

Integrated Multi-Trophic Aquaculture (IMTA) on Growth of Abalones (*Haliotis squamata*), Vannamei Shrimps (*Litopenaneus vannamei*) and Macroalgae (*Ulva lactuca*)

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Abstract: The study about modification of the integrated multi-trophic aquaculture (IMTA) was examined by adding components of scavenger organism namely Vannamei shrimp (*Litopenaeus vannamei*). The purpose of this study is to compare the production abalone per unit volume of water between the integrated system of abalone and macroalgae (*Ulva lactuca*) with the integrated system of shrimp, abalone and ulva. The completely randomized design (CRD) was using with three treatments, i.e. a) biomass ratio of shrimp: abalone: ulva = 4: 1: 40; b) biomass ratio of shrimp biomass: abalone: ulva = 2: 1: 40; and c) biomass ratio of shrimps: abalones: ulva = 0: 1: 40 (as a control), with three replication respectively. The results of the variance analysis (ANOVA) showed that the ulva cultivated in condition of high nitrogen compounds from residual metabolites of shrimp, grew faster than the ulva cultivated without shrimp. Enhancement of the shrimp biomass in the system will increase the growth of ulva. Aquaculture systems with larger shrimp biomass will produce ulva with a higher protein content than ulva harvested from nature. Abalone is consuming ulva in an integrated aquaculture system with larger shrimp biomass, grew faster than abalone no consuming ulva cultivated without shrimp.

Keywords: Integrated, aquaculture, shrimp, abalone, macroalgae.

INTRODUCTION

Intensification of aquaculture has increased the potential for environmental pollution that will be caused. Shrimp or fish that are kept in intensive systems only change about 20-30% of the feed given to meat [1]. Mostly, 70 to 80% is excreted into two main forms: (i) as dissolved nutrients, and (ii) as contaminated organic matter (particulate organic matter).

Remnants of feed, feces and excretion products will experience microbial decomposition which causes various water problems such as increased BOD, decreased dissolved oxygen, eutrophication by nitrogenous compounds, and blooming algae.

The development of environmentally friendly cultivation systems is important to reduce the impact of intensive shrimp and fish farms. The desired environmentally friendly system is that which can reduce the production of nitrogenous waste to a minimum so that it does not cause a decrease in oxygen and an increase in nutrient content in natural waters [2]. There are two strategies that can be taken. First, implement a recirculation system where the remaining aquaculture water is used continuously so that little is discharged into the environment. In this system, the role of decomposing microorganisms in biofilter is very important in order to rapidly decompose highly toxic compounds of ammonia (NH₃) and nitrite (NO₂⁻) into nitrates (NO₃⁻) which are relatively safe for shrimp. Second, maintaining fish whose feed does not use large quantities of fish meal components. Herbivorous fish, for example, only need a low fish meal and produce one-fifth the amount of nitrogenous waste carnivorous fish or shrimp [3]. However, both approaches are relatively difficult to apply to intensive cultivation of penaeid shrimp such as *Litopenaeus vannamei*. Although the recirculation system may convert ammonia (NH₃) compounds in the final product of nitrate (NO₃⁻) by not replacing water, but nitrate compounds will still accumulate in water and, eventually reach a deadly level for shrimp [1, 4]. In other words, the current recirculation system needs to be improved so that it does not cause accumulation of certain compounds, which, although in low concentrations are not dangerous, but lethal effects at high concentrations.

Efforts to perfect the recirculation aquaculture system have been carried out since the beginning of the year 2000. For example utilizing phytoplankton to absorb formed NO_3 [5]. However, phytoplankton utilization is relatively difficult because the type and biomass that grows cannot be controlled [4]. Therefore, the use of macroalgae as nitrogen stripper is more developed because it is easier to control and harvest than phytoplankton [3, 6]. In closed recirculation systems that utilize macroalgae, large amounts of algal biomass are produced, and this has the potential to include herbivorous organisms that can consume the resulting algae. Thus, in the same recirculation system can be produced several products of cultivated organisms and known as an integrated cultivation system or integrated multi-trophic aquaculture (IMTA).

In this study, the modification of IMTA was examined by adding components of carnivorous animal namely vannamei shrimp, with the hope that, besides being a high-value harvest, this organism would also increase the concentration of nitrogen in the water, which stimulated ulva growth which would be used as abalone feed. The purpose of this study is to compare the growth rates of ulva and biomass production performance as feed suppliers for abalone in the ratio of shrimp biomass: abalone: different macroalgae; and the next goal is to compare the production of abalone per unit volume of water between systems that rely on ulva produced from the integration system of abalone-macroalgae with the shrimp-abalone-macroalgae integration system.

MATERIALS AND METHODS

This research has been carried out for 3 months starting from October 2015 to December 2015. The Research Site is a Field Laboratory in Oebobo District, Kupang City. Proximate analysis was also carried out at the Nutrition and Feed Laboratory, Faculty of Agriculture, Bogor Agricultural Institute (IPB).

Materials and Equipment

The equipment used in this study are wooden tub with tarps for abalone culture sized 120 x 50 x 20 cm³ (9 units); wooden tub for ulva culture sized 120 x 80 x 40 cm³ (9 units), plastic PVC Tank for shrimp culture 160 liters in capacity (9 units), plastic buckets as a biological filter composed of broken coral, ceramics and sponges (9 units). Magnetic pumps, sand filters, paralon pipes, water quality equipment (DO-meter, pH-meter, thermometer, refractometer), precision electric scales 0.1 g for measuring the weight and caliper to measure the body length of the test animal.

Sampling Procedure

The research is experiment using the basic design of the IMTA system designed in previous research [6]. This study was arranged in a completely randomized design (CRD) consisting of three (3) treatments and three (3) replications using the ratio as follows: IMTA1 with a ratio of shrimp biomass: abalone: macroalgae (4: 1: 40); IMTA2 ratio of shrimp biomass: abalon: macroalgae (2: 1: 40); IMTA3 ratio of shrimp biomass: abalon: macroalgae (0: 1: 40).

Macroalgae (*Ulva lactuca*) was collected from Pasir Panjang Beach - Kupang City, which is adjacent to the experimental site. The sea water used is sea water filtered with sand filter. Outdoor cultures use natural light. Macroalgae tubs are placed outdoors and shaded with 60% paranet. Water lost due to evaporation is replaced with new water and salinity is cultivated in the range of 30-32 ppt. Each algal tank was loaded with Ulva macroalgae as much as 300 grams of ulva/tub. Harvesting and weighing are done every 2 weeks by looking at the difference between the initial weight and the final weight using an electric scale. Juvenile abalone *Haliotis squamata* originating from the hatchery. Each tub is scattered abalone with a weight ranging from 0.3 to 1.5 gr/shell length 1.22 - 2.20 mm with a density of 10 juveniles/tub. Abalones were placed in wooden tarpaulin plastic tubs measuring 120 cm x 80 cm x 20 cm, placed outside the paranet roofed room without walls. Feeding was carried out every 2 days, using Ulva sp from the ulva culture. Feeding is ± 0.6 grams or 80% of body weight per day. The study used shrimp juvenile measuring 1.2 - 2.0 cm in length for the treatment of IMTA 1 density of 70 shrimps/tank with a weight of ± 30 grams (seed size 0.3 - 0.6 gr/shrimp) and treatment IMTA 2 density of 40 shrimps/tank with a weight of ± 15 grams. Shrimp was cultivated in a 160-liter plastic PVC tank. Shrimp culture tanks are placed adjacent to abalone snail culture. Shrimp are fed with formulations with 35% protein content every day as much as 5% of biomass with the frequency of feeding twice a day. Temperature and salinity water quality measurements were carried out 2 times a day while pH and DO measurements were carried out every 2 weeks.

Variables Analysis

The ulva growth rate is determined based on the weekly wet weight gain. Production of Ulva. per unit area (g/m²/day) is calculated by the formula [7].

$$P = \frac{(Wf - Wi)}{SA \times d}$$

Where: Wf is the final wet weight (g) and Wi is the initial wet weight (g) for each 1 week period, SA is the surface area of the algal culture tank (m²), and d is the length of the growth period (days).

The abalone growth rate is determined on a daily shell length and percentage changes in daily weight changes, with formula:

$$\Delta SL = (Lf - Li)/d$$

Where: ΔSL is the increase in shell length ($\mu\text{m} / \text{day}$) L_f is the end of the abalone L_i shell length is the initial length of the abalone shell and d is the duration of the experiment (days).

$$SGR = 100 \times (\ln W_f - \ln W_i)/d$$

Where: SGR is the specific growth rate ($\% / \text{day}$), W_f is the final weight (g), W_i is the initial weight (g), d is the duration of the experiment (days).

The weight ratio (BW) and abalone shell length (SL) using the formula:

$$Ratio = \frac{BW}{SL}$$

The survival rate (SR) abalone and shrimp are calculated with the following formula:

$$SR = \frac{N_t}{N_0} \times 100\%$$

Where: SR is the survival rate ($\%$), N_t is the number of test animals at the end of the experiment, N_0 is the number of test animals at the beginning of the experiment.

Analysis of Statistic

To find out the treatment effect on the growth and survival of *Ulva*, shrimp and abalone analysis of variance (ANOVA) was carried out using the SPSS 24 application. The differences between treatments were tested by the Tukey Test.

RESULTS AND DISCUSSION

Ulva (Ulva lactuca) Growth Rate in IMTA

The results of study showed that growth rate of *ulvas* were obtained range between 0.017 ± 0.13 to 0.147 ± 0.11 g/m^2 (Table 1). The results showed that the highest *ulva* growth rate occurred in the treatment of IMTA 1 which had the highest shrimp biomass, followed by the control treatment (IMTA 3) and treatment of IMTA 2. The rather biased results were in the treatment of IMTA 2 which although had shrimp, but the growth rate, the rate is lower than the control treatment without shrimp. Furthermore, variance analysis showed that the treatment of shrimp ratio: abalone: *ulva* had a significant effect ($P < 0.05$) on the rate of *ulva* growth, thus the higher the shrimp biomass the faster the rate of *ulva* growth.

Table-1: *Ulva (Ulva lactuca)* Growth Rate in IMTA

Treatments	Initial Weigth (g)	Final Weigth (g)	SGR (g/m^2)
IMTA 1	300 ± 0	475 ± 127.33	0.147 ± 0.11
IMTA 2	300 ± 0	336 ± 135.86	0.017 ± 0.13
IMTA 3	300 ± 0	378 ± 138.39	$0,051 \pm 0.13$

In this study, *ulva* can grow rapidly in the IMTA system even though the container used to cultivate *ulva* is relatively small (384 liters). Data shows that the growth rate ranges from $0.017 - 0.147$ $\text{g}/\text{m}^2/\text{day}$. These results are higher than the previous study which get a growth rate ranging from $0.098 - 0.139$ $\text{g}/\text{m}^2/\text{day}$ [8]. Although this value is much lower than previous study which uses *Gracilaria* algae where the growth rate is 23.33 $\text{g}/\text{m}^2/\text{day}$. In addition to the differences in factors of environmental factors, especially the duration of irradiation of sunlight affects the growth rate of algae in this study. The position of the algae tank that gets less light after 14.00 has resulted in a relatively low growth rate. In addition, this study uses a closed IMTA system where different water changes are not carried out by the open IMTA system which changes water to 200 L/hr/day [9]. This study also showed that the *ulva* cultivated under conditions of high nitrogen compounds due to the inclusion of *vannamei* shrimp into the system grew faster than without shrimp, especially in the highest density of 70 shrimps/tank. The thing that is anomaly in this study is the treatment of IMTA 2 which has shrimp, which should put more N into the system to stimulate *ulva* growth, but the *ulva* growth rate in this treatment is lower than the IMTA 3 control treatment that does not have shrimp. This is thought to be unrelated to the content of nitrate or phosphate, but other factors such as the position of the experimental unit are less subject to sunlight

so the growth rate is sluggish even though there is enough nutrients. In terms of proximate content, there are differences between ulva grown in the IMTA system and those harvested directly from nature. Ulva cultivated in the IMTA system has a higher water content, lower ash content, and higher fat and protein. For carbohydrates, the IMTA treatment that incorporates shrimp in the system has a lower carbohydrate content, while the IMTA - control that does not involve shrimp has a relatively similar carbohydrate content with ulva from nature. The results of measurement of the proximate content possessed by ulva from the results of this IMTA study are similar to those reported in previous research that Ulva cultivated under conditions of high-hydrogen media, such as those originating from shrimp or fish culture effluent, tend to have high protein content [5, 9].

Based on this explanation, it can be said that the IMTA system applied during this study was able to support ulva growth so that it was suitable for use as a nutrient stripper in the cultivation of abalone and vanamei shrimp. With the nutrient content, especially higher protein than the wild Ulva, the cultivation in the IMTA system is suitable to be used as an alternative to the use of ulva harvested from nature which can disrupt the availability of these algae in natural ecosystems.

Abalone (*Haliotis Squamata*) Growth Rate in IMTA

Abalone growth rates were determined based on changes in the daily shell length and percentage of daily weight change (Table 2 and Table 3). After a 43 day cultivated period, the specific abalone growth rate shows a range of values of 0.5 to 1.1% body weight per day. The highest specific growth was owned by abalone in IMTA 1, followed by abalone IMTA 3 and IMTA 2. The high value of abalone specific growth velocity in the IMTA 1 system was thought to be due to abalone in IMTA 1 consuming ulva which had a higher protein content than IMTA2 and IMTA3. The IMTA system developed in this study showed that IMTA with the intervention of vanamei shrimp could increase the ulva protein content in the system which had an effect on the abalone weight gain. Fish get energy from food. Food energy must be digested and absorbed before growth takes place [11].

Table-2: The Weight Growth Rate of Abalone (*Haliotis Squamata*) in the IMTA System

Treatments	Initial Weigth (gr)	Final Weigth (gr)	SGR (% weigth/day)
IMTA 1	0.707 ± 0.31	1.17 ± 0.47	1.060 ± 0.991
IMTA 2	0.707 ± 0.26	0.843 ± 0.28	0.457 ± 0.632
IMTA 3	0.707 ± 0.28	1.033 ± 0,37	0.928 ± 0.606

The ANOVA results showed a significant difference between treatments ($P < 0.05$). After Tukey HSD's further test analysis, it was found that the treatment of IMTA 2 was significantly different from IMTA 1 and 3. Different things found in the treatment of IMTA 2 were thought to be due to the water quality conditions of the IMTA treatment 2. Where the ability of ulva as a biofilter to maintain water quality not functioning properly. This is consistent with the main function and role of macroalgae biofilter in a cultivated system is the taking and conversion of toxic metabolites and pollutants. Environment plays an important role in the growth of abalone, the dirty environment causes water quality to decrease [5]. One indicator of decreased water quality is water brightness or transparency of water. The low condition of abalone water quality in IMTA 2, especially the water brightness level, causes abalone to become stressed and under stress conditions the rate of feed consumption decreases, consequently the growth of abalone becomes disrupted.

Table-3: Abalone Length Increase (*Haliotis Squamata*) in the IMTA System

Treatments	Initial Length (cm)	Final Length (cm)	Δ SL(μ m/day)
IMTA 1	1.865 ± 0,21	2,074 ± 0.23	48,45 ± 45,13
IMTA 2	1.750 ± 0,21	1,850 ± 0.17	23,26 ± 22,56
IMTA 3	1.783 ± 0,27	1.936 ± 0,31	35,58 ± 35,37

The results of the calculation of abalone shell length increments, it was found that the abalone in the treatment of IMTA 1 had a larger shell length increase of 12.87 μ m/day compared to abalone in the treatment of IMTA 3 and greater 25.29 μ m/day of the IMTA 2 treatment. However the results ANOVA showed that the growth of abalone shell for the three treatments of the IMTA system is no significantly different ($P < 0.05$). It can be concluded that the ulva food produced by IMTA consumed by abalone had more influence on the rate of abalone weight gain than the rate of increase in shell length. This condition is similar to previous study which examines the effect of feed types on abalone growth stating that abalone consuming feed from seaweed species *Ulva* sp has the lowest absolute long growth rate compared to other types of seaweed, and the growth of the length of the abalone seed shell was very slow and began to show up after a cultivated period of 8 to 10 months, namely the growth reached 1.5-3.0 mm per month [10].

Shrimp (*Litopenaeus vannamei*) Growth Rate in IMTA

Shrimp growth speed is determined based on the percentage of daily weight changes and changes in shrimp body length. Shrimp growth rate during 43 days cultivated period. (Table 4 and Table 5)

Table-4: The Weight Specific Growth Rate of Shrimp (*Litopenaeus vannamei*) in IMTA

Treatments	Initial Weigth (g)	Final Weigth (g)	SGR (g/m ²)
IMTA 1	0.437 ± 0.085	1.897 ± 0.574	3.361 ± 0.85
IMTA 2	0.400 ± 0	0.942 ± 0.22	0.942 ± 1.26
IMTA 3	0.00	0.00	0.00

The measurement results showed the growth rate of the specific weight of shrimp in the treatment of IMTA 1 was greater than the shrimp in the treatment of IMTA 2. Shrimp in IMTA 2 had a lower growth rate of weight compared to shrimp in IMTA 1. This was suspected because IMTA 2 using a shrimp culture container which is a used pesticide tank which may contain pesticide residue left on the wall so that the cultivation media is contaminated with the residue of the pesticide, consequently the treatment of IMTA 2 shrimp is stressed. Based on the results of visual observations, it was found that the condition of the shrimp seemed to be uneasy and swim continuously. In this condition the shrimp experiences an active metabolic rate. The active metabolic rate relates to the level of oxygen consumption coinciding with what is called maximum continuous swimming activity. This condition affects the speed of growth and even the survival of it. Good water quality conditions can support better organism conditions, in utilizing nutrient sources and water-soluble gases [11].

Based on the ANOVA results it is known that there are significant treatment differences with a P value of <0.05 and the value of F hit > F tab. Tukey HSD's further tests showed that the treatment of IMTA 1 and treatment of IMTA 2 were in different sub-sets. This means that there are significant differences in treatment between the treatment of IMTA 1 and treatment of IMTA 2.

Table-5: The Length Growth of Shrimp (*Litopenaeus vannamei*) in IMTA

Treatments	Initial Length (cm)	Final Length (cm)	ΔSL(μm/day)
IMTA 1	1.560 ± 0.154	7.140 ± 0.743	1298 ± 170
IMTA 2	1.420 ± 0.137	3.240 ± 2.378	423 ± 534
IMTA 3	0.00	0.00	0.00

The measurement results show that the IMTA system developed by shrimp also experiences body length increase. The increase in body length of the shrimp in the treatment of IMTA 1 was greater than that of shrimp in the treatment of IMTA 2. Shrimp kept in the treatment of IMTA 1 experienced growth in both body weight and body length. Shrimp can grow in a cultivation activity besides being influenced by the nutritional content of the feed also influenced by the water quality conditions of the cultivation media. The use of ulva as a biofilter in the treatment of IMTA 1 has proven to be able to maintain the quality of water in the media so that it is in a condition of water quality that is good for the growth of shrimp.

The findings of this study have implications for natural resource management. The finding that needs to be underlined is that in environmentally sound aquaculture technology, the recycling process or utilization of leftover organic matter and sewage is very important in reducing organic waste that is wasted into the waters and not utilized. All organic waste is expected to be reduced and even used to increase the productivity of marginal waters and maintain environmental stability. IMTA in this study was able to show that wastewater or waste can be reused for the cultivation of other organisms, for the development of environmentally friendly and water-efficient shrimp farming. Waste water from the shrimp and abalone cultivation is recycled or recirculated for abalone and ulva aquaculture activities, so that the fulfillment of the needs of aquaculture water is lower than general cultivation activities. The results also show that the quality of filtration water that passes through the ulva bath and is reused into abalone and shrimp tubs has water quality parameters within tolerant limits of cultivation so that ulva, abalone and shrimp cultivated in the IMTA system can experience growth in both weight and length. In addition, in order to fulfill the macroalgae needs in large quantities as the abalone food of the IMTA system, it is feasible to be developed as an alternative fulfillment of feed needs in abalone cultivation, to reduce dependence on natural macroalgae and over-exploitation which can disrupt the availability of macroalgae in nature.

CONCLUSION

Based on the results of the study it can be concluded the following matters: 1) Ulva which is cultivated under conditions of high nitrogen compounds due to the inclusion of the vannamei shrimp into the IMTA system grows faster than the ulva, which is cultivated without shrimp, and the greater the shrimp biomass the faster the ulva growth rate; 2)

The IMTA system with larger shrimp biomass can produce Ulva with a higher protein content than ulva harvested from nature; 3) Abalone who consumes Ulva from IMTA cultivation with larger shrimp biomass (IMTA 1) grows faster than abalone which does not consume ulva cultivated without shrimp intervention (IMTA 3).

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