

1 **ACCEPTED MANUSCRIPT**

2

3 A DIETARY MICROCAPSULE BASED ON SINGLE CELL PROTEIN (*Spirulina platensis*)
4 FOR MILK FISH, CHANOS-CHANOS, LARVAE

5

6 Sukardi P, Yansah N, Winanto T, Marnani S, Prayogo NA, Harisam T, Sudaryono A

7

8 DOI: 10.11598/btb.2019.26.3.1103

9

10 To appear in : BIOTROPIA Vol. 26 No. 3 December 2019 Issue

11

12 Received date : 20 July 2018

13 Accepted date : 06 December 2018

14

15 **This manuscript has been accepted for publication in BIOTROPIA journal. It is unedited,**
16 **thus, it will undergo the final copyediting and proofreading process before being published in**
17 **its final form.**

ACCEPTED MANUSCRIPT

18 **A DIETARY MICROCAPSULE BASED ON SINGLE CELL PROTEIN (*Spirulina platensis*)**
19 **FOR MILK FISH, CHANOS-CHANOS, LARVAE****

21 **Purnama Sukardi^{1*}, Noprie Yansah¹, Tjahyo Winanto³, Sri Marnani¹, Norman A. Prayogo²,**
22 **Taufan Harisam³ and Agung Sudaryono⁴**

23 ¹Department of Aquaculture Universitas Jenderal Soedirman, Purwokerto 53123, Indonesia

24 ²Department of Water Resource Management, Purwokerto 53123, Indonesia

25 ³Department of Marine Science, Universitas Jenderal Soedirman, Purwokerto 53123, Indonesia

26 ⁴Department of Aquaculture, University of Diponegoro, Semarang, Indonesia.

27 *Corresponding author: purnamas@unsoed.ac.id

28 **This paper was presented at the 2nd Scientific Communication in Fisheries and Marine Sciences
29 (SCiFiMaS 2018), 07-09 May 2018, Purwokerto, Central Java, Indonesia

31 Running title: Single cell protein diet for milk fish larvae

32
33 **ABSTRACT**

34 The purpose of this study was to evaluate differences between spirulina-based
35 microcapsules and commercial diets on absolute, daily and specific growth and survival rates of
36 milkfish larvae. *Spirulina platensis* was as a core diet in microcapsules with different matrix
37 (walls). The first capsule wall was gelatin and fish oil, while the second capsule wall was gelatin,
38 fish oil and whole egg. The control group was commercial diet. A total of 1200 larvae were used in
39 this experiments using recirculation systems. Larvae are fed three times a day and increased
40 regularly when the size of the larvae increases. The results showed that the effect of both spirulina-
41 based microcapsule diets on the absolute growth rate (AG), specific growth rate (SGR) and average
42 daily growth rate (ADGR) of Chanos-chanos larvae fed spirulina-based microcapsule same as them
43 which to be fed using commercial diet. The survival rate was as 80.6±11.171%; 84.6±8.443%;
44 83.8±16.496%, respectively. This study showed that Spirulina-based microcapsules had the same
45 effect as commercial feed on the growth of milkfish larvae which means that this diet could replace
46 commercial diet.

47
48 **Keywords:** microcapsule wall, Spirulina, Chanos chanos

49
50 **INTRODUCTION**

51 Milk fish (*Chanos-chanos* Forskal), Orange-spotted grouper (*Epinephelus coioides*), hard-
52 lipped barb (*Osteochilus hasselti*) and giant gouramy (*Osphronemus gourami* Lacepede) are
53 particularly favored in Indonesia especially in Java because they are easy to breed and their flesh is
54 favored (Yuwono and Sukardi, 2009; Prayogo et al. 2016^a,2016^b and Sukardi, et al., 2018). In the
55 brakishwater, fish, crustaceans and other aquatic organisms larvae consume a variety of micro and
56 macro-algae which has good nutritional composition such as protein, lipids, fatty acids, and
57 vitamins. These components are essential to promote growth and immune enhancers (van Dam, et
58 al. 2002; Ju et al., 2009; Kuhn et al., 2010; Supamattaya et al., 2005; Van Der Meeren et al., 2007,
59 Sudaryono et al., 2018). In the brakishwater, fish, crustaceans and other aquatic organisms larvae
60 consume a variety of micro-algae which has good nutritional composition such as protein, lipids,

61 fatty acids, and vitamins. These components are essential to promote growth and immune
62 enhancers (van Dam, et al. 2002; Ju et al.,2009; Kuhn et al.2010; Supamattaya, et al. 2005; Van
63 Der Meeren et al., 2007).

64 Milk fish larvae, like other fish species, after yolk sac absorption, need sufficient and
65 continuous source of live food such as rotifer, *Brachionus plicatilis* and *Artemia*, therefore in the
66 hatchery applied green-water in which consisted of phyto-and zooplankton (Tamaru et al., 1994;
67 van Dam, 2002; Soomro, et al. 2015). Formulated microcapsul diets using single cell protein-base
68 ingredients represent an alternative approach to improve the delivery of essential nutrients to the
69 larvae. Microencapsulation is a technique which allows the manufacture of stable small capsules
70 that may prevent nutrient leaching, easy to handle, and environmentally friendly (Aragao, et
71 al.,2014; Dubay et al., 2009; Umer, et al., 2010, Wilson and Shah, 2007). Microencapsulated diets
72 appear to be a good option to over come these limitations. Microcapsule diet substitution for live
73 prey is therefore important for lowering production cost and ensuring sustainable supply of high
74 quality fish seed. A number of different formula of microencapsulated diets have been developed
75 and experienced extensively for several species of crustaceans include *Penaeus japonicus* Bate (Xie,
76 et al.,2010), bivalve lions-paw scallop, *Nodipecten subnodosus* (Saucedo, et al.,2013), and fish,
77 larval Halibut (*Hippoglossus hippoglossus*). The purpose of this research was to evaluate the
78 difference of spirulina-based microcapsules and commercial feed to absolute, daily and specific
79 growth and survival rate of milkfish larvae and difference of microcapsule wall types on the fish
80 growth.

81 82 **MATERIALS AND METHODS**

83 **Writing the Materials and Methods**

84 A recirculating system were applied in which every tanks aerated with air stones. Three
85 group experiments were carried out wherein each group consisted of three cylinder tanks (50 L)
86 contained 100 fish with size of 1-2 cm and weight of 0,11-0,21g (equivalent to a fish density of 2 L
87 water volume) maintained at 27-29⁰C, for feeding trials. Each of the group was conducted using
88 three tanks randomly. Microencapsulated diets were designed using two different wall materials, the
89 first spirulina capsule (treatment 1) was designed use wall consisted of gelatin and fish oil, whereas
90 the second one (treatment 2) was eggs, gelatin and fish oil. **Fish oil used as an attractant flavor.**
91 The control group (treatment 3) was commercial feed. The alga species, *Spirulina platensis*, was
92 cultured as described previously (Sukardi, et al. 2014). The algal species as inclusion materials of
93 microencapsulated diets were harvested when reach stationary phases at a density of **73442 x 10⁴**
94 **cell^{mL}** *Spirulina platensis*. Capsule particles produced by a modification method of the thermal

95 cross-linking technique, as described Sukardi, et al. (2014, 2018). Microcapsules were prepared by
96 mixing one part of wall (matrix) with one part of inclusion and the ratio was described as follows.

97

98

99 Table 1. Composition of microencapsulated diet for feeding experiment (treatment 1)

No.	Diet components	% composition by weight
1.	Matrix : (60%/w)	
	Gelatin	42
	fish oil	18
2.	Inclusion (40%/w)	
	Spirulina platensis	32
	Vitamin mix	4
	Lysine	4

100

101 Table 2. Composition of microencapsulated diet for feeding experiment (treatment 2)

No.	Diet components	% composition by weight
1.	Matrix : (60%/w)	
	Eggs	42
	Gelatin	12
	fish oil	6
2.	Inclusion (40%/w)	
	Spirulina platensis	32
	Vitamin mix	4
	Lysine	4

102

103 Fish larvae were cultured with a series of microencapsulated and commercial diets in brackish
104 water (15-25 ppt). The diets were fed to fish larvae three times daily for 42 days. During the first
105 several days, feeding rates were based on observation of feeding behavior of fish and increased
106 periodically as the larvae increased in size.

107

108 **Growth parameter**

109 Absolute Growth = weight gain (g), $AG(g) = W_t - W_i$, where W_t is final weight (g), W_i is
110 initial weight (g). Average daily growth rate= $ADGR = \frac{W_t - W_i}{T}$, where W_t is final weight (g),
111 W_i is the weight of fish at time 0 and T is a culture periode in days of experiment. Specific Growth
112 Rate= $SGR (\%/d) = 100 \frac{(\ln W_t - \ln W_i)}{T}$, where W_t is final weight (g), W_i is the weight of fish at
113 time 0, T is a culture periode in days of experiment. Survival($\%$)= $(\frac{\text{Total number of fish survived}}{\text{Total number of fish stocked}}) \times 100$.

114

115 **Statistical analysis**

116

117 The arch-sine square root transformation was applied to all percentage data prior to analysis.
118 A one way analysis of variance (ANOVA) was used to determine whether significant differences
119 existed among treatments. Then, Tukey's procedure used when significant difference found
120 amongst the treatments. Statistical analysis fulfilled using SPSS for Window (V.24).

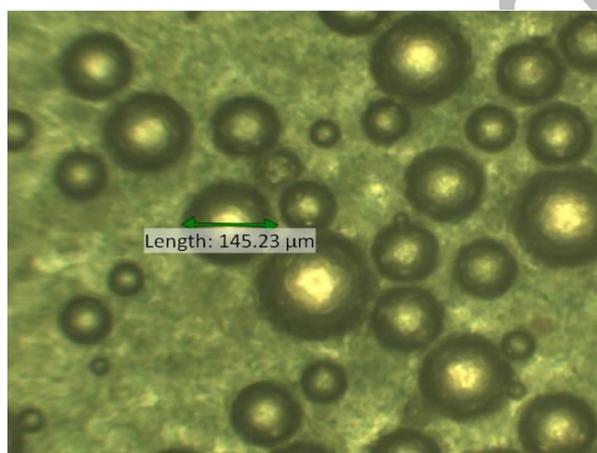
121

122

RESULTS AND DISCUSSION

123 The capsules were measured microscopically and the diameters ranged from about 100,98 -
124 187,94 μm and the average was 145,93 \pm 20.95 μm . Spirulina microcapsules were adequate shape and
125 size, stability in the brackish, as well (Fig.1). The length of larvae was about 2-2.5cm. The first
126 capsule and the second had a final composition of 57.4% and 47.5% crude protein, respectively,
127 whilst the control feed was 41%.

128

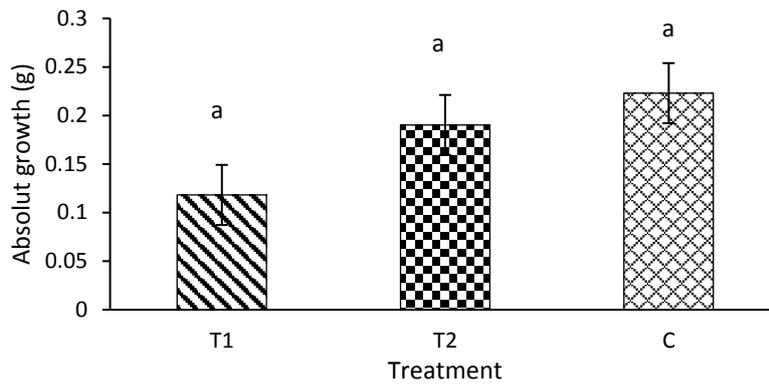


129

130 Figure 1 A Microphotograph showing the spirulina microcapsule (light microscope Boeco 10 x 10)

131

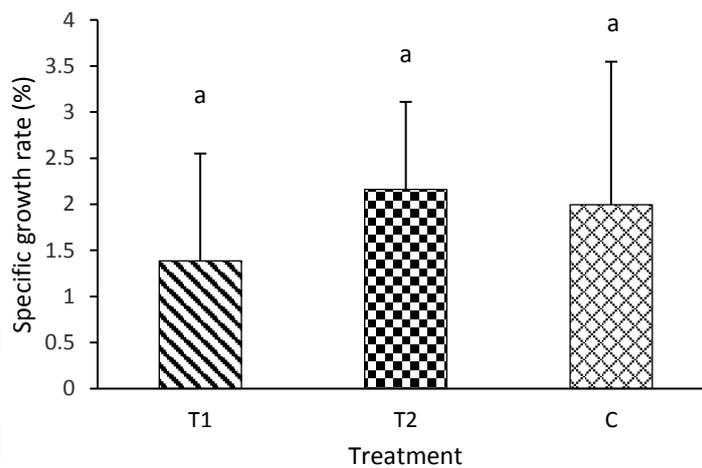
132 The absolute growth of *Chanos chanos* for period of 42 days showed in Fig.2. It was
133 observed that upon the the harvest the fish in the treatment 1, treatment 2 and control group reached
134 a weight of 0.1182 \pm 0.055 g; 0.1902 \pm 0.043 g and 0.2230 \pm 0.086 g, respectively. There was not
135 significantly different ($P > 0.05$) in the absolute growth of *Chanos chanos* larvae which were fed
136 microcapsule based on *Spirulina platensis*. It indicate that the nutritional components of spirulina-
137 based microcapsule fulfilled requirements for growth of *Chanos-chanos* larvae same as the
138 commercial diet.



139

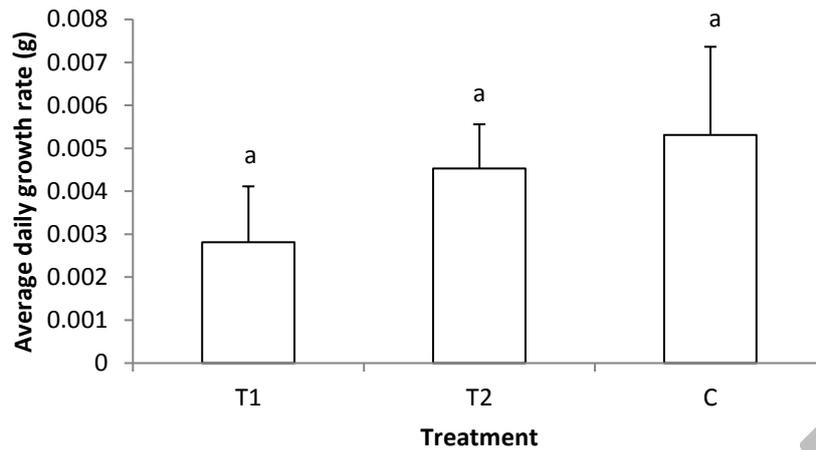
140 Figure 2 Absolute growth of *Chanos-chanos* larvae reared during 42 days of culture. Bars
 141 represented by same superscript letters indicate not significantly different values ($P >$
 142 0.05).
 143

144 It can be seen (Fig. 3) that specific growth rate of *Chanos-chanos* fed spirulina microcapsule
 145 1, 2 and the control was $1,39 \pm 1,16\%/d$; $2,16 \pm 0,95\%/d$; and $2,00 \pm 1,55\%/d$, respectively. The
 146 SGR of *Chanos chanos* fed both spirulina microcapsule diets and the control were not significantly
 147 different ($P > 0.05$).
 148



149

150 Figure 3 The specific growth rate (SGR) performance of *Chanos chanos* during 42 days of culture
 151

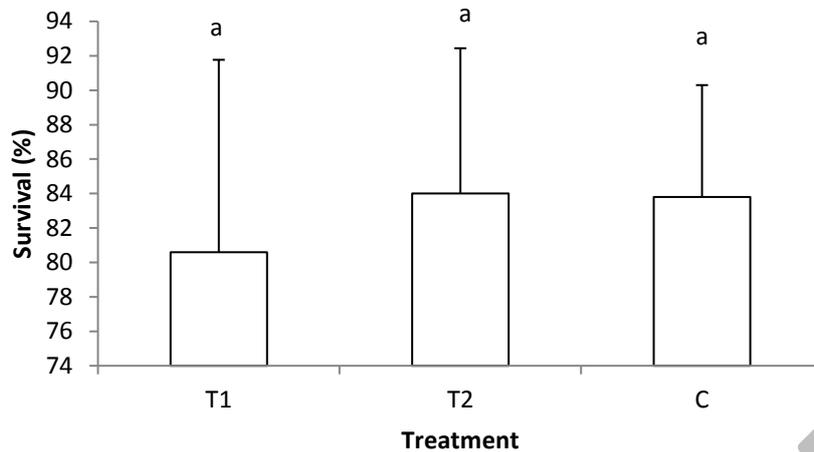


152

153 Figure 4 Average daily growth rate (ADGR) performance of milk-fish, *Chanos chanos* larvae
 154 during 42 days of culture. Bars represented by same superscript letters indicate not
 155 significantly different values ($P > 0.05$).
 156

157 ADGR of *Chanos-chanos* larvae which fed spirulina-based microcapsule on the treatment 1,
 158 2 and control group was $0,0028 \pm 0,001$ g/day; $0,0050 \pm 0,001$ g/day; dan $0,0053 \pm 0,003$ g/day,
 159 respectively (Fig.4). The effect of both spirulina-based microcapsule diets on the absolute growth
 160 rate (AG), specific growth rate (SGR) and average daily growth rate (ADGR) of *Chanos-chanos*
 161 larvae same as the commercial diet. It means that nutritional component inside the maicrocapsule
 162 matched the requirements of the larvae for their growth. In some studies showed that good larval
 163 growth were only achieved with micro-diets if feeding with live prey takes place. Live feed
 164 enrichment could improve the utilization of micro-diets. Larval red sea bream, *Pagrus major*, and
 165 Japanese flounder, *Paralichthys olivaceus* fed micro-diet together with live feed could keep up the
 166 growth and survival (Kanazawa, et al. 1989). Microdiet prepared using an internal gelation method
 167 was used to partially substitute the traditional live food (*Artemia*) for larval Atlantic halibut,
 168 *Hippoglossus hippoglossus* L. Microcapsule can be used to partially substitute the live food,
 169 *Artemia*, for Atlantic halibut, *Hippoglossus hippoglossus* L. larvae (Murray, et al. 2010). In the
 170 rearing marine fish larvae, gilthead sea bream, *Sparus aurata* L., live food could be substitute with
 171 microencapsulated diets, however, only limited growth was achieved (Langdon 2003; Yúfera et al.
 172 1999). For Giant-gouramy *Osphronemus gouramy*, a micro-diet together with *Tubifex* worm was
 173 only effective if introduced 22 days post hatching (Sukardi et al, 2018). A kappa-carrageenan-based
 174 micro-diet was also suitable for *Penaeus japonicus* larvae (Koshio et al., 1989).

175



176

177 Figure 5 Survival rates of Chanos-chanos larvae reared during 42 days of culture. Bars represented
 178 by same superscript letters indicate not significantly different values ($P > 0.05$).
 179

180 It can be seen in Fig. 4, the survival of Chanos chanos larvae was $80,6 \pm 11,17$ %;
 181 $84,6 \pm 8,44$ %; $83,8 \pm 16,50$ %. More than 80% survival of milk fish larvae was achieved in this
 182 experiment, which was higher as compared to the survival (32.7%) larvae fed phytoplankton,
 183 rotifers and brine shrimp nauplii (Eda, et al.1990). However, it was lower compared to Chanos-
 184 chanos larvae (94-97%) fed diets contained white fish meal and zein supplemented with amino
 185 acids (Borlongan and Benitez, 1990).

186

187

188

189

CONCLUSION

190 Microencapsulated diet showed prospect as a larval diet in milk-fish, Chanos-chanos,
 191 although not entirely successful. Growth is still limited to fish as in other micro-diets. Ever-
 192 changing the physical properties and chemical composition and the formulation of micro-capsules,
 193 such as particle size, amino acid composition, will improve the quality and health of milk fish
 194 larvae.

195

196

ACKNOWLEDGEMENTS

197 This study supported by a grant from The Ministries of Research, Technology, and Higher
 198 Education Republic of Indonesia under “Hibah Bersaing-Grant” and Jenderal Soedirman
 199 University, Indonesia.

200

201

REFERENCES

- 202 Aragão, C., R. Colen, S. Ferreira, W. Pinto, L.E.C. Conceição, J. Dias. 2014. Microencapsulation of
203 taurine in Senegalese sole diets improves its metabolic availability Senegalese sole (*Solea*
204 *senegalensis*) *Aquaculture* 431 (2014) 53–58.
- 205 Borlongan, I. G., L.V. Benitez. 1990. Quantitative lysine requirement of milkfish (*Chanos chanos*)
206 juveniles. *Aquaculture*, 87, 3-4, 341-347
- 207 Dubey, R., T.C. Shami, K.U. Bhasker Rao, 2009. Microencapsulation Technology and Applications.
208 *Defence Science Journal*. **Vol.59, No.1, pp.82-95.**
- 209 Eda, R., Murashige, B., Eastham, L., Wallace, P., Bass, C.S., Tamaru, C.S., Lee, 1990. Survival and
210 growth of milkfish (*Chanos chanos*) larvae in the hatchery. I. Feeding *Aquaculture* 89,3-4:
211 233-244
- 212 Ju, Z.Y., Forster, I.P., Conquest, L., Dominy, W., 2008. Enhanced growth effects on shrimp
213 (*Litopenaeus vannamei*) from inclusion of whole shrimp floc or floc fractions to a
214 formulated diet. *Aquaculture Nutrition* 14, 533–543.
- 215 Ju, Z.Y., Forster, I.P., Dominy, W.G., 2009. Effects of supplementing two species of marine algae
216 or their fractions to a formulated diet on growth, survival and composition of shrimp
217 (*Litopenaeus vannamei*). *Aquaculture* 292, 237–243.
- 218 Kanazawa, A., S.Koshio, S.Tesima. 1989. Growth and Survival of Larval Red Sea Bream *Pagrus*
219 *major* and Japanese Flounder *Paralichthys olivaceus* Fed Microbound Diets. *Journal of*
220 *World Aquaculture Soc.* **Vol.20,2,31-37.** DOI:10.1111/j.1749-7345.1989.tb00521.x
- 221 Koshio, S., A.Kanazawa, S-I. Teshima, J.D.Castell. 1989. Nutritional evaluation of crab protein for
222 larval *Penaeus japonicus* fed microparticulate diets. *Aquaculture*, **Vol.81, 2,145-154.**
- 223 Kuhn, D.D., Lawrence, A.L., Boardman, G.D., Patnaik, S., Marsh, L., Flick, G.J., 2010. Evaluation
224 of two types of bioflocs derived from biological treatment of fish effluent as feed
225 ingredients for Pacific white shrimp, *Litopenaeus vannamei*. *Aquaculture* 303, 303, 28–33.
- 226 Langdon, C. 2003. Microparticle types for delivering nutrients to marine fish larvae. In *Aquaculture*
227 (Vol. 227, pp. 259–275). [https://doi.org/10.1016/S0044-8486\(03\)00508-8](https://doi.org/10.1016/S0044-8486(03)00508-8)
- 228 Murray, H. M., Lall, S. P., Rajaselvam, R., Boutilier, L. A., Flight, R. M., Blanchard, B., Douglas,
229 S. E. (2010). Effect of early introduction of microencapsulated diet to larval Atlantic halibut,
230 *Hippoglossus hippoglossus* L. assessed by microarray analysis. *Marine Biotechnology*,
231 12(2), 214–229. <https://doi.org/10.1007/s10126-009-9211-4>
- 232 Prayogo, N. A., Wijayanti, G. E., Sulistyono, I., & Sukardi, P. 2016^a. Cloning and expression *cgnrh-ii*
233 and *sgnrh* genes in hard-lipped barb (*Osteochilus hasselti* c.v.). *Biodiversitas*, 17(29),
234 5230530.
- 235 Prayogo, N. A., Siregar, A., & Sukardi, P. 2016^b. The disruptive effect mercury chloride (HgCl) on
236 gene expression of *cGnRH-II*, *sGnRH*, and estradiol level in Silver Sharkminnow
237 (*Osteochillus hasseltii* CV). *Turkish Journal of Fisheries and Aquatic Sciences*, 16(4), 1003-
238 1009.
- 239 Saucedo P.E, A. González-Jiménez, H. Acosta-Salmón, J.M. Mazón-Suástegui, J.A. Ronsón-
240 Paulín. 2013. Nutritional value of microalgae-based diets for lion-paw scallop (*Nodipecten*
241 *subnodosus*) juveniles reared at different temperatures. *Aquaculture* 392-395, 113–119
- 242 Soomro, M.H., A.J.A.F. Memon, M. Zafar, A.B. Daudpota, M.A. Soomro, A.M. Ishqui. 2015. To
243 evaluate growth performance of Milkfish, *Chanos chanos* (Fingerling) applied range of food
244 treatments in captivity. *Journal of Interdisciplinary and Multidisciplinary* vol.2, 6, 168-173.
- 245 Sudaryono, A., Sukardi, P., Yudiarti, E., Hardi, E.H., Hastuti, S. and Susilowati, T. 2018. Potential
246 of using tropical brown macroalgae *Sargassum cristaeifolium* meal in the diets for juvenile

- 247 white shrimp (*Litopenaeus vannamei*). 1st International Conference on Tropical Studies and
248 Its Application (ICTROPS). IOP Publishing. IOP Conf. Series: Earth and Environmental
249 Science **144** (2018) 012049 doi :10.1088/1755-1315/144/1/012049
- 250 Sukardi, P., Hana, N.A. Prayogo, T. Harisam and P.H.T. Soedibyo. 2018. A Lipid-walled
251 microcapsule diet as co-feed for early weaning the *Osphronemus gouramy* Lacepede larvae.
252 Acta Scientiarum. Animal Sciences, 40, e38335, 2018. Doi:
253 10.4025/actascianimsci.v40i1.38335
- 254 Sukardi, P., T. Winanto, Hartoyo, T.B. Pramono. 2014. Microencapsulation of single-cell protein
255 from various microalgae species. Jurnal Akuakultur Indonesia 13 (2), 115–119.
- 256 Supamattaya, K., Kiriratnikom, S., Boonyaratpalin, M., Borowitzka, L., 2005. Effect of a *Dunaliella*
257 extract on growth performance, health condition, immune response and disease resistance in
258 black tiger shrimp (*Penaeus monodon*). Aquaculture 248, 207–216.
- 259 Tamaru, C.S., R. Murashige, L. Cheng-Sheng. 1994. The paradox of using background
260 phytoplankton during the larval culture of striped mullet, *Mugil cephalus* L. Aquaculture,
261 Vol.119, 2-3, 167-174
- 262 Umer H., H.Nigam, A.M.Tamboli, M.S.M. Nainar, 2011. Microencapsulation: Process, Techniques
263 and Applications. Review Paper. International Journal of Research in Pharmaceutical and
264 Biomedical Sciences. Vol.2(2)April-June.www.ijrpbsonline.com
- 265 van Dam, A.A., Beveridge, M.C.M., Azim, M.E., Verdegem, M.C.J., 2002. The potential of fish
266 production based on periphyton. Reviews in Fish Biology and Fisheries 12, 1–31.
- 267 Van Der Meeren, T., Mangor-Jensen, A., Pickova, J., 2007. The effect of green water and light
268 intensity on survival, growth and lipid composition in Atlantic cod (*Gadus morhua*) during
269 intensive larval rearing. Aquaculture 265, 206–217.
- 270 Wilson, N. and N.P. Shah, N.P. 2007. Microencapsulation of Vitamins. ASEAN Food Journal 14
271 (1): 1-14.
- 272 Xie, Z., Wang, F., Liu, H., Guo, S., Zhu, A., Niu, H., 2010. Gelatin-walled microencapsulated diet
273 for larval shrimp (*Penaeus japonicus* Bate) manufactured using the fluidized bed coating
274 process. Aquaculture Research 42, 65–73. doi:10.1111/j.1365-307 2109.2010.02557.
- 275 Yúfera, M., E. Pascual and C. Fernández-Díaz. 1999. A Highly Efficient Microencapsulated Food
276 for Rearing Early Larvae of Marine Fish. Aquaculture 177: 249–256.
- 277 Yuwono, E. and Sukardi, P. 2009. Development of an environment-friendly feeding management
278 for pond-reared fish species in the Segara Anakan region. Regional Environmental Change
279 9(4): 329-333.