JURNAL PERIKANAN
UNIVERSITAS GADJAH MADA
Terakreditasi Ristekdikti No: 158/E/KPT/2021

ISSN: 2502-5066 (Online)
ISSN: 0853-6384 (Print)
Vol. 23 (2), 197-206
DOI 10.22146/jfs.80976

Effects Density and Salinity of Artificial Brackish Water on the Growth and Physiological Perfomance of Whiteleg Shrimp (*Litopenaeus vannamei* Boone, 1931)

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Submitted: 05 January 2023; **Revised:** 15 September 2023; **Accepted :** 03 November 2023

ABSTRACT The increase in shrimp culture causes problems with the quality of seawater which is a source of water for whiteleg shrimp culture it self. The use of artificial brackish water as a culture medium is an effort to increase the availability of water sources for whiteleg shrimp culture. This study aims to (1) determine the optimum stocking density of whiteleg shrimp culture using artificial brackish water salinity based on carrying capacity, (2) determine mineral content (Mg, Ca, Na and K) in artificial brackish water, (3) study the effect of salinity on the growth and blood glucose concentration of whiteleg shrimp cultivated in artificial brackish water, (4) examine the effect of salinity on hemolymph osmolarity of whiteleg shrimp cultivated in artificial brackish water. This study used an experimental method consisting of two stages. Each stage was arranged using a completely randomized design (CRD). Obtained stocking densities of SD-50% (20 shrimps/50L), SD (40 shrimps/50L) and SD+50% (60 shrimps/50L). The best growth and performance were obtained in the treatment of 20 individuals, namely survival (SR) 87%, specific growth rate (SGR) 3.26%/day and hemolymph glucose concentration of 30.73 mg/dl. In the next stage of the research, the salinity treatments tested were seawater, 10 ppt, 15 ppt, 20 ppt and 25 ppt. The best shrimp performance was obtained in treatment 20 shrimps/50 L, namely survival rate 70.00%, specific growth rate (SGR) 1.55%/day, feed conversion ratio (FCR) 1.72, glucose haemolymp 73.44 mg/dl. The optimal osmotic work level (OWL) value is obtained at a salinity of 15-20 ppt.

Keywords: Artificial brackish-water; osmoregulation; oxygen consumption; response stress; whiteleg shrimp

INTRODUCTION

White leg shrimp (*Litopenaeus vannamei*) has several advantages, namely, they can be reared with a wide range of salinity (0.5-45 ppt), and can live in the water column, so the stocking density is higher than tiger prawns (*Penaeus monodon*). The presence of whiteleg shrimp in Indonesia can replace the existence of tiger shrimp (*P. monodon*) which has decreased in production because it is more susceptible to disease (Fadillah *et al*., 2019).

During the January-April 2019 period, white leg shrimp production was 33,500 tons or USD 258.5 million, lower than in 2018 in the same period, namely 36,200 tons or USD 337.5 million (BPS, 2019). These results decreased by 700 tons and in terms of value decreased by 90 million USD. Based on these data, it is necessary to intensify culture to continue to increase the production of whiteleg shrimp farming.

Whiteleg shrimp culture in Indonesia is mostly carried out in ponds, whereas in areas far from seawater sources it is still rarely practiced. Shrimp culture in the interior can be done by moving seawater from the coast to the inland areas (Roy *et al*., 2010). However, removing seawater on a large scale can increase production costs. To deal with this problem, it is necessary to engineer artificial brackish water as a culture medium, so that it is no longer dependent on seawater (Bull *et al*., 2020). Efforts to engineer artificial brackish water for shrimp farming in the inland areas can be carried out by adding unrefined salt. Unrefined salt is the result of crystallization from the drying process, so it has a constituent component that resembles seawater.

The use of artificial brackish water is an innovation in whiteleg shrimp culture, so it is necessary to study several aspects that affect growth and physiological performance. The main aspect that needs to be tested is the oxygen content. It is feared that the salinity of artificial brackish water can affect oxygen solubility. Even though the oxygen content in water is the basis for calculating the ability of the water environment to support the shrimp being reared or carrying capacity (Losordo & Westersmo, 1994). However, the determination of stocking density for whiteleg shrimp culture using artificial brackish water has never been done so far. So it is necessary to study the determination of the best stocking density of whiteleg shrimp cultivated using artificial brackish water based on the container's carrying capacity which is calculated using the level of oxygen consumption of whiteleg shrimp.

Another factor affecting growth is the salinity of the water. Anggoro & Nakamura (2005) said whiteleg shrimp had a direct effect on salt levels which worked through an osmotic effect on osmoregulation and absorption of food. The higher the salinity, the greater the osmotic pressure the higher (Mc Connaughey & Zottoli, 1983). This opinion is based on the results of research on seawater, while research on osmoregulation and stress response of whiteleg shrimp cultivated using artificial brackish water has never been done.

Based on the description above, it is necessary to study the effect of salinity and mineral content of artificial brackish water on the growth and physiological performance of whiteleg shrimp.

MATERIALS AND METHODS

Shrimp

The research was carried out using an experimental method, which consisted of two phases. The first research determined the best stocking density (SD), which was then used in the second research to determine the effect of artificial brackish water salinity on the growth and physiology of whiteleg shrimp. The first experiment consisted of 3 treatments, namely: best stocking density (SD), SD-50%SD and SD+50%SD in five replicates. Shrimp stocking density is determined based on carrying capacity calculations. Shrimps (1.77 g) were obtained from a previous research and cultured in container with water volume of 50 l. The second research consisted of five salinity treatments: 10, 15, 20, 25 ppt and sea water salinity (35 ppt) as control in three replicates each. Shrimps were cultured for 60 days in tanks aerated using a blower. The feeds were given 5% of biomass/day (five times a day).

Stocking density

Initial research was treatment of stocking densitiy. To determine stocking density was carried out with oxygen consumption of shrimp. The calculation is continued to determine carrying capacity. Carrying capacity was calculated on Elliotts (1969).

$$CC = \frac{(DO_n - DO_{Min}) \times V}{OC}$$

CC = Carrying capacity (kg)
DO_n = Average available dissolved oxygen content (mg/L)
DO_{Min} = Minimum dissolved oxygen content for shrimp culture (mg/L)
V = Volume of water (L)
OC = Oxygen consumption (mgO_2/g/hour)

Treatment of stocking density were designed with SD calculation of carrying capacity, SD-50% was population of carrying capacity with diminished half of populations and SD+50% was carrying capacity with add half of populations. The calculate of SD:40 shrimps/50L, SD-50%: 20 shrimps/50L, SD+50%: 60 shrimps/50L.

Salinity

After the shrimp were stocked, salinity measurements were taken to determine the initial salinity before adjusting the salinity to the treatment. Artificial brackish water as a medium for cultivating whiteleg shrimp was made by dissolving refined salt in fresh water from a well: for every 1 ppt water salinity requires 1.26 g of refined salt salt in 1 liter of fresh water. The process of adding and decreasing the salinity level in the treatment was carried out in stages, namely by

$$S2 = \frac{a \times S1}{n + a}$$

increasing/decreasing the salinity level by 2 ppt/day. This was done to avoid stress levels that can cause shrimp death. Making salinity based on the equation from Anggoro (1992).

Observation parameters

Oxygen consumption

Calculation of oxygen consumption using a respirometer. Shrimp samples were taken randomly from 20 different size groups.

Oxygen consumption was calculated on Liao & Huang's (1975):

$$OC = \frac{V \times (DO_{to} - Do_{tn})}{W \times T}$$

OC = Oxygen consumption ($mg O_2$/g/hour)
V = Volume of water in the container (L)
DO_{to} = Concentration of dissolved oxygen at the initial observation (mg/L)
DO_{tn} = Concentration of dissolved oxygen at the end observation (mg/L)
W = Weight of test animal (g)
T = Observation period (hours)

Hemolymph glucose level

Hemolymph glucose was measured using the Gluco Test (Gluco Dr). Hemolymph in the microtube was taken with capillary and dropped on glucose strip. The result of glucose concentration was read digitally.

Osmoregulations

Osmoregulation was measured using the osmolarity of hemolymp and osmolarity of water. Measurements of hemolymph osmolarity and medium osmolarity were carried out on the 7th day of treatment and the 21st day of the second phase of the research.

The technique of measuring the osmotic of the medium and the hemolymph of shrimp followed the method that used by Anggoro *et al*. (2018) with the help of the Automatic Micro-osmometer Roebling.

Osmotic working level mOsm/L H_2O = osmolarity of water – osmolarity of hemolymph

Shrimp Growth

Measurement of weight and length were carried out at the initial, middle and end of research by taking 30% shrimp samples in each treatment container. The number of live shrimps was counted at the end of research to calculate of survival rate.

SGR = 100 (LnW2 – LnW1) / t
FCR = total feed (kg) / increase in shrimp biomass (kg)
SR = (Nt / No) x 100%

Statistical analysis

All data were analyzed by ANOVA (Analysis of Variance) at the significance level of 0.05. A Duncan's Multple Range Test (DMRT) was used to examine significant differences among treatments using SPSS IBM 20 software. A response between treatment and result curve was used of orthogonal polynomials which are obtained from Microsoft Excel 2019. Before the analysis, all data were analyzed on the normality and homogeneity test.

RESULTS AND DISCUSSION

Research phase 1

Dissolved oxygen and oxygen consumption

The results of observations of dissolved oxygen content

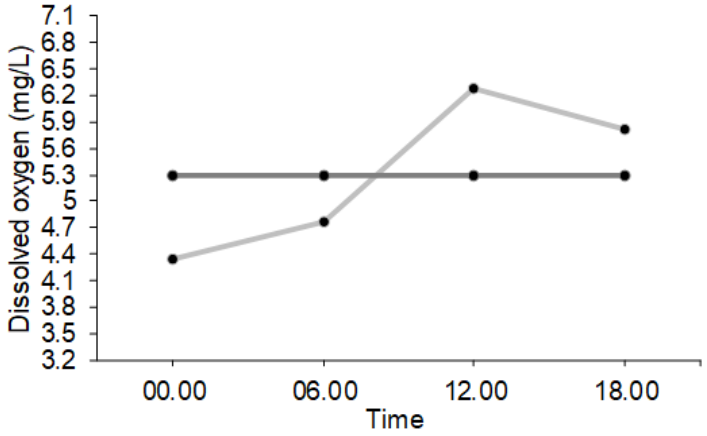

Figure 1. Graph of dissolve oxygen content and its average availbility for 24 hours after aeration turned on.

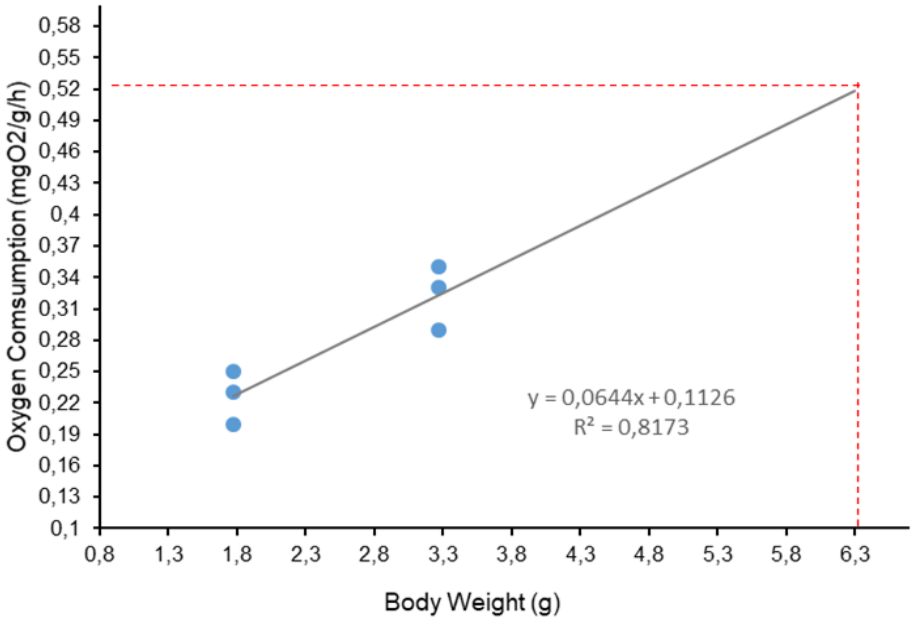

Figure 2. The relationship between average shrimp body weight and oxygen consumption.

when aeration is turned on for 24 hours are shown in Figure 1. Observations were made for 24 hours with an interval of 6 hours. The results of observations at 00.00 – 18.00 WIB were 4.34, 4.77, 6.28, and 5.82 mg/L. The measurement results indicate that the average dissolved oxygen (O_2) available in the treatment medium is 5.3 mg/L.

Shrimp weighing 5.8 g/shrimp is the target harvest for research phase 1. The reference for determining the target weight for shrimp is 5.8 g based on Widodo *et al*. (2011). Setting a target of 5.8 g to prove the difference in oxygen consumption of shrimp in seawater and artificial brackish water. The results of the measurement of oxygen consumption are presented in Figure 2. These data show an increase in oxygen consumption with increasing shrimp body weight. The estimated oxygen consumption of 5.8 g shrimp was obtained at 0.49 mgO_2/kg/h. This estimate becomes a reference for calculating the carrying capacity and stocking density.

Carrying capacity (CC) and stocking density (SD)

Calculation of carrying capacity (CC) is carried out to determine the maximum limit of the number of shrimps that can be accommodated in the treatment tub based on the availability of dissolved oxygen available. Oxygen consumption (OC) used in CC calculations is presented on Table 1.

Table 1. Carrying capacity (CC) calculation.

Carrying capacity (CC)
$CC = \frac{(DO_o - Do_{tn}) \times V}{OC}$
$CC = \frac{(5{,}3 - 3) \times 50}{0{,}49}$
$CC = \frac{23{,}5 \times 50}{0{,}49}$
$CC = \frac{115}{0{,}49}$
CC = 234.69 g

The CC calculation results in Table 1 are 234.69 g. Calculation of stocking density based on the equation below:

Stocking density = CC / shrimp target size
= 234.69 g / 5.8 g
= 40.46 shrimps or 40 shrimps (rounding)

Shrimp performance

The survival rate of white leg shrimp after 28 days of treatment is shown in Table 2. The data shows the highest sur-

Table 2. Survival rate, specific growth rate and glucose hemolymph of white leg shrimp with different densities.

Density (shrimps/50L)	Survival rate (%)	Spesific growth rate (%/day)	Glucose hemolymp (mg/dl)
20	87%±8.36[a]	3.26±0.76[a]	30.73±5.28[c]
40	63.5%±15.16[b]	3.23±0.32[a]	55.67±19.53[b]
60	52.32%±13.41[b]	3.18±0.63[a]	82.73±15.81[a]

Notes: average value followed by different letter superscript indicate significant different.

vival rate of white leg shrimp in the 20 shrimps/50L treatment, which is 87%±8.36[a]. Survival rate in 60 shrimps/50L treatment showed the lowest result at 52.32%±13.41[b], followed by 40 shrimps/50L treatment at 63.5%±15.16[b]. Statistical results for survival rate data show that there is a significant effect (P<0.05) between stocking density in the survival rate and glucose haemolymp of white leg shrimp. DMRT showed that the results between the 60 shrimps/50L and 40 shrimps/50L treatments did not show a significantly different effect, but the 20 shrimps/50L treatment gave a significantly different effect. The best treatment was shown in the 20 shrimps/50L treatment with an average of 87%±8.36[a].

The average specific growth rate (SGR) of whiteleg shrimp ranges from 3.18-3.26%/day (Table 2). The highest SGR was shown in the treatment with a stocking density of 20 shrimps/50L with an average SGR of 3.26%/day. The lowest SGR was shown in the treatment with a stocking density of 60 shrimps/50L with average 3.18%/day. Based on the results of the ANOVA test, it was shown that each treatment had no significantly different (P>0.05) effect on the SGR of whiteleg shrimp.

Observation of hemolymph in the first stage of the experiment proved that stocking density influenced the stress level of white leg shrimp. The lowest concentration is obtained in the 20 shrimps/50L treatment, which is 30.67± 5.28c mg/dl, increases with the treatment of the number of spreads. The highest concentration of hemolymph was found in the 60 shrimps/50L treatment (Table 2). The results of statistical tests for hemolymph glucose data showed a significant effect (P > 0.05) between stocking density and hemolymph glucose concentration of white leg shrimp. Based on DRM, glucose hemolymp results show the results that the three treatments have a significantly different effect. The best treatment is shown in the 20 shrimps/50L treatment with an average of 30.73 mg/dl±5.28.

Water quality

The results of dissolved oxygen observations in 20 shrimps/50L treatment are known to be dissolved initially at 5.01 mg/l decreases to 3.68 mg/l (Table 3). Based on the results of the measurement of dissolved oxygen, it was decided that the treatment of 20 shrimps/50L became the stocking density used in the next trial. Selection of the stocking density of 20 shrimps/50L to be used in the next trial, namely paying attention to the availability of oxygen for the treatment process in the 2nd trial. Water quality measured during the experiment was suitable for the growth of white shrimp as suggested by Boyd & Tucker (1992).

Research Phase 2

Shrimp performance

The survival rate of whiteleg shrimp after 30 days of treatment is shown in Table 4. Table 4 shows that the highest survival rate was in the seawater treatment, namely 86.67% ± 2.89[a], and the lowest survival rate was in 10 ppt salinity of 58.33% ± 7.63[b]. The results of ANOVA is significant effect on whiteleg shrimp survival (P<0.05). However, DMRT results showed that seawater and 20 ppt did not show a significant difference. The results were not significantly different among 10, 15, and 25 ppt treatments. The best treatment was shown in seawater with an average of 86.67% ± 2.89[a] followed by 20 ppt treatment of 70.00% ± 5.00[ab].

Specific Growth Rate (SGR) (Table 4) shows that the highest value is in seawater, which is 2.06 ± 00a %/day, while in the artificial brackish water salinity treatment it is aimed at a salinity of 20 ppt with an average 1.55 ± 001b %/day. The lowest SGR value was obtained at 10 ppt salinity, namely 1.31 ± 001d %/day, that the highest value is in seawater, which is 2.06±00a %/day.

The FCR value in each treatment can be said to be high, this is caused by slow growth and high mortality rates in whiteleg

Table 3. The quality of water in different stocking density treatment.

Test Parameters	Density (shrimps/50L)								
	1st day			15th day			30th day		
	20	40	60	20	40	60	20	40	60
Temperature (°C)	25.9	25.9	26.1	28.8	28.2	28.1	29.8	29.7	29.7
Salinity (ppt)	20	20	20	17	18	18	16	18	18
pH	7.2	7.1	7.1	6.9	6.3	6.7	6.2	6.1	6.5
O_2 dissolved (mg/L)	5.01	4.89	4.81	4.32	4.12	3.87	3.68	3.29	3.12
Alkalinity (mg/L)	63.9	70.3	70.3	-	-	-	149.9	124.9	187.3
Nitrate (mg/L)	113.88	111.1	112.57	-	-	-	163.82	158.52	174.7
CO_2 (mg/L)	97.5	84.2	97.5	-	-	-	47.9	47.9	39
Ammonia (mg/L)	0.138	0.141	1.143	-	-	-	0.101	0.161	0.216

Table 4. Whiteleg shrimp performance with different salinity.

Salinity (ppt)	Survival rate (%)	Spesific growth Rate (%/day)	Feed convertion ratio
35	86.67±2.89^{a}	2,06±000^{a}	1.32±0.12^{c}
10	58.33±7.63^{b}	1,31±001^{d}	2.01±0.17^{a}
15	66.67±17.56^{b}	1,49±004^{bc}	1.95±0.27^{ab}
20	70.00±5.00a^{b}	1,55±001^{b}	1.72±0.15^{b}
25	61.6±7.63^{b}	1,43±002^{c}	2.00±0.21^{ab}

shrimp. The highest FCR value was found in the 10 ppt treatment, namely 2.01 ± 0.17^{a} and the lowest in seawater, namely 1.32 ± 0.12^{c}. meaning that the best FCR was in seawate r (35 ppt)

White leg shrimp glucose concentration

The whiteleg shrimp hemolymph glucose concentration is shown in Figure 3. The hemolymph glucose level after 30 day of treatment has increased and after a stress test using 5 ppt salinity for 3 hours. Hemolymph glucose levels on day 1 of treatment were within the range of 37.78 ± 2.16^{c} - 65.78 ± 3.65^{a} mg/dl. Day 15 of treatment, hemolymph glucose was in the range of 54.22 ± 7.68^{b} - 81.89 ± 4.14^{a} mg/dl. Hemolymph glucose levels increased after the stress test, which ranged from 100.67 ± 1.2^{a} - 105.88 ± 2.5^{a} mg/dl.Hemolymph glucose values in all treatments increased after the stress test.

The results of the ANOVA test showed no significant effect (P>0.05) of salt water salinity treatment on hemolymph glucose concentrations on day 1, but DMRT tests showed significantly different effects on seawater, 10 and 25 ppt. On the 15th day of treatment, seawater was significantly different (P<0.05) at 10 to 25 ppt salinity, but at 10 to 25 ppt salinity there was no significant difference in whiteleg shrimp hemolymph glucose concentrations. The stress test using 5 ppt salinity on whiteleg shrimp showed an increase in hemolymph concentration and there was no significant difference in each treatment.

White leg shrimp osmolarity

Observations of hemolymph and water osmolarity on day 1, day 15 and stress test are presented in Figure 4. hemolymph osmolarity measurements on day 1 were 478±1.73^{d} - 549±1.00^{a} (mOsm/l H_2O) and water osmolarity ranged from 293±1.00^{e} - 1026±0.00^{a} (mOsm/l H_2O). Based on the results of these measurements, there was a significant difference in the osmolarity value of hemolymph on day 1 at 10.15 and 25 ppt salinity (P<0.05), but in seawater and 20 ppt there was no significant difference. The osmolarity of the water in each artificial brackish water salinity variable on day 1 was significantly different (P<0.05).

After the 15th day of treatment, the osmolarity value of whiteleg shrimp hemolymph reared at various artificial brackish water salinity levels ranged from 483 ± 1.00^{d} - 550 ± 0.00^{a} (mOsm/l H_2O) and the water osmolarity ranged from 294±1.00^{e} – 1026±0.00^{a} (mOsm/l H_2O). The results of statistical analysis showed that the osmolarity value of whiteleg shrimp hemolymph in seawater and 15 ppt water salinity did not show a significant difference (P>0.05), while the water osmolarity showed significantly different results (P<0.05) in each artificial brackish water salinity levels. On the 30th day of treatment a stress test was performed using a salinity of 5 ppt. The results of the hemolymph osmolarity of white leg shrimp showed that treatments seawater, 10, and 25 ppt did not show a significantly different effect (P>0.05), but the treatments with 20 and 15 ppt had a significantly different effect (P<0.05). The osmolarity value of the artificial brackish water with 5 ppt salinity showed that the results were not significantly different from both the control and treatment (P>0.05).

On the 30th day of treatment, a stress test was carried out using 5 ppt of salinity. The results of the whiteleg shrimp hemolymph osmolarity values during the stress test used a 5 ppt salinity, which ranged from 455 ± 0.00^{c} - 464 ± 1.00^{a} (mOsm/l H_2O). The measurement results showed that the 15 and 20 ppt treatments were significantly different effect (P<0.05) and the other treatments were not significantly different (P>0.05). The results of the water osmolarity measurements were 146 ± 0.00^{c} - 147 ± 0.00^{c}

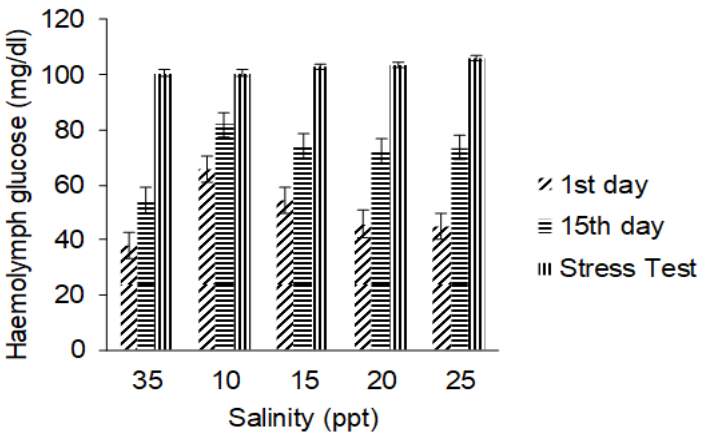

Figure 3. The concentration of hemolymph glucose in white leg shrimp in different unrefined salt salinity treatment and after stress test with 5 ppt salinity for 3 hours.

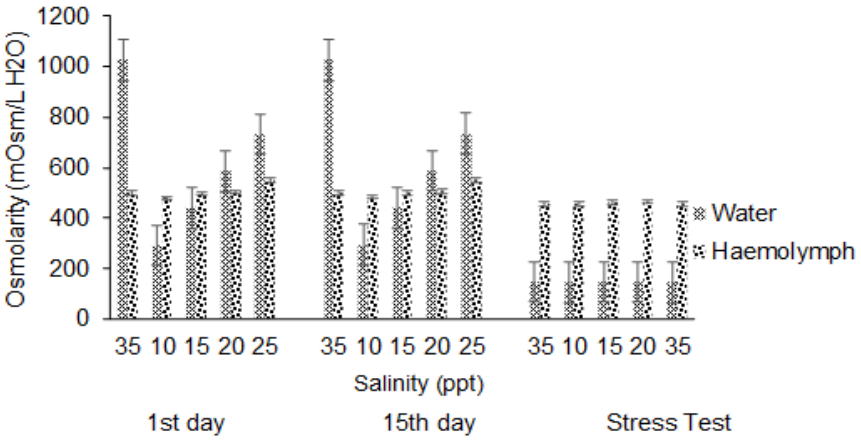

Figure 4. Osmotic pressure on whiteleg shrimp in salinity of artificial brackish water.

(mOsm/l H_2O) and showed no significantly different effect (P>0.05) in each treatment. The results of measuring the osmolarity of the water during the stress test did not show a significant difference due to the same salinity value used.

The best artificial brackish water salinity was determined based on the difference between the hemolymph osmolarity and the water osmolarity. This difference is the osmotic load or osmotic work level (OWL) of the shrimp in the process of osmoregulation. The OWL of whiteleg shrimp in each treatment, namely sea water (525 mOsm/l H2O) equivalent to hypo-osmotic, 10 ppt (189 mOsm/l H_2O) equivalent to hyper-osmotic, 15 ppt (59 mOsm/l H_2O)) iso equivalent to hyper-osmotic, 20 ppt (81 mOsm/l H_2O) iso equivalent to hypo-osmotic, and 25 ppt (184 mOsm/l H_2O) iso equivalent to hypo-osmotic. The osmotic work level of whiteleg shrimp during the stress test was carried out using a 5 ppt salinity, namely 309 – 317 mOsm/l H_2O and was hyper-osmotic. The results of OWL measurements for each salinity treatment showed that the best salinity for whiteleg shrimp farming using artificial brackish water was 15-20 ppt close to iso-osmotic.

Mineral content

The measurement results show that the Ca and Mg content in the artificial brackish water is lower than that in seawater. The function of the Ca content is for the formation of whiteleg shrimp carapace, while Mg functions as a growth modulator in whiteleg shrimp.

Water quality

Observation of water quality parameters during the 2nd study had a good level of water quality with adequate water quality eligibility categories (Table 6). The results of research that have been carried out on the parameters of Temperature (°C), salinity (ppt), pH, dissolved oxygen (O_2), alkalinity (mg/L), nitrate (mg/L), CO_2 (mg/L), and ammonia (mg/L). Table 6 shows the average temperature value of 28.5 °C, pH 6.80, salinity 10-35 ppt, dissolved O_2 4.86 mg/L, alkalinity 63.4 mg/L, nitrate 71.4 mg/L, CO_2 18.6 mg/L and ammonia 2.24 (mg/L). During the observation,

Table 5. Mineral content of Ca, Mg, Na, K in each treatment.

Parameter	Salinity (ppt)				
	35	10	15	20	25
Ca (mg/L)	408	172	156	176	212
Mg (mg/L)	1273.32	138.51	187.11	230.85	272.16
Na (mg/L)	4054	4365	4990	6549	7017
K (mg/L)	69	71	106	122	186

Table 6. The quality of water in different salinity treatment.

Test Parameter	Treatment									
	1st day					30th day				
Temperature (°C)	27.1	27.5	27.2	27.3	27.2	27.8	28.2	28.4	27.9	28.5
Salinity (ppt)	35	10	15	20	25	35	10	15	20	25
pH	7.4	7.5	7.4	7.6	7.4	7.1	6.9	7.2	7.1	6.8
Dissolved Oxygen (mg/L)	3.98	4.06	3,92	4.11	3.94	3.01	3.21	3.19	3.10	3.18
Alkalinity (mg/L)	76,6	57,5	70,3	89,4	89,4	63,9	51,1	44,7	51,1	63,9
Nitrate (mg/L)	51,2	61,6	43,5	39,4	38,1	70,1	116,4	101,7	105,8	125,5
CO_2 (mg/L)	12,4	13,3	7,1	8,9	8,9	17,7	17,7	21,2	30,1	44,3
Ammonia (mg/L)	0.005	0.001	0.001	0.001	0.002	0.100	0.011	0.017	0.021	0.020

the water quality parameter values were still within the range suggested by Boyd & Tucker (1992).

The use of unrefined salt is a new innovation in shrimp culture so it needs to be studied in several aspects that affect the survival of shrimp. The main aspect studied is the oxygen content in the water which is the basis for calculating the Carrying Capacity (CC). Observations showed that the average dissolved O_2 content in the water culture was 5.3 mg/L (Figure 1). The result of shrimp oxygen consumption is 0.49 mg02/kg/h and this value is stated to be sufficient to maintain shrimp with a target harvest weight of 5.8 g/shrimp. The data also shows that the increase in oxygen consumption is proportional to the increase in shrimp weight (Figure 2). This statement is supported by Budiardi *et al*. (2005) that the level of oxygen consumption of white leg shrimp depends on the size of the shrimp. Ambeng *et al*. (2006) also said that the low dissolved O_2 content will affect the level of oxygen consumption and can reduce production, this is because shrimp spend more energy to survive than to grow.

The calculation results show a positive correlation between the availability of dissolved O_2 and Carrying capacity (CC). The CC result was 234.69 g, then this value was divided by the weight of the target size, which was 5.8 g/shrimp. The calculation results showed that the optimal stocking density during the study was 40 shrimp. In this study, the increase in carrying capacity can be proven in the performance conditions of the survival rate of shrimp and the stress response of shrimp. One of the studies by Stigebrandt (2011), stated that the optimal stocking density can be determined by measuring the carrying capacity. The measured carrying capacity reviews the conditions that are acceptable in the water quality standard and the impact on the environment. Ariadi *et al*. (2022) stated that the carrying capacity will affect the performance of shrimp to be able to grow well.

The performance of the shrimp tested in Phase 1 is the survival rate (Table 2). From the calculation results, it is known that the best treatment is shown in the 20 shrimps/50L treatment, namely 87%±8.36^a. The results of statistical calculations also showed that there was a significant effect (P>0.05) between stocking density and white leg shrimp survival. So it is known that the higher the stocking density, the lower the shrimp survival rate value. This is because the increase in density affects the space for movement, food needs, and environmental conditions. This statement is supported by Lama *et al*. (2020) the lower the stocking density, the higher the chance of shrimp survival due to the wide range of motion so there is no space competition. Another study conducted by Novriadi *et al*. (2020) also stated that the optimum density will provide better bearing capacity.

The specific growth rate (SGR) (%/day) of whiteleg shrimp at a stocking density of 60 shrimps/50L was lower than that of the treatments with a stocking density of 20 and 40 shrimps/50L (Table 2). Based on the ANOVA test, it was shown that stocking density did not have a significantly different effect on growth. A higher stocking density causes an increase in the intensity of treatment which has an impact on increasing shrimp competition in getting space, feed or oxygen which causes uneven growth and high mortality rates (Rakhfid *et al*., 2017).

The stress response in this study was seen from changes in glucose levels (Table 2). The lowest concentration was found in the 20 shrimps/50L treatment, which was 30.67± 5.28^c mg/dl, increasing with the number of stocking treatments. From the test results, it is known that increasing stocking density will increase glucose levels in the hemolymph which indicates the condition that the shrimp is under stress. Stress is a survival response in shrimp to stressors. Glucose levels will increase when stressed due to homeostatic processes or automatic mechanisms for self-defense (Septiningsih *et al*., 2015). This statement is in accordance with the research of Arifin *et al*. (2014) which states that the energy needs to improve homeostasis during stress are met by the processes of glycogenolysis and gluconeogenesis that produce glucose. Another study by Yustiati *et al*. (2017) also stated that an increase in glucose concentration was related to the mobilization of energy storage under stress conditions as a fuel source for anaerobic metabolism resulting in the production and accumulation of lactate.

During the observation of water quality parameters observed temperature, salinity, pH, dissolved O_2, alkalinity, nitrate, CO_2, and ammonia (Table 3) were still within the range suggested by Boyd & Tucker (1992). The concentration of ammonia and nitrate obtained tends to increase with the increasing stocking density used. The need for dissolved O_2 increased in accordance with the increase in the mass of shrimp, thus making the availability of dissolved O_2 increasingly depleted in all treatments on the 30th day of treatment.

Based on the results of observations in the first phase of the study after 28 day of culture, it was shown that the remaining dissolved oxygen content in the 20 shrimps/50L treatment had a higher concentration than the other treatments, namely 3.68 mg/L. The survival rate in the 20 shrimps/50L treatment also showed the greatest value and was significantly different compared to the other treatments. There was no significant difference in the specific growth rate of each treatment, but the glucose concentration in the 20 shrimps/50L treatment was lower compared to the other treatments. Based on these results it was decided that a stocking density of 20 shrimps/50L was used for the next phase of the trial.

Next trials focused more on the use of unrefined salt as a substitute for seawater. Observations were made for 30 days with salinity concentrations of 10 ppt, 15 ppt, 20 ppt, and 25 ppt. Some of the parameters observed were spe-cific growth rate (SGR), survival rate (SR), feed conversion ratio (FCR), stress response as measured by glucose concentration, whiteleg shrimp osmolarity, and mineral content.

In this study, the minerals observed were calcium (Ca), magnesium (Mg), sodium (Na), and potassium (K) (Table 5). It was found that the highest Ca and Mg content in the seawater (control), which was 408 mg/L and 1273.32 mg/L respectively, while in artifisifial brackish water, the Ca and Mg value increased with increasing salinity treatment. This result is in accordance with the research of Yunus & Haris (2020), the main minerals composition in seawater salt mostly calcium (Ca) and magnesium (Mg). The

main minerals are needed by crustaceans for the continuity of basal metabolism and growth. Meanwhile, in artificial brackish water, the mineral content is lower than seawater because in making un-refined salt from sea water, many minerals are lost by evaporation Arwiyah *et al*. (2015). Because this reason white leg shrimp sea water is better than artificial brackish water.

The result of whiteleg shrimp performance is the highest SR can be seen from Table 4, which was obtained in the seawater treatment, namely 86.67% ± 2.89^{a} followed by salinity of 20 ppt is 70.00% ± 5.00^{ab}. The other treatments did not have much difference between the treatments. Statistical test results on survival rates also showed a significant difference between seawater and each treatment. The percentage value of the SGR in this study shows that there is an effect of each salinity treatment on whiteleg shrimp SGR. The results showed that the best growth value between the artificial brackish water at salinity 20 ppt it was 1.43%/day ± 001^{b}. However, when compared to seawater, the SGR value of seawater is the highest value, namely 2.06%/day ± 00a. Good SRI and FCR in shrimp during this study followed the previous parameters, namely in the treatment of seawater. Table 4 shows the FCR value also found in seawater, namely 1.32 ± 0.12^{c}. This FCR value was followed by the 20 ppt treatment, which was 1.72 ± 0.15^{b}. From the results of SGR, survival and FCR measurements it can be concluded that the best growth performance was found in seawater followed by 20 ppt salinity treatment.

In the previous discussion, it was stated that mineral content affects growth and survival. This statement is following the research of Yunus & Haris (2020) the main minerals in the constituents of seawater salt include calcium (Ca) and magnesium (Mg). The main minerals are needed by crustaceans including whiteleg shrimp for the continuation of basal metabolism and growth. In the study of Dwiono *et al*. (2018) the ratio between minerals in the water is considered the ideal ratio found in seawater. The ideal ratio of minerals can increase the survival and growth of shrimp. Other research also stated that the growth of whiteleg shrimp was influenced by two factors, namely the frequency of moulting and growth at each moult. Provides Ca and Mg which are needed by whiteleg shrimp in the formation of their shells (Atmomarsono *et al*., 2014).

The stress response in 2nd study was also reviewed from the concentration of glucose content in the shrimp's body. The results of observing glucose concentrations are presented in Figure 3. The results of the observations showed that the glucose concentration increased with increasing observation time and experienced another increase during the stress test using 5 ppt salinity for 3 hours. In addition, the results also show a relationship, increasing salinity decreases the value of glucose concentration. Hemolymph glucose levels on day 1 of treatment were within the range of 37.78 ± 2.16^{c} - 65.78 ± 3.65^{a} mg/dl. Day 15 of treatment of hemolymph glucose was in the range of 54.22 ± 7.68^{b} - 81.89 ± 4.14^{a} mg/dl. Hemolymph glucose levels increased after the stress test, which ranged from 100.67 ± 1.2^{a} - 105.88 ± 2.5^{a} mg/dl. Hemolymph glucose values in all treatments increased after the stress test. Glucose levels increase during stress due to homeostatic processes or automatic mechanisms for self-defence (Septiningsih *et al*., 2015).

The osmotic work level (OWL) is the difference between the osmotic pressure of the hemolymph and the osmolarity of the water used by the shrimp in the osmoregulation process. In this study, measurements were made for each treatment of artificial brackish water. The observation results are presented in Figure 4, which shows the osmolarity measurements of hemolymph on day 1, namely 478±1.73$_{d}$ - 549±1.00$_{a}$ (mOsm/l H_2O) and water osmolarity ranging from 293±1.00^{b} - 1026±0.00^{a} (mOsm/l H_2O). The results of measurements on the 15th day of the hemolymph osmolarity values of whiteleg shrimp reared at various levels of artificial brackish water salinity ranged from 483±1.00^{d} - 550±0.00^{a} (mOsm/l H_2O) and water osmolarity ranged from 441±1.00^{d} - 1026±0.00^{a} (mOsm/l H^2O). The results of the hemolymph osmolarity measurements during the stress test ranged from 455±0.00^{c} - 464±1.00^{a} (mOsm/l H_2O), while the water osmolarity was 146±0.00^{c} - 147 ± 0.00^{c} (mOsm/l H_2O).

The OWL of whiteleg shrimp in each salinity treatment, namely seawater (525 mOsm/l H_2O) equivalent to hypo-osmotic, 10 ppt (189 mOsm/l H_2O) equivalent to hyper-osmotic, 15 ppt (59 mOsm/l H_2O) iso-equivalent to hyper-osmotic iso, 20 ppt (81 mOsm/l H_2O) iso-equivalent to hypo-osmotic and 25 ppt (184 mOsm/l H_2O) iso-equivalent to hypo-osmotic. The OWL results during the stress test ranged from 309-317 mOsm/l H_2O which is hyper-osmotic. The pattern of hyper-osmotic osmoregulation is a condition where the shrimp has a higher body osmotic pressure compared to the environment. This condition forces the shrimp to excrete fluids in the body to match the osmotic pressure with the surrounding environment. The process of ionic traffic in shrimp will use up the portion of the available energy so that the portion of energy that should be used for the growth process will be reduced or not even left. This is what makes shrimp slow growth and can even experience stress.

From the observations, it can be seen that the osmotic pressure comparison between hemolymph and the water shows that the osmotic pressure in the treatment medium is lower than that of hemolymph. So the whiteleg shrimp osmoregulation pattern is hyperosmotic. Based on the results of calculating the osmotic work level, it is shown that the ideal salinity of artificial brackish water for whiteleg shrimp reared in artificial brackish water is ideal at 15-20 ppt. The low OWL at this salinity can minimize the shrimp using energy for the osmoregulation process so that the available energy can be used optimally for growth.

Maghfiroh *et al*. (2019) stated that the form of the relationship between the level of osmotic work is inversely related to hemolymph osmolarity and parallel to water osmolarity. According to Anggoro *et al*. (2018) hyperosmotic or hypoosmotic regulation is aimed to maintaining the ability of osmolality and the balance system between body fluids and fluid of the water. In the process of osmotic regulation in the shrimp body, the higher the water salinity, the higher the whiteleg shrimp's workload. This workload is to balance the osmolarity pressure and balance the electrolyte content so that the energy that is discharged towards osmotic performance is even greater (Rachmawati

et al., 2012).

Observation of water quality parameters during the 2nd study had a good level of water quality with adequate water quality eligibility categories (Table 6). The results of research that have been carried out on the parameters of temperature (°C), salinity (ppt), pH, dissolved oxygen (mg/L), Alkalinity (mg/L) Nitrate (mg/L), CO^2 (mg/L), and ammonia (mg/L). Table 6 shows the average temperature value of 28.5 °C, pH 6.80, salinity 10-35 ppt, dissolved O_2 4.86 mg/L, Alkalinity 63.4 mg/L, Nitrate 71.4 mg/L, CO_2 18.6 mg/L and ammonia 0.021 (mg/L). During the observation, the water quality parameter values were still within the range suggested by Boyd & Tucker (1992). Parameters of temperature, salinity, pH to dissolved O_2 did not have a significant difference from the first day until the stress test. While the concentration of Alkalinity tends to decrease, this is because alkalinity can be influenced by CO_2. Meanwhile, the concentration of nitrate, CO_2 and Ammonia increased because they come from the decomposition of accumulated feed organic matter.

CONCLUSION AND RECOMMENDATION

Conclusion

The best stocking density based on carrying capacity for whiteleg shrimp culture in artificial brackish water is 20 shrimps/50L. Mineral content of Ca, Mg, Na and K in artificial brackish water is still low compared to seawater, but it able to support the shrimps survive. The best growth and lowest glucose hemolimph of whiteleg shrimp reared in artificial brackish water was found at salinity of 20 ppt. The lowest osmotic work level in whiteleg shrimp reared in artificial brackish water was found at a salinity of 15-20 ppt, which is close to iso-osmotic.

Recommendation

Based on the results of research on the growth of whiteleg shrimp in artificial brackish water treatment, the results were lower compared to seawater due to the low mineral content of Ca and Mg. So, in future research, is expected to add Ca and Mg minerals to artificial brackish water treatment to maximize good shrimp growth.

AUTHOR'S CONTRIBUTIONS

The contributions of each author, including ideas, data generation, data analysis, manuscript preparation and funding, must be listed here using their initials only, e.g., SBS is doing research. RR is written manuscript etc.

ACKNOWLEDGEMENT

Thank you to the Dean of the UGM Faculty of Agriculture who funded the Collaborative Research of Lecturers and Students for Regular Undergraduate and Postgraduate Programs in 2022.

REFERENCES

Anggoro, S. 1992. Osmotic effects of various levels of media salinity on egg hatchability and vitality of black tiger prawn larvae, Penaeus monodon Fabricius.

Anggoro, S & K. Nakamura, K. 2005. Osmotic response and fedding pettern of kuruma shrimp (*Penaeus javanicus*) at various molting stages. Research Report. Lab of Propagation Physiology. Sciencetific article 11. Fisheries fac. Kagoshima University, Kagoshima.

Anggoro, S., D. Suprapto & F. Purwanti. 2018. Osmoregulation pattern of fingerling whiteleg shrimp (*Litopenaeus vannamei*) rearing in three molt stage iso-osmotic media. 23 (3): 119-122. https://doi.org/10.14710/ik.ijms

Ariadi, H., B.D. Madusari & D. Mardhiyana. 2022. Analysis of the Influence of the Carrying Capacity of the Cultivation Environment on the Growth Rate of Whiteleg Shrimp (*L. vannamei*). Enviro Scienteae. 18 (1): 29-37. http://dx.doi.org/10.20527/es.v18i1.12976

Arifin, M.Y., E. Supriyono & D. Widanarni. 2014. Total hemocytes, glucose and survival rate of mantis shrimp (*Harpiosquilla raphidea*) post-transportation with two different systems. Jurnal Kelautan Nasional. 9 (2): 111-119.

Arwiyah, A., M. Zainuri & M. Efendy. 2015. Study of NaCl Content in raw water and produced salt and salt land productivity using different salt table media. Jurnal Kelautan. 8 (1): 1-9. https://doi.org/10.21107/jk.v8i1.804

Atmomarsono, M., S. Supito, M. Mangampa, H. Pitoyo, L. Lideman & H. Tjahyo. 2014. WWF (Better Management Practice "Whiteleg Shrimp Cultivation - Semi-Intensive Ponds with Waste Water Treatment Plants." www.wwf.or.id.

Boyd, C.E & C.S. Tucker. 1992. Water quality and pond soil analyses for aquaculture. Water quality and pond soil analyses for aquaculture.

Budiardi, T., T. Batara & W. Wahjuningrum. 2005. Oxygen consumption levels of whiteleg shrimp (*Litopenaeus vannamei*) and oxygen management models in intensive ponds. Jurnal Akuakultur Indonesia. 4 (1): 89-96. http://journal.ipb.ac.id/index.php/jai

Bull, E.G., C.D.L. Cunha & A.C. Scudelari. 2020. Water quality impact from shrimp farming effluents in a tropical estuary. Water Science and Technology. 83 (1): 123-136. https://doi.org/10.2166/wst.2020.559

Dwiono, A., Widigdo, B., & Soewardi, K. 2018. Influence of groundwater mineral composition on the physiology and histology of whiteleg shrimp (*Litopenaeus vannamei*). Jurnal Ilmu dan Teknologi Kelautan Tropis. 10 (3): 535-546. https://doi.org/10.29244/jitkt.v10i3.21049

Elliot, J.W. 1969. The oxygen requirements of Chinook salmon. Progressive Fish-Culturist. 31 (2): 67-73. https://doi.org/10.1577/1548-8640(1969)31[67:TOROCS]2.0.CO;2

Fadillah, N., S. Waspodo & F. Azhar. 2019. Addition of Rhizophora apiculata mangrove leaf extract to whiteleg shrimp (*Litopenaeus vannamei*) feed to prevent vibriosis. Journal of Aquaculture Science. 4 (2): 91-101.

Lama, H.A.W., D. Darmawati & F. Wahyu. 2020. Optimization of stocking density on the growth and survival of whiteleg shrimp (*Litopenaeus vannamei*) using a recirculation system. Octopus : Jurnal Ilmu Perikanan. 9 (1): 48-52.

Liao, I.S & H.J. Huang. 1975. Studies on the respiration of economic prawn in Taiwan. I. Oxygen consumption and lethal dissolved oxygen of egg up to young prawn

of *Penaeus monodon* Fabricus. Journal of the Fishries Society of Taiwan. 4 (1): 33-50.

Losordo, T.M & H. Westers. 1994. System Carrying Capacity and Flow Estimation. In: Timmmons, M. B. & T. M. Losordo (Eds) Aquaculture Water Reuse System. Engineering Design and Management. Elsevier Press. Amsterdam

Maghfiroh, A., S. Anggoro & P.W. Purnomo. 2019. Osmoregulation pattern of condition factors for whiteleg shrimp (*Litopenaeus vannamei*) cultivated in the Mojo Ulujami intensive pond, Pemalang. Journal of Maquares. 8 (3): 177-184. https://doi.org/10.14710/marj.v8i3.24253

Mc Connaughey, B.H & R. Zottoli. 1983. Introduction to Marine Biology. 4th ed. The C.V. Mosby Company, London.

Novriadi, R., K.N. Alfitri, S. Supriyanto, R. Kurniawan, D. Deendarlianto, R. Rustadi & S. Rahardjo. 2020. Effect of stocking density and use of venturi injector on growth rate of shrimp (*Litopenaeus vannamei*) in concrete tanks. Jurnal Perikanan Universitas Gadjah Mada. 22 (2): 141-147. https://dx.doi.org/10.22146/jfs.60516

Rachmawati, D., J. Hutabarat & S. Anggoro. 2012.The effect of different media salinity on the growth of tiger snails (*Babylonia spirata* L.) in the domestication process. Ilmu Kelautan. 17 (3): 141-147. https://doi.org/10.14710/ik.ijms.17.3.141-147

Rakhfid, A., B. Nur, B. Muh & F. Fendi. 2017. Growth and survival of whiteleg shrimp (*Litopenaeus vannamei*) at different stocking densities. Akuatikisle: Jurnal Akuakultur, Pesisir dan Pulau-Pulau Kecil. 1 (2): 1-6. https://doi.org/10.29239/j.akuatikisle.1.2.1-6

Septiningsih, E., B.R. Tampangallo, D. Hidayat & S. Suwoyo. 2015. Changes in hematological concentrations due to partial harvest of whiteleg shrimp (*Litopenaeus vannamei*) in superintensive culture. Prosiding Forum Inovasi Teknologi Akuakultur. 1117-1122.

Yunus, R., A. Haris & H. Hamsah. 2020. Effect of adding dolomite lime and tohor lime in the maintenance media on moulting, growth and survival of whiteleg shrimp. Octopus : Jurnal Ilmu Perikanan. 9 (1) : 39-47. https://doi.org/10.26618/octopus.v9i1.4000

Yustiati, A., S.S. Pribadi, A. Rizal & W. Lili. 2017. The effect of density in transportation at low temperatures on glucose levels and live blood of nile tilapia (*Oreochromis niloticus*). Jurnal Akuatika Indonesia. 2 (2): 137-145. https://doi.org/10.24198/jaki.v2i2.23424

How to cite this article Prawira, H.G., R. Rustadi & S.B. Priyono. 2023. Effects Density and Salinity of Artificial Brackish Water on the Growth and Physiological Perfomance of White Leg Shrimp (*Litopenaeus vannamei* Boone, 1931). Jurnal Perikanan Universitas Gadjah Mada. 23 (2): 197-206. DOI https://doi.org/10.22146/jfs.80976