The effects of season, aeration and light intensity on the performance of pacific whiteleg shrimp (Litopenaeus vannamei) polycultured with seaweed (Gracilaria verrucosa)

T Susilowati, Desrina, J Hutabarat, S Anggoro, M Zainuri, Sarjito, F Basuki and T Yuniarti

Aquaculture Department. Fisheries and Marine Science Faculty, Diponegoro University. Jl. Prof. Soedharto SH, Lingkar Tembalang, Semarang, Central Java, Indonesia.

E-mail :susilowatibdp@gmail.com

Abstract. This study was aimed to determine impact of stocking season, additional oxygen supply and light intensity on performance of pacific white leg shrimp (Litopenaeus vannamei) polycultured with seaweed Gracilaria verrucosa. Three sets of experiments were used and each experiment was conducted separately in 3 different season (factor W). Three factors and the interaction, that is, stocking seasons (W1: March-June; W2 : July-October and W3 : November-February), Oxygen supply (O, with or without supply aeration of 6.5 ppm) and light intensity (with or without addition of light 640 lux) was observed. The experiment was conducted in 16 polyethilen net place in concrete tanks (1.2 m³). Shrimp (average weight 0.1 g and lenght 1.2 cm) with density 94 shrimp/m³ and seaweed 2.188 g/m³, cultured for 94 days. Data collected included absolute growth, specific growth rate (SGR), survival rate (SR) and biomass production of shrimp and seaweed. The result showed that culture period March to June, additonal light and suply DO gave the best result with shrimp absolute growth 13.23 g, SGR 5.09 %/day, SR 99.64 % and biomass production 1.256.36 g/m³. Absolute growth of G. verrucosa was 5.223.75g, SGR 268 %/day and biomass production 12.608.55 g/m³.

1. Introduction
Polyculture of pacific whiteleg shrimp (Litopenaeus vannamei) and seaweed (Gracilaria verrucosa) is a common practice in Southeast Asia. Pacific whiteleg shrimp (L. vannamei) are suitable for this type of polyculture because they are highly adaptive to different environmental conditions. For example, L. vannamei are cultured in “ponds”, formerly excavated sand, from which 4 tons of L. vannamei are produced after 90 days of culture [1]. The use of G. verrucosa in the polyculture system benefits the environment by trapping excess nutrients normally occurring in shrimp ponds but possibly leaving negative effects on shrimp’s health. G.verrucosa also absorbs toxic substances from organic waste while producing oxygen supply during the day. Hence, it reduces waste contamination and converts the waste contamination into nutrients for its growth. Most of the time, polyculture is usually applied in extensive ponds. However, it is also applicable in semi-intensive shrimp ponds with additional oxygen supply. In
addition, the season for farming can also affect shrimp’s survival, growth and, in turn, production. Season affects the pond environment, including the salinity, dissolved oxygen, temperature and concentration of N (nitrate, nitrite and ammonia) in the water, which in turn, contributes to the shrimp’s well-being and seaweed growth. Selecting the right period for stocking and culturing shrimp is important for the success of the polyculture of shrimp and seaweed.

2. Methodology
The study was conducted in the Laboratory of Coastal Development, Faculty of Fisheries and Marine Sciences, Universitas Diponegoro. The experiment was done in different seasons in three periods, namely March-June (post-wet season), July-October (dry season) and November-February (wet season). The experiment used 16 polyethelene nets (1x1x1.2 m in size) placed in four concrete tanks (each tank was 6x5x1.2 m in size). For each period shrimp (average weight of 0.1 g/individual average length of 1.2 cm) were stocked at a density of 94 shrimp/m3 along with seaweed at a density of 2.188 g/m3. The experiment was carried out in split plot in time design with quadreplicate. There were three factors used in this study, namely factor W (season), O (oxygen supply) and I (light intensity). Factor W was the season in which the experiment was started and the stocking was conducted (W1: March-June; W2: July-October; and W3: November-February). Factor O consisted of oxygen supply (2 levels, with or without aeration) and light intensity (with or without additional light). The experiment was designated as follows: O1 (an addition of light intensity of 640 lux without oxygen supply); O2 (an addition of oxygen supply of 1.5 m/second, equal to 6.5 ppm and without additional light); O3 (an addition of oxygen supply of 1.5 m/second and a light intensity of 640 lux) and O4 (without supply aeration and light (control)). A TL lamp was used as the source of illumination in this study because it emits light similar to the sunlight [1].

The shrimp and the seaweed were weighted once every 12 days for 92 days. The growth parameters measured were the absolute growth, specific growth, survival rate and production. The data were analyzed statistically using Analysis of Variance and Duncan’s Multiple Range Test (DMRT) [2].

3. Results and Discussions
3.1 Results
3.1.1 Growth, survival rate and production of pacific white leg shrimp (L. vannamei)
The average absolute weight growth of shrimp (L. vannamei) ranged from 8.93 ± 0.0 g (the lowest, treatment W3O4) to 13.13 ± 0.1 g (the highest, treatment W1O3). The specific growth rate (%/day) ranged from 4.68 ± 0.00 (treatment W3O4) to 5.09 ± 0.00 (treatment W1O3). The survival rate (%) ranged from 40.36 (W3O4) to 99.64 (W1O3). The production (g) ranged from 376.87 ± 0.34 (W3O4) to 1,005.08 ± 2.47 (table 1).
Table 1. Average absolute growth, specific growth rate, survival rate and production of pacific white shrimp (*L. vannamei*) measured in this study.

| Treatment | Average absolute growth (g) | Specific growth rate (%/day) | Average survival rate (%) | Average production (g) |
|-----------|-----------------------------|-----------------------------|---------------------------|------------------------|
| W1O1      | 11.42±0.020                 | 4.94±0.002                  | 80.00±0.290               | 865.85±0.760           |
| W1O2      | 12.71±0.040                 | 5.05±0.003                  | 93.22±0.120               | 964.71±0.220           |
| W1O3      | 13.23±0.000                 | 5.09±0.000                  | 99.64±0.180               | 1005.08±2.470          |
| W1O4      | 9.95±0.0100                 | 4.79±0.001                  | 50.36±0.180               | 467.30±0.380           |
| W2O1      | 11.28±0.000                 | 4.92±0.000                  | 85.00±0.180               | 851.53±0.680           |
| W2O2      | 12.63±0.050                 | 5.04±0.004                  | 73.22±0.180               | 944.37±1.370           |
| W2O3      | 13.13±0.000                 | 5.08±0.000                  | 93.93±0.180               | 972.46±0.080           |
| W2O4      | 9.85±0.0100                 | 4.78±0.001                  | 45.00±0.200               | 394.98±2.610           |
| W3O1      | 10.75±0.000                 | 4.90±0.013                  | 67.50±0.180               | 509.90±0.620           |
| W3O2      | 11.83±0.010                 | 4.97±0.001                  | 81.25±0.630               | 592.97±0.320           |
| W3O3      | 12.57±0.000                 | 5.04±0.000                  | 90.36±0.340               | 646.80±0.220           |
| W3O4      | 8.93±0.000                  | 4.68±0.000                  | 40.36±0.030               | 376.87±0.340           |

3.1.2. Effect of season-oxygen supply interaction on the growth of Pacific whiteleg shrimp (*L. vannamei*)

The results of the Analysis of Variance showed that there was no effect of season and oxygen supply on the absolute growth and specific growth rate of the experimental shrimp in this study, but there was an effect only on the survival rate (figure 1).

![Figure 1](image-url)

3.1.3. Absolute growth, specific growth rate and production of *G. verrucosa*

The average absolute growth of seaweed ranged from 2,496.5 ± 0.32 g/m$^3$ (the lowest, treatment W3O4) to 5,223.75 ± 0.31 g/m$^3$ (the highest, treatment W1O3). The specific growth rate of seaweed obtained in this study ranged from 1.750 ± 0.00 %/day (treatment W3O4) to 2.68 ± 0.00 %/day (treatment W1O3). The average production of seaweed ranged from 3,916.39 ± 0.64 g (W3O4) to 10,086.85 ± 0.79 g (treatment W1O3) (table 2).
### Table 2. Average absolute growth, specific growth rate, and production of sea weed *G. verrucosa* in present study.

| Treatment | Average absolute growth (g) | Specific growth rate (%/day) | Production (g) |
|-----------|-----------------------------|-----------------------------|----------------|
| W1O1      | 3024±0.200                  | 1.97±0.000                  | 4994.19±0.430  |
| W1O2      | 4895±0.540                  | 2.59±0.000                  | 9273.08±1.320  |
| W1O3      | 5223.75±0.310               | 2.68±0.000                  | 10086.85±0.790 |
| W1O4      | 2663.25±0.130               | 1.82±0.000                  | 4249.98±0.250  |
| W2O1      | 2823.25±0.430               | 1.89±0.000                  | 4576.35±0.880  |
| W2O2      | 4721.75±0.380               | 2.54±0.000                  | 8850.98±0.910  |
| W2O3      | 5064±7.170                  | 2.64±0.000                  | 9724.65±0.590  |
| W2O4      | 2518±2.190                  | 1.76±0.000                  | 3950.08±0.640  |
| W3O1      | 2638.25±3.880               | 1.82±0.000                  | 4215.66±0.480  |
| W3O2      | 4611±0.200                  | 2.51±0.000                  | 8583.67±0.490  |
| W3O3      | 4887.5±0.140                | 2.59±0.000                  | 9372.50±8.690  |
| W3O4      | 2496.5±0.320                | 1.75±0.000                  | 3916.39±0.640  |

#### 3.1.4. Effect of season-oxygen supply interaction on the specific growth rate of the seaweed *G. verrucosa*

The interaction between season and oxygen supply had a significant effect on the absolute growth of Pacific whiteleg shrimp (*L. vannamei*), but no effect on the specific growth rate of seaweed (*G. verrucosa*) (figure 2).

![Figure 2](image-url)
3.1.5. Water quality during the study
The water quality parameters measured were the dissolved oxygen (DO), temperature, salinity, pH, transpareancy, nitrate, nitrite, phosphat, CO₂ and total ammonia (table 3).

| Parameters          | W1O1 | W1O2 | W1O3 | W1O4 | W2O1 | W2O2 | W2O3 | W2O4 | W3O1 | W3O2 | W3O3 | W3O4 |
|---------------------|------|------|------|------|------|------|------|------|------|------|------|------|
| DO (ppm) (night)    | 5.7  | 6.6  | 6.5  | 3.3  | 5.7  | 6.7  | 6.7  | 3.3  | 5.7  | 6.4  | 6.7  | 2.3  |
| DO (ppm) Day        | 6.7  | 6.7  | 6.7  | 6.6  | 6.9  | 6.9  | 6.9  | 6.9  | 6.7  | 6.7  | 6.7  | 6.7  |
| pH                  | 7.5-8.2 | 7.3-8.1 | 7.5-8.2 | 7.2-7.8 | 7.3-7.2 | 7.2-7.2 | 7.1-7.7 | 7.2-7.1 | 7.2-7.7 | 7.2-7.7 | 7.2-7.7 | 7.2-7.7 |
| Temperature (°C)    | 27.5-31.1 | 27.1-31.2 | 27.5-31.2 | 27.4-31.2 | 28.7-31.2 | 27.7-31.2 | 28.7-31.2 | 27.2-31.2 | 27.2-31.2 | 26.8-31.2 | 27.2-31.2 | 25.1-30.1 |
| Salinity (ppt)      | 30   | 30   | 30   | 30   | 30   | 30   | 30   | 30   | 30   | 30   | 30   | 30   |
| NO₃ (ppm)           | tt   | 0.005 | tt   | 0.006 | tt   | 0.004 | tt   | 0.00   | tt   | 0.00   | tt   | 0.00   |
| Total NH₃ (ppm)     | 0.011-0.01 | 0.01-0.02 | 0.01-0.01 | 0.01-0.02 | 0.01-0.01 | 0.01-0.02 | 0.01-0.03 | 0.01-0.03 | 0.01-0.03 | 0.01-0.03 | 0.01-0.03 | 0.01-0.03 |
| Fosfat (ppm)        | 0.21-0.32 | 0.32-0.43 | 0.32-0.35 | 0.28-0.33 | 0.22-0.31 | 0.33-0.43 | 0.33-0.44 | 0.29-0.35 | 0.29-0.35 | 0.29-0.35 | 0.29-0.35 | 0.29-0.35 |
| CO₂ (ppm)           | 0.44-3.98 | tt   | tt   | 0.44-3.98 | tt   | 0.45 | tt   | 0.44-3.98 | tt   | 0.44-3.98 | tt   | 0.44-3.98 |
| Transpareancy (cm)  | 80   | 80   | 80   | 80   | 80   | 80   | 80   | 80   | 80   | 80   | 80   | 80   |
| Nitrite mg/l         | 0.025 | 0.023 | 0.015 | 0.084 | 0.03 | 0.02 | 0.02 | 0.09 | 0.0 | 0.02 | 0.0 | 0.098 |

3.2. Discussion
Overall, the results showed that stocking and rearing shrimp and seaweed in the post-wet season (March–June) was advantageous for the cultured organisms. Stocking and culturing shrimp and seaweed in the
period March-June with oxygen supply and light (W1O3) resulted in the highest absolute growth. This result was higher than that of the other seasons with and without oxygen supply and light. The lowest absolute growth was obtained in the period November-February without oxygen supply and light (W3O4). This may be related to water quality, especially the DO and CO₂ at night. The DO at nighttime in the treatment with the highest absolute growth was much higher (6.7 ppm), and the CO₂ was undetected. Quite the contrary, the treatment with the lowest absolute growth had low DO (2.3 ppm) and CO₂ of 0.42-3.98 ppm. Low concentration of DO at night usually causes a problem in shrimp farming [3]. Low DO in treatment W3O4 may have caused the shrimp to spend extra energy to compensate for the dissolved oxygen condition to meet physiological needs. For example, this may be done by pumping more water through the gills, lowering the oxygen partial pressure (pO₂), regulating the amount of gill surface used for respiration, which may affect the osmoregulation [4]. During the experiment, the salinity was maintained at 30 ppt, which was isosmotic for shrimp. Therefore, the energy spent for osmoregulation was minimal. This may contribute to better growth because at isosmotic condition, the Na⁺/K⁺-ATPase activity of shrimp is low, and the food utilization will be efficient [5]. It is possible for oxygen supply and additional light to increase water temperature, which increases the metabolic activities and moulting frequency and results in higher absolute growth of the shrimp [6, 7].

Water temperature is naturally the lowest in the period November-February, averaging 25.1–30.1 °C. This is lower than the optimal temperature for the survival rate (SR) of Pacific whiteleg shrimp, showing a parallel result with that of growth of shrimp. Previous study showed that the SR of shrimp is affected by stocking time and oxygen supply [7, 8, 9]. The mortality of shrimp in this study may be related to season, which in turn, will affect the water temperature and dissolved oxygen concentration that is less than optimal.

During wet season (November to February) water temperature ranges between 25.1 °C and 27.1 °C. On the other hand, high day temperature in the dry season can also cause high mortality shrimp production is population weight gain over certain period of time [10] determined by growth rate, mortality rate and biomass in a certain period of time [11]. One of the natural factors determining production is stocking season. The addition of oxygen supply and light in this study aimed to alleviate the low DO at night. Light is necessary for facilitating photosynthesis by seaweed for it to provide extra DO for shrimp. The gain in treatment W3O4 (wet season, no oxygen supply and no additional light) showed how all of the three factors could impact shrimp production negatively [12]. Seaweed growth is affected by environmental conditions such as light, temperature, pH and nutrient. Light intensity and the length of lighting period are important factors for the culture of algae, including seaweed. The results of this study showed that oxygen supply and additional light had no significant effect on seaweed growth despite longer period of photosynthesis (W1O3). However, the light intensity of 3.500 lux was optimum for the production of good absolute growth of seaweed. In addition, salinity of 30 ppt is suitable for seaweed as it is the salinity of the coast from which the seaweed used in this study originated. The lack of light for the seaweed cultured in November-February, and the lack of additional light resulted in low weight gain. Light intensity influences spore production and the growth of ectocarpus because tetraspora and karpospora production occurs only in the summer [13, 14].

4. Conclusion
This study showed that the most suitable period for the polyculture of the shrimp *L. vannamei* and the seaweed *G. verrucosa* is March to June. An additional light intensity of 640 lux and oxygen supply of 6.5 ppm can produce high growth rate, survival rate and production of shrimp and seaweed.
5. References

[1]. Trobos 2013 *The Magazine Agribisnis of Marine and Fisheries* **12** (1) 24-26

[2]. Srigandono B 1990 Experimental Design Faculty of Husbandry UNDIP Semarang 178 p

[3]. Zonneveld N, Huisman E A and J H Boon 1991 Principles of fish culture (Jakarta: Gramedia Pustaka Utama) 54 p (In Indonesia)

[4]. Fujaya Y 2004 Basic of Fish Fisiology for Developing Fisheries (Jakarta: Technology Rineka Cipta) 179 p (In Indonesia)

[5]. Anggoro S 2000 Osmotic regulatory patterns and enzyme work Na-K-ATPase tiger shrimp (*Penaeus monodon*) of various molting phases *J. Aquac.Ind.* **1** (2) : 15-20. (In Indonesia)

[6]. Vinatea L W, Muedas, R Arantes 2011 The Impact of Oxygen Consumption by Shrimp *Litopenaeus vannamei* According to Body Weight.temperature, Salinity and Stocking Density on Pond Aeration *a Simulation Maringi* **33**(2) 125-132

[7]. Spanopoulos-Hernandez M, C A Martinez-Palacios, R C Vanegas-Perez, C Rosas and L G Ross 2005 The Combined Effects of Salinity and Temperature on The Oxygen Consumption of Juvenile Shrimps Litopenaeus stylirostris (Stimpson 1874) *J. Aquac* **244**:341-348

[8]. Anongponyoskun M A Choksuchart, J Salaeno and P Aranyakananda 2012 *J. Nat. Sci.* **46**(5) : 751-758

[9]. Ruiz-Velazco J M J, A Hernandez-Llamas .and V M Gomez-Munoz 2010 *J. Aquac. Engineering* **43**: 114-119

[10]. Edmonson W T and G G Winberg 1971 *A manual and methods for assesment of secondary productivity in freshwater* (London : IBP Blackwell Sc l Pub) 358 p

[11]. Taqwa F H 2008 The effect of potassium addition on adaptation time of salinity decrease and time of natural feed replacement by artificial feed to performance pasca larva vannamei Shrimp (*Litopenaeus vannamei*) (Bogor : Bogor Agricultural Institute) 83 p (In Indonesia)

[12]. Salisbury F B dan C W Ross 1995 *Plant physiology* Volume 2 (Bandung : Institute of Technology Bandung) 173 p (In Indonesia)

[13]. Masyahoro and Mappiratu 2010 Respons from a Variety of Different Dept Againts Seeds of Seaweeds and Harvest Time *Eucheuma cottonii*) in Palu Bay. *The Magazine of Litbang Sulteng* **3**(2) 104-111. (In Indonesia)

[14]. Alamsjah M A, N O Ayuningtias and S Subekti 2010 *J. Fish. Mar. Sci.* **2** 21-30