Bioencapsulation Single Cell Protein from *Chlorella vulgaris* Extract on Survival rate and Mortality of Barramundi Larvae, *Lates carcarifer*, Bloch 1790

Anugerah Saputra[1] , Muhammad Yusri Karim[2], Zainuddin[2]

[1]Masters Program, Postgraduate School, Hasanuddin University, Makassar, Indonesia
[2] Faculty of Marine Sciences and Fisheries, Hasanuddin University, Makassar, Indonesia

Correspondence Author Email : anugerahsaputra52@gmail.com

DOI: 10.29322/IJSRP.10.11.2020.p10770
http://dx.doi.org/10.29322/IJSRP.10.11.2020.p10770

Abstract- Barramundi is one of the cultivated commodities which is being developed at this time. One of the challenges in Barramundi Aquaculture is the low survival rate due to internal mortality during the larval phase. Adequate feed supplementation, both in quantity and dose can be a solution in preventing mortality in fish larvae. The research objective was to analyze the effect of SCP on survival and mortality in barramundi larvae. This study used a plastic tub with a round shape and filled with 25 liters of water. The study was designed using a completely randomized design (CRD) method with 4 treatments and 3 replications. The treatment doses used is 0, 100, 200, and 300 mg / L. The results of the analysis of variance showed that the dose had a significant effect (P <0.05) on the survival and mortality of barramundi larvae. The results showed that using SCP at a dose of 100 mg / L increased the survival rate of 80.00% and significantly decreased the mortality rate of barramundi larvae.

Key words: Barramundi larvae, Bioencapsulation, *Chlorella vulgaris*, Singel cell protein, Supplementation.

I. INTRODUCTION

The main problem in the treatment of barramundi larvae (*Lates calcarifer*) during the production process is the low survival rate. Previous research from Nurmasyitah et.al., (2018) and Darosman et.al. (2019) obtained the results of research on survival rate barramundi larvae is still around 20-50%. The low survival rate in barramundi larvae can be caused by stress factors arising from lack of nutritional needs due to low feed quality (Basford *et al.*, 2020), and environmental conditions that are less than optimal in larval rearing (Ribeiro et al., 2015). Inadequate nutritional requirements in live food are an important contributor to mortality in larvae during the production period. To increase the survival rate in barramundi larvae, it is necessary to improve water quality and good maintenance management as well as supplementation of live food to improve quality. Supplementation of protein and lipid forms is needed by marine fish larvae to support normal growth and improved survival (Pangkey, 2011). Supplementation that has the potential to be done is through the application of single-cell protein.

Single-cell Protein (SCP) is a biotechnology product that refers to microbial biomass or protein extract that is extracted as a food additive ((Bharti *et.al*, 2014). In addition to protein, SCP has the potential for lipid content in the form of fatty acids reaching 35.13% (Kurnia *et al*, 2018). SCP has been successfully developed from several microalgae extracts such as *Spirulina* sp (Sharma *et.al*, 2019). However, the use of Spirulina sp as SCP is considered inappropriate due to high production and management costs. Therefore, an alternative is needed that could potentially to replace *Spirulina* sp, and *Chlorella vulgaris* can be recommended. Research on SCP has been carried out on several species of fish and shrimp larvae, among others is tilapia fish (Hussein *et.al*, 2013), zebra fish (Şişman *et al*., 2013), salmon fish (Eichner *et.al*, 2018), and vannamei shrimp (Hamidoghli *et al.*, 2019). However, information on the use of SCP on barramundi larvae has not been found.

The utilization of SCP in barramundi larvae is hindered by the carnivorous nature of the fish, so methods such as bioencapsulation are necessary. Bioencapsulation is nutritional enrichment using additives to natural feed to improve the quality and quantity so that the nutritional quality of natural feed can be increased (Sarmudianto et.al., 2015). Based on this background, research on the benefits of *Chlorella vulgaris* as an SCP product needs to be done. This study aims to analyze the effect of SCP on survival and mortality in barramundi larvae.

II. METHOD

This research was conducted from June to September 2020 at the Center for brackish water fisheries development Air, Takalar regency, south Sulawesi province. indonesia. Single- cell protein (SCP) production process at the Mini Fisheries and Water Quality Laboratory, Faculty of Marine and Fisheries Sciences, Hasanuddin University. Analysis of test samples at the Laboratory of Animal Feed Chemistry, Faculty of Animal Husbandry, Hasanuddin University.

This study used a plastic tub with a round shape, measuring 40 liters and filled with 25 liters of water for the treatment test of barramundi larvae. Treatment animals/objects used barramundi D-16 (16 days after hatched) and 50 larvae were stocked at a density of 2 Ind / L. The treatment was carried out for 10 days of maintenance. Barramundi larvae were obtained from Center for brackish water fisheries development Air, Takalar regency, south Sulawesi province. indonesia. Bioencapsulation media using live feed with *Artemia salina*. The test material is using Single Cell Protein (SCP) from *Chlorella vulgaris* and as a solvent using seawater with 30 ppm of salinity. SCP processing requires a starter material for microalgae (*Chlorella vulgaris)* and liquid tofu waste from the waste products of the tofu factory in the Karanganyar area, Mariso District, Makassar. The nutritional content of SCP is showed in Table 1.

Table 1. Proximate analysis of SCP

Ingredients	Composition (%)
water	20.86
Protein	8.55
Fat/lipid	0.87
fiber	0.86
BETN	12.82
Ash	76.91

The study was designed using a completely randomized design with 4 treatments and 3 replications. As treatment using different doses of SCP 0, 100, 200, and 300 mg/L. The parameters observed were the retention of nutrients absorbed in the larvae of barramundi (dissolved protein, lipids, and energy), survival, and mortality during the treatment period for barramundi larvae.

Dissolved protein retention is calculated using the following formula (Dewi and Tahapari, 2018):

$$RPt = \frac{\text{Ptb}}{\text{Kpt}}\ x\ 100$$

Note: RPt is dissolved protein retention (%), Ptb is the increase in dissolved protein in the body (%), and Kpt is the dissolved protein content in feed (%)..

Lipid retention is calculated using the following formula (Dewi and Tahapari, 2018) :

$$\text{RL} = \frac{\text{Ltb}}{\text{Lp}}\ x\ 100$$

Note: RL is the lipid retention (%), Ltb is the increase in lipids in the body (%), and Lp is the lipid content in the feed consumed (%).

Energy retention is calculated using the following formula (Tung and Shiau, 1991):

$$\text{RE} = \frac{(\text{Eend} - \text{Estart})\ \text{x}\ 100}{\text{FE}}$$

Note: RE is energy retention (%), Eend is final body energy (Kcal), Estart is initial body energy (Kcal), FE is total feed energy given (Kcal).

Survival rate is calculated using the following formula (Gunadi *et al*, 2016) :

$$SR = N_t/N_o\ x\ 100$$

Note: SR is the survival rate (%), Nt is the total number of live fish at the end of the study (tails), and No. The total number of fish at the beginning of the study (tails).

Mortality is calculated by comparing the total larvae at the start of the study with the total larvae that died at a certain period in the treatment period. Larval mortality data were obtained in 3 time periods, the period being the 4th day of maintenance (D-4), the 7th day of maintenance (D-7), and the 10th day of rearing (D-10). Larval mortality was calculated using the following formula (Ribeiro, 2015):

$$PM = \frac{(Ndead / Nstart)}{\Delta t} \times 100$$

Note: PM is the mortality of larvae in a certain period (%), Ndead is the number of dead fish larvae in a certain period (tail), Nstart is the total number of fish at the beginning of the rearing period (tails), and Δt is the time interval / maintenance period (days).

During maintenance, several parameters of the water quality of the maintenance media were measured, including thermal temperature, water temperature, salinity, dissolved oxygen, pH, and ammonia. Thermal temperature was measured using a thermal thermometer, water temperature using a water thermometer, salinity using a hand refractometer, dissolved oxygen using a Digital DO meter, pH using a portable Digital pH meter and ammonia measured using a spectrophotometer. Thermal temperature and water temperature parameters were measured three times a day during the morning, afternoon and evening. Salinity, dissolved oxygen, and pH were measured daily during treatment. Ammonia parameters were measured by taking samples 3 times during treatment at the beginning, half of treatment and end of treatment.

The data obtained included dissolved protein retention, lipid retention, energy retention and survival rate for barramundi larvae, analyzed using the Analysis of Variance (ANOVA) method. Because the results have a real effect so it is continued with the W-Tuckey Test. The SPSS version 23.0 package was used as a tool for analysis. The mortality data for barramundi larvae were analyzed descriptively based on the pattern on the graph showed.

III. RESULTS

Retention of dissolved protein, lipid and energy content in barramundi larvae

Retention of dissolved protein, lipid and energy content in barramundi larvae showed in table 2.

Table 2. Nutrient retention in the body of the barramundi larval

Treatment dose (mg/L)	Retention (%)		
	Dissolved protein retention/ RPt	Lipid retention/ RL	Energy retention/ RE
Control (0)	1.26 [a]	1.86 ± 1.85 [ab]	3.47 [a]
100	1.07 [a]	3.54 ± 0.81 [a]	7.2 [a]
200	0.3 [a]	0.85 ± 0.16 [b]	7.39 [a]
300	2.15 [a]	0.4 ± 0.33 [b]	3.44 [a]

Values of the same row with different letters were significantly different (p <0.05)

The retention of dissolved protein content showed insignificant results (P <0.05) between treatments (Table 2). The ANOVA test results showed no significant effect or effect on the treatment given. The highest percentage value was obtained at the treatment dose of 300 mg / L with a value of 2.15% while the other treatments each obtained values of 1.26%, 1.07% and 0.30% for each treatment dose control (0), 100 mg / L and 200 mg / L.

Lipid retention in the body of barramundi larvae showed significant results between treatments (P <0.05) (Table 2). The results at the treatment dose of 100 mg / L showed a significant or significant difference to the treatment at the dose of 200 mg / L and 300 mg / L, but the control treatment did not give a significant impact. The highest lipid retention value was obtained at the SCP dose of 100 mg / L with a value of 3.54%, then respectively in the control treatment, 200 mg/L, and 300 mg / L resulted in retention values of 1.86%, 0.85% and 0.4%.

Energy retention in barramundi larvae showed insignificant results (P <0.05) between treatments (Table 2). The ANOVA test results showed no significant effect or effect on the treatment given. The highest percentage value was obtained at the SCP treatment dose 200 mg / L and 100 mg / L with a value of 7.39%. and 7.20%. Then in the control dose treatment (0) and 300 mg / L, the percentage values were 3.47% and 3.44%.

Survival of barramundi larvae

Survival of barramundi larvae is showed in table 3

Table 3. Survival in Barramundi larval

Treatment dose (mg/L)	Survival rate of barramundi larvae (%) + STD
Control (0)	47.33 ± 9.01 ^{b}
100	80.00 ± 6.00 ^{a}
200	67.33 ± 3.05 ^{ab}
300	68.00 ± 12.16 ^{ab}

Values of the same row with different letters were significantly different (p <0.05)

The value of the survival rate of barramundi larvae showed a significant difference between treatments (P <0.05) (Table 3). The ANOVA test results show that there is a significant effect or effect on the treatment given. Treatment with a dose of SCP 100 mg / L can give a significant result or significantly different effect on control treatment (0). However, the results given did not show any significant results for other treatment doses of 200 mg / L and a dose of 300 mg / L. These results indicate that the treatment of SCP dosage as a feed supplement for barramundi larvae can give better results than treatment without SCP.

larval mortality

The development mortality of barramundi larvae is showed in Figure 1.

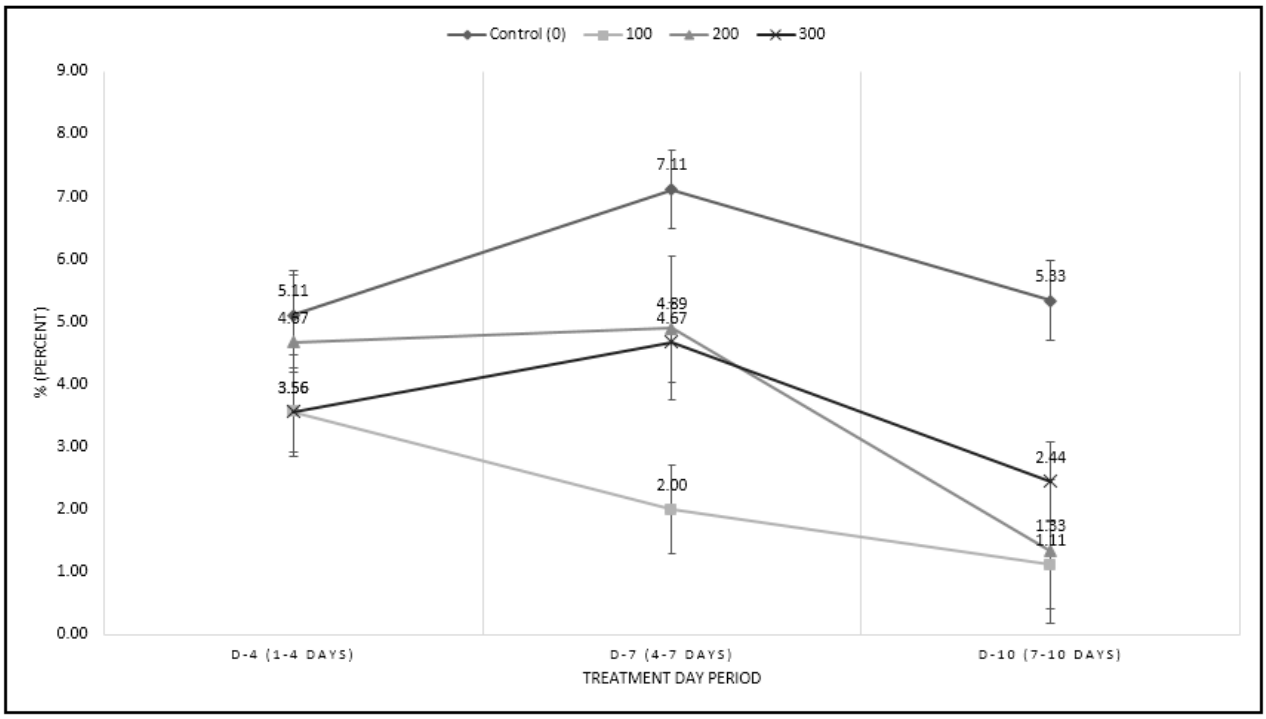

Figure 1. Mortality in Barramundi larvae during treatment

The development of mortality of barramundi larvae (Fig. 1) showed treatment with different doses of SCP can have an effect on mortality rates during larval rearing. The data show that the larval mortality rate has increased on the 7th day (D-7) of the maintenance period, where almost all treatments have resulted in a significant increase in mortality during that period. However, the treatment doses of 100 mg / L and 200 mg / L showed a decrease in the mortality rate during that period. The mortality of larvae at the treatment doses of 100 mg / L and 200 mg / L showed a trend or pattern that slowly decreased in each maintenance period including the D-7 period. In other treatments, the trend graph was showed to be different at the control dose (0) and the dose of 300 mg / L, where both treatments showed an increase in mortality on day 7 (D-7) of the maintenance period. This period as well as being the highest peak of mortality in that treatment during larval rearing. In the control treatment, the decrease in the mean mortality value obtained did not show a lower value than the initial maintenance period.

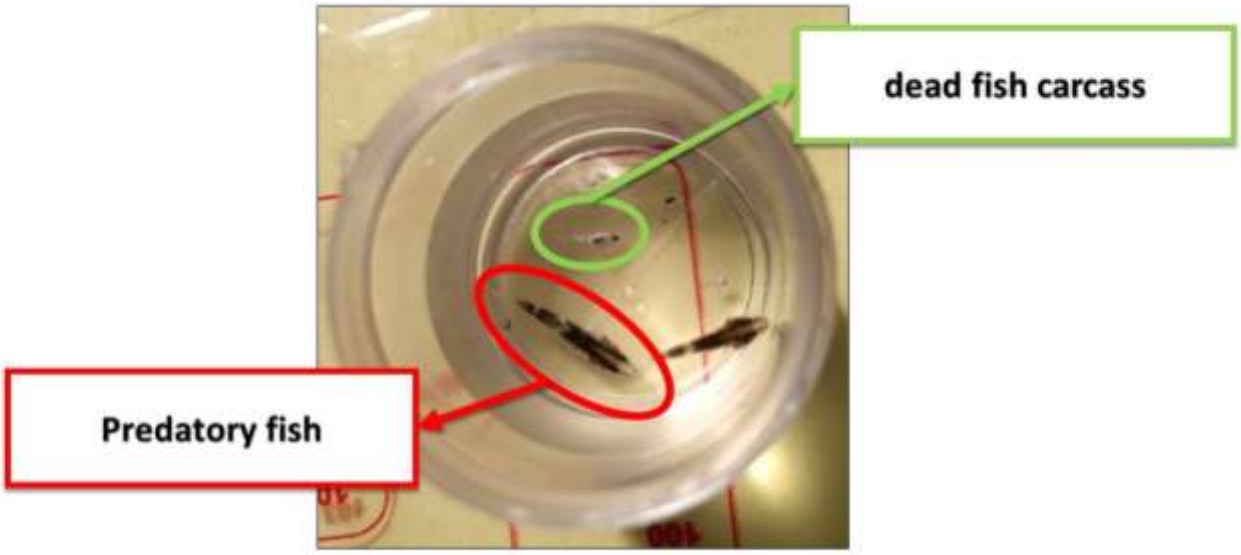

Figure 2. Cannibalism in barramundi larvae

Environmental water quality treatment of barramundi larvae

Analysis of the quality of treatment water is showed in Table 4.

Table 4. water analysis treatment

Parameter test	Treatment dosage (mg/L)				SNI 6145.3:2014 (NSA, 2014)
	Control (0)	100	200	300	
Thermal temperature (°C) :	28.5 - 41.2				-
Water temperature (°C) :	27.5 - 33.5	2.71 - 33.1	27.5 - 33.4	27.5 - 33.1	28-32
Salinity (mg/L)	31-32	31-32	31-32	31-32	28-33
Dissolved oxygen (mg/L)	5.2 - 6.3	5.3 - 6.5	5.1 - 6.5	5.3 - 6.7	min. 4
Water pH	6.8 - 7.8	6.6 - 7.4	6.5 - 7.8	6.5 - 7.7	7,5-8,5
Water ammonia (mg/L)	0,001	0,002	0,003	0,003	max. 1

Based on Table 4, the water quality measurement results obtained did not show any significant differences in each treatment. The value recorded in each parameter shows the results by the standards for managing the water quality of barramundi fish larvae that have been set by National standardization agency (NSA) following SNI number 6145.3: 2014 which was published in 2014. For thermal temperature parameters in raising barramundi larvae have not been standardized by National standardization agency.

IV. RESULT

Based on the results of the study in Table 2 regarding the retention of nutrients such as dissolved protein, lipid and energy, it is indicated that the administration of SCP dosage as a bioencapsulation material for *Artemia salina* on certain parameters did not show a significant effect on treatment ($P < 0.05$). As in the result of the dissolved protein retention value parameter, this can be influenced by the substitution value of the protein contained in SCP 8.55% or 0.085 g (Table 1). The result of this protein content is much less than the production of SCP without the compaction/drying process with a percentage value of 52.24% (Putri *et.al*, 2018). This result is also lower than the protein hydrolyzate value in *chlorella* sp in powder form, which is 46.3 g / 100g or 0.46 g (Grossmann et.al, 2018).

The results of the energy retention in Table 2 also did not show a significant effect on treatment ($P < 0.05$). This can occur as a result of the high value of ash content in the resulting SCP, which is 76.91% (Table 1). Ash content is a collection of minerals that have properties as inorganic materials. Where this substance is not completely absorbed by the body. In the process, the main obstacle in mineral absorption is the ability of food to present enough minerals in ionized form to the apical membrane of the

enterocytes for transcellular absorption, so that not all mineral nutrients can be absorbed (Goff, 2018). As a reference, one of the studies related to bioencapsulation using a microalgae source with the type of *Spirulina* sp has a fairly low ash content value of 2.99% (Suyanto et.al, 2019). the ash content produced in this study is very high.

The results of the lipid retention test in table 2 showed a significant effect on treatment (P <0.05). The value of lipid retention is thought to have a significant effect between treatments due to the effect of lipid substitution on SCP, although the resulting lipid value in this study was still low at only 0.87% (Table 1). When compared with SCP products with microalgae sources from *spirulina* sp, it can produce a lipid percentage of 7.4% (Moreira et.al, 2011). However, the resulting lipid value is proven to be used properly as a nutrient in the bioencapsulated feed for barramundi larvae. part of the lipids that are important for the body and can directly play a role in the formation of body energy are fatty acids, one of which plays an important role in the body is EPA (Suyanto et.al, 2019).

Based on the survival rate data in Table 3, the survival value in the SCP dose treatment ranges from 67.33-80.00%. In terms of the results of research related to the test of the benefits of microalgae as an enrichment material in barramundi larvae, it shows the percentage of survival rate of larvae ranging from 42.75 - 55.50 % (Darosman et al., 2019). Then another study showed that the average survival rate obtained in barramundi larvae obtained values ranging from 20.75 - 72.50 % (Nurmasyitah et al., 2018). Based on the results showed from previous studies, the results achieved in this study showed a much higher increase in survival rate in barramundi larvae.

Survival rate in barramundi larvae is caused by many factors. The factors that cause it can come from the adequacy of nutrients from the enrichment process of food and environmental factors that contribute to each other (Kusumawati et.al, 2019). Enrichment in food/feed, especially lipid levels and energy retention levels in the body of barramundi larvae can be used as a reference in determining the effectiveness of increasing survival rate in larvae. The results of lipid retention or lipid absorption in the body of barramundi larvae that were given different doses of SCP resulted in a percentage range of 0.40% - 3.54% (Table 2). Then the lipid retention values commonly found in juvenile barramundi fish range from 0.45 to 1.59%. (Phan et al., 2019). Specifically, there is no reference regarding lipid retention found in the larval phase of barramundi, but based on the reference value of retention found in the juvenile phase, in this study the lipid retention value obtained was quite high.

The important component in lipids/lipids is lipid acid, where barramundi fish larvae require intake of lipid acids such as DHA with the recommended amount of 10 g / Kg in feed (Glencross and Rutherford, 2011). However, excess nutrients in the form of lipid acids such as DHA and ARA which are given in feed substitutions where there is also one or more nutrients that do not meet the minimum nutritional needs of barramundi larvae, it can cause a decrease in survival rate in barramundi larvae (Thépot *et.al*, 2016). Therefore the dose in the substitution of feed supplements has a very important role for the larvae of barramundi. There is no more specific reference regarding the correct SCP dose in barramundi larvae, so based on the findings of this study, a treatment dose of 100 mg/L can be recommended.

Based on the data in graph 1, death during the treatment of barramundi larvae can be caused by many factors. According to Ribeiro, (2015) the factors that cause mortality in fish are due to the heterogeneity of growth so that it can cause quite high cannibalism and death from wounds and suffocation. These factors are also the causes of death found in this study. The cause of death in this study indicated a link with the nutritional substitution in larvae feed. Nutrients such as dissolved protein can be indicated to play a role in the development and life of larvae. Dissolved protein is a source of nutrition consisting of oligopeptide bonds that are easily absorbed by the organism's body (Mardhika, et al, 2020). The Oligopeptide content has the properties or functions as antihypertensive, antimicrobial, immunomodulatory and antioxidant which is quite prominent (Jia et al., 2010) As immunomodulators and antioxidants, when substituted in sufficient quantities, it can improve health performance in fish (Dwinanti and Sasanti, 2019). Therefore, it is strongly suspected that the protein substituted by administering SCP at a certain dose can supply sufficient oligopeptides into the body of the larvae.

Based on the evidence in Figure 2, it shows that in this study the mortality that contributed the most was indicated as a result of cannibalism that occurred. This is based on findings on days at the 7th rearing period (D-7) or at the age of larvae 20-22 (D-21 to D-22) days. Besides, other findings in this study also indicated that the cannibalism that occurs mostly has the same pattern as the study from Fehér *et al*., (2013) no findings of dead fish (carcasses) where cannibalism is responsible for the death of the barramundi fish. Several studies have showed cases of cannibalism in barramundi fish are generally found to occur in the juvenile phase where the larval age ranges from 67-112 days (after hatching) (Jesu Arockiaraj and Appelbaum, 2011). Recent studies have showed cases of cannibalism begin to appear in the juvenile phase when the fish are 35 (D-35) days after hatching (Pham *et.al*, 2020). The results regarding cannibalism in susceptible larvae aged 20-22 days after hatching have not been found in several articles regarding studies that have been conducted on barramundi fish. This is new information, especially in raising barramundi larvae.

Based on the data in table 4 regarding the test results of the water quality value parameters in the rearing of barramundi fish larvae, they comply with the standards set by NSA. Recent research shows the suitability of the results of water quality parameters in raising barramundi larvae where the water quality parameter values are deformed, such as water temperature in the range of 26 - 28.5 ° C, pH values in the range of 7.5 - 8.3 and salinity levels with range 32 - 34.5 and dissolved oxygen levels> 5 (Darosman dkk., 2019). The value obtained in this study indicates the conformity of the water quality value obtained, although parameters such as water temperature in the research conducted are slightly higher. The thermal temperature has not been standardized by NSA.

However, the thermal temperature tolerance value has been tested on barramundi fish in open waters. The tolerance value is obtained at the highest value of 40 ° C (Newton *et.al,* 2010). Barramundi fish can tolerate a wide temperature range of 14–40 ° C, where this species tends to be cultivated in water with a temperature range of 22 to 35 ° C (Tucker, *et.al* (2002); Thépot and Jerry, 2015). The intensive cultivation of barramundi sometimes exposes individuals to temperatures that reach above thermal tolerance temperatures. So with this reference, the value of room temperature or thermal temperature obtained in the range of 28-42 ° C can be tolerated by the larvae of barramundi, although in some conditions such as during the day the temperature value showed is 2 ° C higher than the tolerance value stated (Newton *et.al,* 2013).

V. CONCLUSIONS

The conclusions in this study indicate that administering SCP doses to barramundi larvae by bioencapsulation method gives the best results at a dose of 100 mg / L. Treatment with SCP 100 mg / L can provide a survival rate of up to 80% and a decrease in mortality during treatment.

REFERENCES

[1] Basford, A. J., Mos, B., Francis, D. S., Turchini, G. M., White, C. A., & Dworjanyn, S. 2020. A microalga is better than a commercial lipid emulsion at enhancing live feeds for an ornamental marine fish larva. Aquaculture, vol. 523, no. 735203.

[2] Bharti, V., Pandey, P. K., & Koushlesh, S. K. 2014. Single Cell Proteins: a Novel Approach in Aquaculture Systems. World Aquaculture, vol 45, no. 4: 62–63.

[3] Darosman, T. C., Muhammadar, A. A., & Satria, S. 2019. Enrichment of Rotifers (Brachionus plicatilis) with *Chlorella* sp. For the Feed of Barramundi fish (*Lates calcarifer*). Jurnal Ilmiah Mahasiswa Kelautan Dan Perikanan Unsyiah, vol. 4, no. 2: 124–133.

[4] Dewi, R. R. S. P. S., & Phaseari, E. 2018. Utilization of Commercial Probiotics in Catfish (Clarias gariepinus) Rearing. Journal of Aquaculture Research, vol 12, no. 3: 275.

[5] Dwinanti, S. H., & Sasanti, A. D. (2019). Utilization of Vitamin C to Improve Immunity Performance of Snakehead Fish (Channa Striata) Seeds. Indonesian Journal of Swamp Aquaculture, 7 (1), 67–76.

[6] Eichner, C., Dondrup, M., & Nilsen, F. (2018). RNA sequencing reveals distinct gene expression patterns during the development of parasitic larval stages of the salmon louse (Lepeophtheirus salmonis). Journal of Fish Diseases, 41(6), 1005–1029.

[7] Fehér, M., Baranyai, E., Simon, E., Bársony, P., Szucs, I., Posta, J., & Stündl, L. (2013). The interactive effect of cobalt enrichment in Artemia on the survival and larval growth of barramundi, Lates calcarifer. Aquaculture, 414–415, 92–99.

[8] Glencross, B., & Rutherford, N. 2011. A determination of the quantitative requirements for docosahexaenoic acid for juvenile barramundi (Lates calcarifer). Aquaculture Nutrition vol. 17, no. 2: 536–548.

[9] Goff, J. P. 2018. Invited review: Mineral absorption mechanisms, mineral interactions that affect acid–base and antioxidant status, and diet considerations to improve mineral status. Journal of Dairy Science vol. 101, no. 4: 2763–2813.

[10] Grossmann, L., Ebert, S., Hinrichs, J., & Weiss, J. 2018. Effect of precipitation, lyophilization, and organic solvent extraction on preparation of protein-rich powders from the microalgae Chlorella protothecoides. Algal Research vol. 29: 266–276.

[11] Gunadi, B., Robisalmi, A., & Setyawan, P. 2016. Growth Performance and Estimation of Juvenile Heterosis Value of Tilapia (*Orochromis niloticus*), Blue Tilapia (*Oreochromis aureus*) and Their Crosses Raised in Freshwater Ponds, pp. 571– 577. Proceedings of the Aquaculture Technology Innovation Forum, Subang, December 5, 2016. Sukamandi Fish Breeding Research Center, West Java.

[12] Hamidoghli, A., Yun, H., Won, S., Kim, S. K., Farris, N. W., & Bai, S. C. 2019. Evaluation of a single-cell protein as a dietary fish meal substitute for whiteleg shrimp *Litopenaeus vannamei*. Fisheries Science vol. 85, no. 1: 147–155.

[13] Hussein, E. E. S., Dabrowski, K., El-Saidy, D. M. S. D., & Lee, B. J. 2013. Enhancing the growth of Nile tilapia larvae/juveniles by replacing plant (gluten) protein with algae protein. Aquaculture Research vol. 44, no. 6: 937–949.

[14] Jesu Arockiaraj, A., & Appelbaum, S. 2011. Sibling cannibalism in juvenile barramundi, Lates calcarifer (Actinopterygii: Perciformes: Centropomidae), reared under different light conditions. Acta Ichthyologica et Piscatoria vol. 41, no 1: 7–11.

[15] Jia, J., Zhou, Y., Lu, J., Chen, A., Li, Y., & Zheng, G. 2010. Enzymatic hydrolysis of alaska pollack (Theragra chalcogramma) skin and antioxidant activity of the resulting hydrolysate. Journal of the Science of Food and Agriculture, 90(4), 635–640.

[16] Kurnia, D. 2018. Fatty acid analysis of marine microalgae chlorella sp. In modified medium used gas crhomatography-mass spectrometry (gc-ms). Journal of Pharmacopolium vol. 1, no. 1: 1–8.

[17] Kusumawati, D., Asih, Y. N., & Setiawati, K. M. 2019. Increased survival of Sunu Grouper (Plectropomus leopardus) larvae through appropriate maintenance management. Journal of Life Sciences, LIPI vol.18, no. 1: 59-70.

[18] Mardhika, H., Dwiloka, B., & Setiani, B. E. 2020. The Effect of Various Thawing Methods of Frozen Lay Laying Chicken on Protein, Dissolved Protein and Fat Content of Chicken Steak. Journal of Food Technology vol. 4, no. 1: 48–54.

[19] Moreira, L. M., Rocha, A. da S. R., Ribeiro, C. L. G., Soares, Rodrigues, R. da S., & Leonor Almeida de Souza. 2011. Nutritional evaluation of single-cell protein produced by Spirulina platensis. African Journal of Food Science vol. 5, no. 15: 799–805.

[20] National standardization agency. 2014. Barramundi (Lates carcarifer, Bloch 1790) part 3. SNI 6145.3:2014. Marine and Fisheries Ministry Republik of indonesia.

[21] Newton, J. R., Smith-Keune, C., & Jerry, D. R. 2010. Thermal tolerance varies in tropical and sub-tropical populations of barramundi (Lates calcarifer) consistent with local adaptation. Aquaculture, vol. 308: 128–132.

[22] Newton, J. R., Zenger, K. R., & Jerry, D. R. 2013. Next-generation transcriptome profiling reveals insights into genetic factors contributing to growth differences and temperature adaptation in Australian populations of barramundi (Lates calcarifer). Marine Genomics vol. 11: 45–52.

[23] Nurmasyitah, Defira, C. N., & Hasanuddin. 2018. Effect of Different Natural Feeding on Survival Rate of White Snapper (Lates calcarifer) Larvae. Unsyiah Marine and Fisheries Student Scientific Journal vol. 3, no 1: 56–65.

[24] Pangkey, H. 2011. The Need for Essential Fatty Acids in Marine Fish. Journal of Fisheries and Tropical Marine vol. 7, no. 2: 93-102.

[25] Pham, V. K., Truong, H. P., Nguyen, D. K., & Nguyen, N. H. 2020. Genetic component of cannibalism in Asian seabass Lates Calcarifer. Applied Animal Behaviour Science vol 231, no 105074: 1-6.

[26] Phan, L. T. T., Groot, R., Konnert, G. D. P., Masagounder, K., Figueiredo-Silva, A. C., Glencross, B. D., & Schrama, J. W. 2019. Differences in energy utilisation efficiencies of digestible macronutrients in common carp (Cyprinus carpio) and barramundi (Lates calcarifer). Aquaculture vol. 511, no. 734238: 1-8.

[27] Putri, D., Ulhidayati, A., Musthofa, I. A., & Wardani, A. K. 2018. Single cell protein production of Chlorella sp. using food processing waste as a cultivation medium. IOP Conference Series: Earth and Environmental Science vol 131, no. 1: 1-6.

[28] Ribeiro, F. F. 2015. Cannibalism in Barramundi Lates calcarifer: Understanding Functional Mechanisms and Implication to Aquaculture.. [Thesis]. School of Biological Sciences, Faculty of Science & Engineering, Flinders University. South Australia.

[29] Ribeiro, F. F., Forsythe, S., & Qin, J. G. 2015. Dynamics of intracohort cannibalism and size heterogeneity in juvenile barramundi (Lates calcarifer) at different stocking densities and feeding frequencies. Aquaculture vol. 444: 55–61.

[30] Sarmudianto, E., Rosmawati, & Muarif. 2015. Increasing Levels of Omega 3 Fatty Acids in Daphnia Sp with Fish Oil Enrichment. Journal of Mina Sains vol. 1, No. 1: 1–5.

[31]Sharma, A., Kaur, K., & Marwaha, D. 2019. Spirulina Platensis an " Ultimate Food ": A Review. International Journal of Research and Analytical Reviews vol. 6, no. 1: 428–437.

[32]Şişman, T., Gür, Ö., Doğan, N., Özdal, M., Algur, Ö. F., & Ergon, T. 2013. Single-cell protein as an alternative food for zebrafish, Danio rerio: A toxicological assessment. Toxicology and Industrial Health vol 29, no. 9: 792–799.

[33] Suyanto, E., Rahman, Y. S., Suyanto, E., & Rahman, Y. S. 2019. Effect of Bioencapsulation of Artemia salina with Spirulina platensis on Survival Rate of Tilapia (Oreochromis niloticus) Seeds. Biotropics: Journal of Tropical Biology vol. 7, no. 2: 75–81.

[34] Thépot, V., & Jerry, D. R. 2015. The effect of temperature on the embryonic development of barramundi, the Australian strain of Lates calcarifer (Bloch) using current hatchery practices. Aquaculture Reports vol. 2: 132–138.

[35] Thépot, V., Mangott, A., & Pirozzi, I. 2016. Rotifers enriched with a mixed algal diet promote survival, growth and development of barramundi larvae, Lates calcarifer (Bloch). Aquaculture Reports, 3, 147–158.

[36] Tung, P. H., & Shiau, S. Y. 1991. Effects of meal frequency on growth performance of hybrid tilapia, Oreochromis niloticus × O. aureus, fed different carbohydrate diets. Aquaculture vol. 92: 343–350.

AUTHORS

First Author – Anugerah Saputra, graduate student, Hasanuddin university, anugerahsaputra52@gmail.com.
Second Author – Muhammad Yusri Karim, Lecture, Faculty of Marine Sciences and Fisheries, yusri_karim@yahoo.com
Third Author – Zainuddin, Lecture, Faculty of Marine Sciences and Fisheries, zainuddinlatief@gmail.com.

Correspondence Author – Anugerah Saputra, anugerahsaputra52@gmail.com, anugerahbdpunhas2014@yahoo.co.id, 085255872850