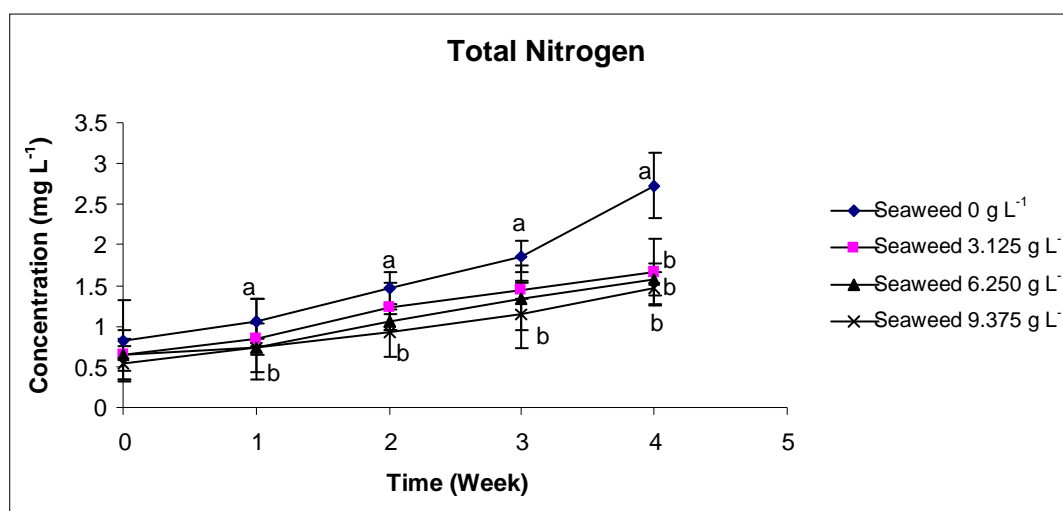


Figure 5. Concentration of ammonium-nitrogen (NH<sub>4</sub><sup>+</sup>-N).

Nitrate showed a different pattern as well. The peak nitrate concentration in all treatments was 0.009 mg L<sup>-1</sup>. Treatment (A) occurred at week 1, treatment (B) at week 2, treatment (C) at week 4, and treatment (D) at week 3 (Figure 6). While nitrite showed

the same pattern in all treatments. The peak concentration of nitrite in all treatments occurred at week 2 and not significantly different ( $p > 0.05$ ). Treatment (A) was  $0.009 \text{ mg L}^{-1}$ , treatment (B)  $0.007 \text{ mg L}^{-1}$ , treatment (C)  $0.008 \text{ mg L}^{-1}$ , and treatment (D)  $0.009 \text{ mg L}^{-1}$  (Figure 7).

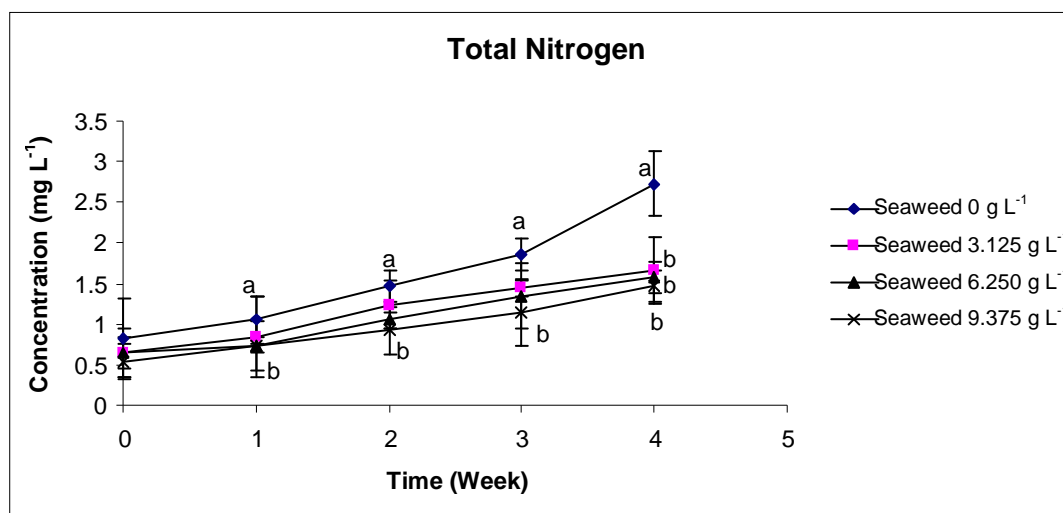


Figure 6. Concentration of nitrate-nitrogen ( $\text{NO}_3^-$ -N).

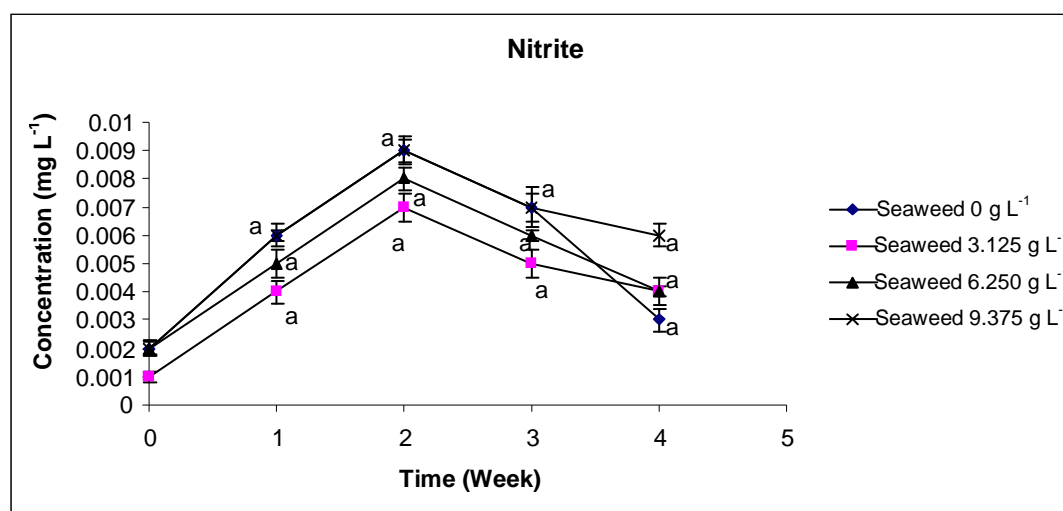


Figure 7. Concentration of nitrite-nitrogen ( $\text{NO}_2^-$ -N).

**Discussion.** Utilization of dissolved nitrogen by seaweed in the water aims to reduce the waste burden in the aquaculture media. Nitrogen content of the treatment using the seaweed was increased but it did not get too high (Figure 4), proving that the seaweed *G. verrucosa* could take up nitrogen. The seaweed could make use of ammonium through a diffusion process using all parts of its body. The higher the ability of seaweed to absorb the dissolved ammonium, the greater its growth. This means that the content of nitrogen will also further increase in the seaweed biomass, which can be seen from nitrogen seaweed bladder increases.

Nitrogen is necessary for the seaweed in the regulation of metabolism and reproduction (Pedra et al 2017). Growth and biomass can be achieved properly if the seaweed receives sufficient nitrogen. Uptake of nitrogen by seaweed *G. verrucosa* is not only a function of external nitrogen concentration, but also of the internal concentration in the plant net. Retrieval and storage of nitrogen by the seaweed can be affected by the concentration of dissolved inorganic nitrogen in water and influenced by ecological



fluctuations of nitrogen in plant tissues. Low nitrogen concentrations in the environment cannot meet the needs of seaweed for nitrogen for further usage. But the seaweed has the ability to assimilate and store nutrients from its surroundings, especially at low concentrations. Nitrogen dry weight content in treatments C and D were lower than treatment B. Presumably, although the amount of nitrogen in the form of nitrate and nitrite in water is high but *Gracilaria* was less able to utilize it. This is consistent with the finding that the most nitrogen absorbed by *Gracilaria* is nitrogen in ammonium form (Liu et al 2016). To meet the need for nitrogen, the reserves stored in the network are used prior to growth.

The ability of seaweed in taking up nitrogen from shrimp aquaculture waste in different treatments during the four weeks of maintenance in treatment B, seaweed was capable of utilizing dissolved nitrogen from shrimp waste up to 14.62 g, so that the weight of seaweed would be increasing twice. If it was calculated per hour, then the seaweed is capable of absorbing dissolved nitrogen at a rate of 0.013 g-N kg<sup>-1</sup> hr<sup>-1</sup>. In phase 2 of the study, the absorption of nitrogen by seaweed was three times greater than the production value of nitrogen excretion of shrimp per hour and kilogram in study phase I (Table 1). This means that dissolved nitrogen excretion of the shrimp can be utilized optimally by the seaweed.

Utilization of ammonium at treatments C (6.250 g L<sup>-1</sup> of seaweed) and D (9.375 g L<sup>-1</sup> of seaweed) is greater than for treatment B (3.125 g L<sup>-1</sup> of seaweed) only at the beginning of the study (first week). This condition does not last as long as the amount of ammonium reduced. To meet nutrient needs, seaweed then utilizes nitrate and nitrite. It can be seen from the steady depletion of nitrate and nitrite content in aquaculture media. In general, seaweed gradually absorbs nitrogen, i.e. ammonium > nitrate > nitrite. Utilization of nitrate and nitrite by the seaweed is less efficient because nitrate and nitrite must first be reduced before used by seaweed. Seaweed using nitrate for the metabolism need involvement of nitrate reductase enzyme. Absorption of nitrate and nitrite by the seaweed is influenced by the concentration of ammonium in the media. Due to the nitrogen used by seaweed in treatments C and D being nitrate and nitrite, then the growth of seaweed was not as fast at the beginning of more research utilizing ammonium. Marinho-Soriano et al (2002) reported the growth of seaweed during the first two weeks as rapid, but that it then declined until the end. Maintenance of seaweed *G. verrucosa* in drain ponds of shrimp in the first 15 days reached 8.8% and then continued to decline.

Seaweed *G. verrucosa* cultivation in shrimp pond effluent water could increase the nitrogen content in the thallus of 1% to 3.5% with a growth rate of 8-9% per day, which is higher than the growth rate of seaweed fed chemical fertilizers i.e. only 4-5% per day. The content of ammonium in treatment A (without seaweed) in the second week decreased drastically. This is due to the oxidation of ammonium into nitrite and then into nitrate. The content of nitrite and nitrate increased until reaching a peak; this is due to the aeration of the culture medium so that the oxygen demand for oxidation processes is met. Boyd et al (2002) described the process of oxidation with ammonium as the energy source, CO<sub>2</sub> as a carbon source and O<sub>2</sub> the source for the oxidation process. The oxidation process also occurred in treatments B, C, and D but in small quantities because the ammonium first utilized by seaweed. Besides that, seaweed also produces oxygen from photosynthesis rest. Xu et al (2008) reported *Gracilaria* cultivation can improve other aspects of water quality instead of increasing DO. Its photosynthesis produces DO, which promotes decomposition of organics. Density raft culture of *G. verrucosa* impedes the water circulation and may reduce chemical oxygen demand (COD) in the water column. In addition, several species of *Gracilaria* can produce oxygen under low light conditions, such as in rainy days and remediate anoxia (Xu et al 2008). Neori et al (2004) reported that seaweed *Gracilaria* sp. is capable of supplying oxygen (DO) of 2.86 mg L<sup>-1</sup> for 24 hours to the maintenance medium using polyculture with milkfish, shrimp, and seaweed.

Ammonium concentrations increased again in all treatments during week 4, with the highest value in treatment (D). This is due to the feeding; the higher residual and fecal excretion issued shrimp and dead seaweed. Additionally, the maximum growth of

seaweed was achieved in the third week. When maximum growth has been achieved then the absorption of nitrogen will decrease.

Water quality greatly affects the growth of shrimp. Good water quality is capable of supporting shrimp life, thereby increasing the appetite of the shrimp. Based on the value of FCR and the retention of each treatment, it is known that the FCR indicates the efficiency level of the feed utilization by shrimp as well as affecting the nutrient waste load discharged into the environment. The smallest FCR occurred in treatment B (1.99) with a biomass of 350.2 g and survival rate of 82.67%. This means that the high feed utilization by shrimp for growth causes the retention value also to be high (2.73 g). In comparison to the global average shrimp feed conversion ratio of around 2.0, the observed feed conversion were quite good (Tacon 2002).

The concentration of ammonium and nitrite in treatment (B) was lower compared with other treatments. To grow the shrimp from 265.95 g to 350.16 g, 15.36 g of nitrogen waste were generated (Table 3). Most of the waste (14.62 g) was absorbed by the seaweed, which enabled it to grow to 1.69 kg, while the remaining 0.74 g of residual waste remained in the water. The smaller the concentration of nitrogen remaining in the water, the more effective the utilization rate of nitrogen by the seaweed (Zhao et al 2018).

**Conclusions.** The seaweed *G. verrucosa* can be cultivated in polyculture together with the shrimp *P. vannamei*. The ability of seaweed in taking up nitrogen from the water would make the farming environment better and support shrimp production. This can be seen from the survival rates (SR) being significantly different ( $p < 0.05$ ).

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Authors:

Yudi Nurul Ihsan, Department of Marine Sciences, Faculty of Fisheries and Marine Sciences, Universitas Padjadjaran, 45363 West Java, Indonesia, e-mail: yudi.ihsan@unpad.ac.id

Subiyanto, Department of Marine Sciences, Faculty of Fisheries and Marine Sciences, Universitas Padjadjaran, 45363 West Java, Indonesia, e-mail: subiyanto@unpad.ac.id

Tri Dewi Kusumaningrum Pribadi, Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Padjadjaran, 45363 West Java, Indonesia, e-mail: tridewi.pribadi@unpad.ac.id

Carsten Schulz, Kiel University, Gesellschaft für Marine Aquakultur mbH, 24098 Kiel, Germany, e-mail: cschulz@tierzucht.uni-kiel.de

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