ACCEPTED MANUSCRIPT

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

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

DOI: 10.11598/btb.2019.26.3.1103

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

Received date : 20 July 2018
Accepted date : 06 December 2018

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

Purnama Sukardi[1*], Noprie Yansah[1], Tjahyo Winanto[3], Sri Marnani[1], Norman A. Prayogo[2], Taufan Harisam[3] and Agung Sudaryono[4]

[1]Department of Aquaculture Universitas Jenderal Soedirman, Purwokerto 53123, Indonesia
[2]Department of Water Resource Management, Purwokerto 53123, Indonesia
[3]Department of Marine Science, Universitas Jenderal Soedirman, Purwokerto 53123, Indonesia
[4]Department of Aquaculture, University of Diponegoro, Semarang, Indonesia.
[*]Corresponding author: purnamas@unsoed.ac.id
[**]This paper was presented at the 2nd Scientific Communication in Fisheries and Marine Sciences (SCiFiMaS 2018), 07-09 May 2018, Purwokerto, Central Java, Indonesia

Running title: Single cell protein diet for milk fish larvae

ABSTRACT

The purpose of this study was to evaluate differences between spirulina-based microcapsules and commercial diets on absolute, daily and specific growth and survival rates of milkfish larvae. *Spirulina platensis* was as a core diet in microcapsules with different matrix (walls). The first capsule wall was gelatin and fish oil, while the second capsule wall was gelatin, fish oil and whole egg. The control group was commercial diet. A total of 1200 larvae were used in this experiments using recirculation systems. Larvae are fed three times a day and increased regularly when the size of the larvae increases. The results showed that the effect of both sprirulina-based microcapsule diets on the absolute growth rate (AG), specific growth rate (SGR) and average daily growth rate (ADGR) of Chanos-chanos larvae fed spirulina-based microcapsule same as them which to be fed using commercial diet. The survival rate was as 80.6±11,171%; 84.6±8.443%; 83.8±16.496%, respectively. This study showed that Spirulina-based microcapsules had the same effect as commercial feed on the growth of milkfish larvae which means that this diet could replace commercial diet.

Keywords: microcapsule wall, Spirulina, Chanos chanos

INTRODUCTION

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

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

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

MATERIALS AND METHODS

Writing the Materials and Methods

A recirculating system were applied in which every tanks aerated with air stones. Three group experiments were carried out wherein each group consisted of three cylinder tanks (50 L) contained 100 fish with size of 1-2 cm and weight of 0,11-0,21g (equivalent to a fish density of 2 L water volume) maintained at 27-29^{0}C, for feeding trials. Each of the group was conducted using three tanks randomly. Microencapsulated diets were designed using two different wall materials, the first spirulina capsule (treatment 1) was designed use wall consisted of gelatin and fish oil, whereas the second one (treatment 2) was eggs, gelatin and fish oil. **Fish oil used as an attractant flavor**. The control group (treatment 3) was commercial feed. The alga species, Spirulina platensis, was cultured as described previously (Sukardi, et al. 2014). The algal species as inclusion materials of microencapsulated diets were harvested when reach stationary phases at a density of **73442 x 10^4 cell^{-mL}** Spirulina platensis. Capsule particles produced by a modification method of the thermal

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

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

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

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

Growth parameter

Absolute Growth = weight gain (g), AG(g)= Wt – Wi, where Wt is final weight (g), Wi is initial weight (g). Average daily growth rate=ADGR= [{Wt-Wi}/T], where Wt is final weight (g), Wi is the weight of fish at time 0 and T is a culture periode in days of experiment. Specific Growth Rate=SGR (%/d)= 100[(lnWt – lnWi)/T], where Wt is final weight (g), Wi is the weight of fish at time 0, T is a culture periode in days of experiment. Survival(%)=(Total number of fish survived/Total number of fish stocked)x100.

Statistical analysis

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

RESULTS AND DISCUSSION

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

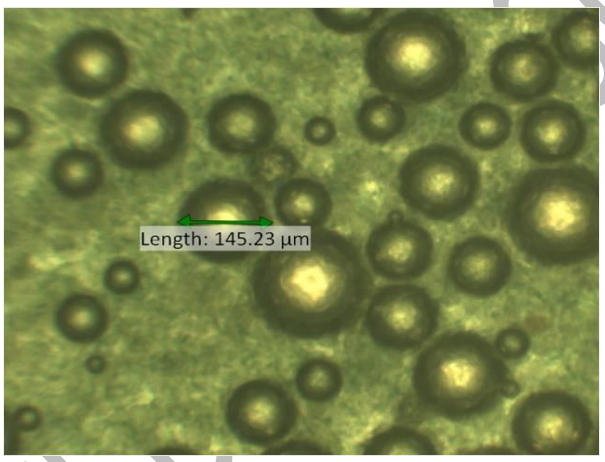

Figure 1 A Microphotograph showing the spirulina microcapsule (light microscope Boeco 10 x 10)

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

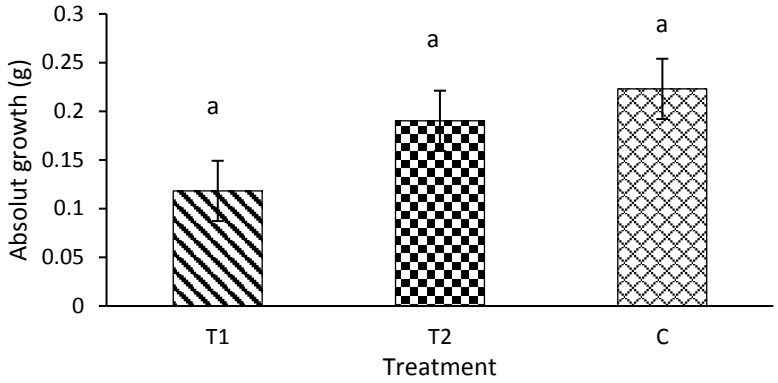

Figure 2 Absolute growth of Chanos-chanos larvae reared during 42 days of culture. Bars represented by same superscript letters indicate not significantly different values (P > 0.05).

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

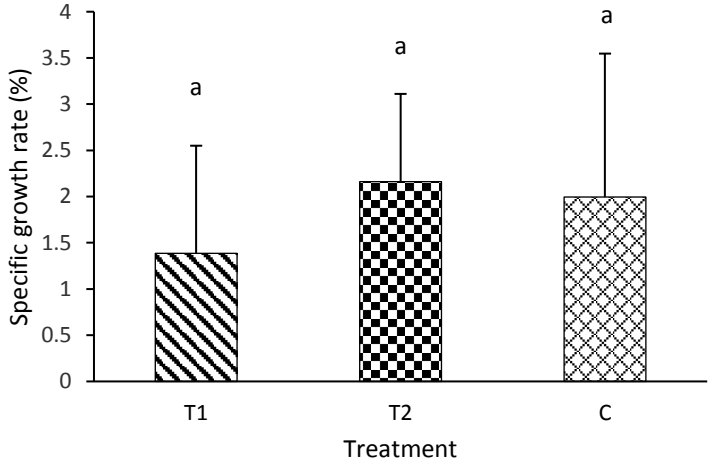

Figure 3 The specific growth rate (SGR) performance of Chanos chanos during 42 days of culture

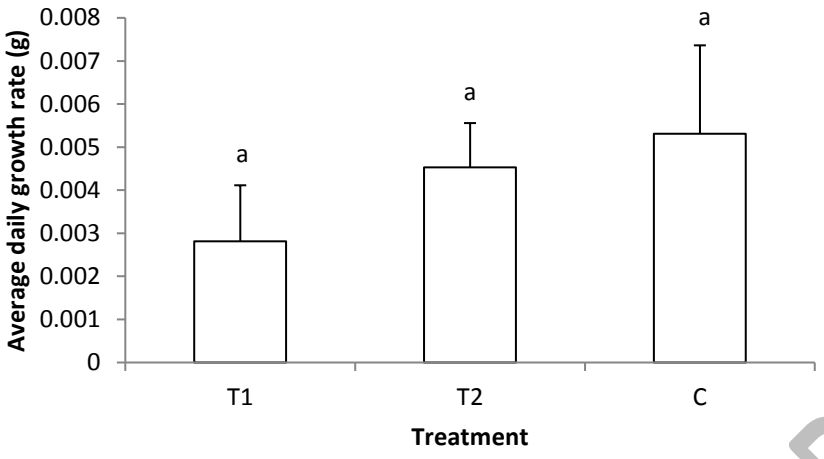

Figure 4 Average daily growth rate (ADGR) performance of milk-fish, Chanos chanos larvae during 42 days of culture. Bars represented by same superscript letters indicate not significantly different values (P> 0.05).

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

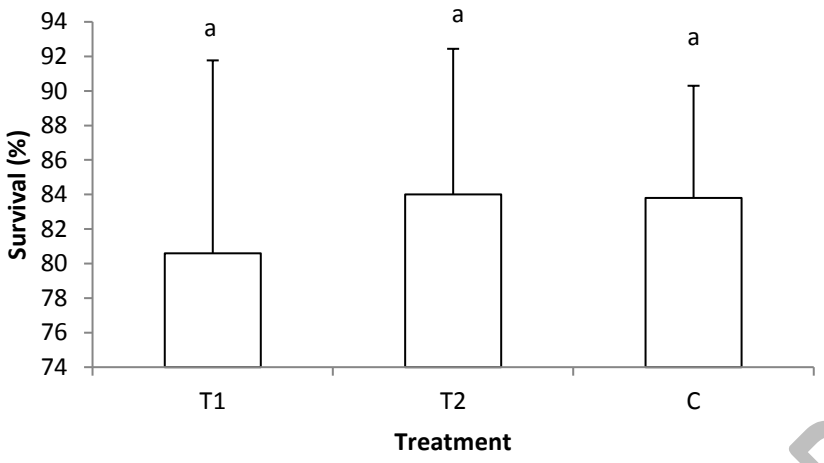

Figure 5 Survival rates of Chanos-chanos larvae reared during 42 days of culture. Bars represented by same superscript letters indicate not significantly different values (P>0.05).

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

CONCLUSION

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

ACKNOWLEDGEMENTS

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

REFERENCES

Aragão,C, R. Colen, S. Ferreira, W. Pinto, L.E.C. Conceição, J. Dias. 2014. Microencapsulation of taurine in Senegalese sole diets improves itsmetabolic availability Senegalese sole (Solea senegalensis) Aquaculture 431 (2014) 53–58.

Borlongan, I. G., L.V. Benitez.1990. Quantitative lysine requirement of milkfish (Chanos chanos) juveniles. Aquaculture, 87, 3-4, 341-347

Dubey, R., T.C. Shami, K.U.Bhasker Rao, 2009. Microencapsulation Technology and Applications. Defence Science Journal. **Vol.59, No.1.pp.82-95.**

Eda, R. Murashige, B. Eastham, L. Wallace, P. Bass, C.S. Tamaru, C.S. Lee, 1990. Survival and growth of milkfish (Chanos chanos) larvae in the hatchery. I. Feeding Aquaculture 89,3-4: 233-244

Ju, Z.Y., Forster, I.P., Conquest, L., Dominy, W., 2008. Enhanced growth effects on shrimp (Litopenaeus vannamei) from inclusion of whole shrimp floc or floc fractions to a formulated diet. Aquaculture Nutrition 14, 533–543.

Ju, Z.Y., Forster, I.P., Dominy, W.G., 2009. Effects of supplementing two species of marine algae or their fractions to a formulated diet on growth, survival and compo- sition of shrimp (Litopenaeus vannamei). Aquaculture 292, 237–243.

Kanazawa, A., S.Koshio, S.Tesima. 1989. Growth and Survival of Larval Red Sea Bream Pagrus major and Japanese Flounder Paralichthys olivaceus Fed Microbound Diets. Journal of World Aquaculture Soc. **Vol.20,2,31-37**.DOI:10.1111/j.1749-7345.1989.tb00521.x

Koshio, S., A.Kanazawa,S-I. Teshima, J.D.Castell. 1989. Nutritional evaluation of crab protein for larval Penaeus japonicus fed microparticulate diets. Aquaculture, **Vol.81, 2,145-154.**

Kuhn, D.D., Lawrence, A.L., Boardman, G.D., Patnaik, S., Marsh, L., Flick, G.J., 2010. Evaluation of two types of bioflocs derived from biological treatment of fish efflu- ent as feed ingredients for Pacific white shrimp, Litopenaeus vannamei. Aquaculture 303, 303, 28–33.

Langdon, C. 2003. Microparticle types for delivering nutrients to marine fish larvae. In Aquaculture (Vol. 227, pp. 259–275). https://doi.org/10.1016/S0044-8486(03)00508-8

Murray, H. M., Lall, S. P., Rajaselvam, R., Boutilier, L. A., Flight, R. M., Blanchard, B.,Douglas, S. E. (2010). Effect of early introduction of microencapsulated diet to larval Atlantic halibut, Hippoglossus hippoglossus L. assessed by microarray analysis. *Marine Biotechnology*, *12*(2), 214–229. https://doi.org/10.1007/s10126-009-9211-4

Prayogo, N. A., Wijayanti, G. E., Sulistyo, I., & Sukardi, P. 2016[a]. Cloning and expression cgnrh-ii and sgnrh genes in hard-lipped barb (Osteochilus hasselti c.v.). Biodiversitas, 17(29), 5230530.

Prayogo, N. A., Siregar, A., & Sukardi, P. 2016[b]. The disruptive effect mercurychloride (HgCl) on gene expression of cGnRH-II, sGnRH, and estradiol level in Silver Sharkminnow (Osteochillus hasseltii CV). Turkish Journal of Fisheries and Aquatic Sciences, 16(4), 1003-1009.

Saucedo P.E, A. González-Jiménez, H. Acosta-Salmón, J.M. Mazón-Suástegui, J.A. Ronsón-Paulín.2013. Nutritional value of microalgae-based diets for lions-paw scallop (Nodipecten subnodosus) juveniles reared at different temperatures. Aquaculture 392-395, 113–119

Soomro, M.H., A.J.A.F. Memon, M. Zafar, A.B. Daudpota, M.A. Soomro, A.M. Ishqui. 2015. To evaluate growth performance of Milkfish, Chanos chanos (Fingerling) applied range of food treatments in captivity. Journal of Interdisciplinary and Multidisciplinary vol.2, 6, 168-173.

Sudaryono, A., Sukardi, P., Yudiarti, E., Hardi, E.H., Hastuti, S. and Susilowati, T. 2018. Potential of using tropical brown macroalgae Sargassum cristaefolium meal in the diets for juvenile

white shrimp (Litopenaeus vannamei). 1st International Conference on Tropical Studies and Its Application (ICTROPS). IOP Publishing. IOP Conf. Series: Earth and Environmental Science **144** (2018) 012049 doi :10.1088/1755-1315/144/1/012049

Sukardi, P., Hana, N.A. Prayogo, T. Harisam and P.H.T. Soedibyo. 2018. A Lipid-walled microcapsule diet as co-feed for early weaning the Osphronemus gouramy Lacepede larvae. Acta Scientiarum. Animal Sciences, 40, e38335, 2018. Doi: 10.4025/actascianimsci.v40i1.38335

Sukardi, P., T. Winanto, Hartoyo, T.B. Pramono. 2014. Microencapsulation of single-cell protein from various microalgae species. Jurnal Akuakultur Indonesia 13 (2), 115–119.

Supamattaya, K., Kiriratnikom, S., Boonyaratpalin, M., Borowitzka, L., 2005. Effect of a Dunaliella extract on growth performance, health condition, immune response and disease resistance in black tiger shrimp (Penaeus monodon). Aquaculture 248, 207–216.

Tamaru, C.S., R. Murashige, L. Cheng-Sheng. 1994. The paradox of using background phytoplankton during the larval culture of striped mullet, Mugil cephalus L. Aquaculture, Vol.119, 2-3, 167-174

Umer H., H.Nigam, A.M.Tamboli, M.S.M. Nainar, 2011. Microencapsulation: Process, Techniques and Applications. Review Paper. International Journal of Research in Pharmaceutical and Biomedical Sciences. Vol.2(2)April-June.www.ijrpbsonline.com

van Dam, A.A., Beveridge, M.C.M., Azim, M.E., Verdegem, M.C.J., 2002. The potential of fish production based on periphyton. Reviews in Fish Biology and Fisheries 12, 1–31.

Van Der Meeren, T., Mangor-Jensen, A., Pickova, J., 2007. The effect of green water and light intensity on survival, growth and lipid composition in Atlantic cod (Gadus morhua) during intensive larval rearing. Aquaculture 265, 206–217.

Wilson, N. and N.P. Shah, N.P. 2007. Microencapsulation of Vitamins. ASEAN Food Journal 14 (1): 1-14.

Xie, Z., Wang, F., Liu, H., Guo, S., Zhu, A., Niu, H., 2010. Gelatin-walled microencapsulated diet for larval shrimp (Penaeus japonicus Bate) manufactured using the fluidized bed coating process. Aquaculture Research 42, 65–73. doi:10.1111/j.1365-307 2109.2010.02557.

Yúfera, M., E. Pascual and C. Fernández-Díaz. 1999. A Highly Efficient Microencapsulated Food for Rearing Early Larvae of Marine Fish. Aquaculture 177: 249–256.

Yuwono, E. and Sukardi, P. 2009. Development of an environment-friendly feeding management for pond-reared fish species in the Segara Anakan region. Regional Environmental Change 9(4): 329-333.