Eicosapentaenoic acid (EPA) and Docosahexaenoic acid (DHA) Mass Production Strategy: Technology to maximize egg production of Nilem fish (*Osteochilis Hasselti*)

R. Umar Hasan Saputra*, Djadja Subardja Sjafei* and D. Sastradipradja**

***Dept. of Aquatic Resources, Fac. of Fisheries and Marine Science, Institut Pertanian Bogor, Kampus IPB Darmaga, Bogor**

****Dept. of Biology, Fac. of Medicine, Krida Wacana Christian University, Jalan Tanjung Duren Raya 4, Jakarta Barat 11470**

Introduction

Feeding the growing world population, particularly in developing countries, is the grand challenge of the future. This holds true not only on a quantitative basis, but qualitative as well. Eicosapentaenoic acid (EPA) and Docosahexaenoic acid (DHA) are highly unsaturated fatty acids (HUFA) of the ω-3 family which are important for brain and nerve development of infants and for the prevention of coronary heart diseases. Since humans are uncapable of synthisizing EPA and DHA, their requirement for those nutrients must be supplied with their food.

In principle, EPA and DHA can only be produced by fish, whether marine or fresh water. EPA and DHA in marine fishes originated from their feed, i.e. micro-organisms and plankton rich in both HUFAs, while fresh water fish have the ability to synthesize both from precursors linolenic (18:3n) and linoleic (18:6n) acids (Kanazawa *et al.*, 1979). In addition, it should be remembered that in fish, most fatty acids are localized in eggs because egg is the place where fat and protein are stored during the process of vitellogenesis. For that reason, for the present, the fastest way of successful EPA and DHA mass production should be through fish egg production. Induced sex manipulation (Donaldson and Benfey, 1987) to make females is desired.

The freshwater nilem fish (*Osteochilis hasselti*) is the most appropriate fish for egg production. Through feminisation technique of nilem fish larvae population with a time period for the formation of eggs, calculated from hatching, being around 6 month and a favourable ratio between egg- and body-weight, should make this freshwater fish an ideal source for mass production of EPA and DHA. The biosynthetic ability to make EPA and DHA of nilem fish should be elucidated. From information on the biosynthetic pathway, the optimal precursor composition in fish ration can be obtained for maximal EPA and DHA production and the respective enzymes involved known. With the advance in biotechnological engineering techniques, the responsible genes for the expression of the enzyme could be identified for use for transgenesis in other fishes of consumer's preference (e.g. gourami, other common carp, eel, etc.). After conclusion of all these stages of studies, we are confident that food products from fish will have high EPA and DHA contents to ensure more intelligent population than is the situation today.

In the present report the authors discuss results of their study to develop a technology to maximize egg production of nilem fish.

Materials and Methods

The study consisted of 4 parts, i.e. (1) Production potential of nilem fish, (2) Environmental factors for survival and growth of nilem larvae, (3) Feminisation of larvae, and (4) Technique to obtain maturity of gonads.

(1) Production potential of nilem fish.

Fish specimens with mature gonads were obtained from 5 locations in West Java. Collection of samples were carried out 3 times, which was by the end of the dry season, at the start and at the middle of the rainy season. Observations were made on Total length (cm), Fecundity (counts), Gonado-Somatic Index, Egg Diameter Distribution (mm) and Smallest size of Mature Gonad (cm).

(2) Environmental factors for survival and growth of nilem larvae.

Five day old larvae resulting from spawning material from Sukabumi were used for the experiments on the influence on salinity (0, 3, 6 and 9 parts per thousand – ppt in aquariums). The influence of Ca^{2+} on survival and growth used concentration treatments 25, 50 and 75 mg/l, while the influence of temperature of the media used 26, 28, 30 and 32^{o}C. Observations were made on survival rate and growth parameters.

(3) Feminisation.

The experiment used larvae at eye formation growth stadium ($\pm$ 40h post hatching). The larvae were immersed in media where estradiol is dissolved at 0, 50, 100 and 200 μg/l. Immersion was applied for 12h.

(4) Technique to obtain maturity of gonads.

In natural condition, gonad maturity stage (GMS) IV is reached at 18 month of age. This experiment attempted to hasten reaching that stage earlier by offering the fishes feed varying in vit. E content (0, 200, 400 and 600 mg/kg feed). The measured parameters were GMS IV and gonado-somatic index (GSI) of the reproducing fish.

Results and Discussion

Results of the study on production potential are presented in Table 1. For resons of early gonad maturity at relative small body size and high GSI, nilem fish from Cianjur appeared to be the best for egg production.

Results of the experiments on environmental factors for survival and growth of nilem larvae are depicted in figures 1 and 2 for salinity and figures 3 and 4 for the influence on temperature of the media. Optimal salinity for survival rate of larvae is 3 ppt (95.66%) and for growth is 0 ppt. At salinity of 3, 6 and 9 ppt, larvae will lost water with the consequence that for compensation of the water loss, the larvae increase drinking resulting in more energy being expended which should otherwize be deposited as growth. The tendency of decrement of body weight is visualized by the exponential curve as shown in figure 2.

Both survival (81.89%) and growth rate (13.53%) are the best when rearing the larvae at 28^{o}C. From the curve presented in Figure 3, an optimal temperature of 29.9 ^{o}C is found forsurvival rate where matabolic reactions will be optimal. For optimal growth rate however, a temperature of 27.75 ^{o}C was obtained (Figure 4).

Table 1. Reproduction potensial of nilem fish from various breeding localities: Sukabumi, Cianjur, Tasikmalaya, Karawang dan Purwakarta

No	Location	Total length (cm)		Fecundity (counts)		Gonado-Somatic Index*		Egg Diameter Distribution (mm)*		Smallest size of Mature Gonad (cm)	
		Range	Mean	Range	Rataan	Range	Rataan	Range	Rataan	Total length	Stand.le
1	Sukabumi	16,3-21,8	19,0	27920-119568	103.786	11,5-19,45	15,31	0,60-1,84	1,25	16,5	12.0
2	Cianjur	10,5-18,3	13,97	31828-139816	66.060	16,61-26,83	21,72	0,84-1,68	1,24	13,3	9.2
3	Tasikmalaya	15,9-25,5	19,9	55290-137941	92.269	18,38-24,42	21,40	0,72-1,91	1,26	17,2	12.2
4	Garut	12,3-26,7	18,9	33928-163823	83.440	4,49-24,53	14,51	0,60-1,40	0,99	17,0	12.1
5	Karawang	12,4-31,7	19,9	75787-225282	123.215	9,45-29,49	19,47	0,80-1,78	1,26	21,7	14.6
6	Purwakarta	10,5-23,0	20,33	16431-160163	86.978	7,79-25,27	16,53	0,64-1,60	1,12	18,5	13.6

* = for fish gonad maturity stage GMS III or IV

Table 2. Survival and growth rates of nilem larvae as influenced by addition of Ca^{2+}.

	Treatment			
Parameter	0 mg/l	25 mg/l	50 mg/l	75 mg/l
Mean survival rate (%)	22.78	56.11	85.67	77.67
Daily growth rate (mg/d)	16.18	13.17	12.05	12.18

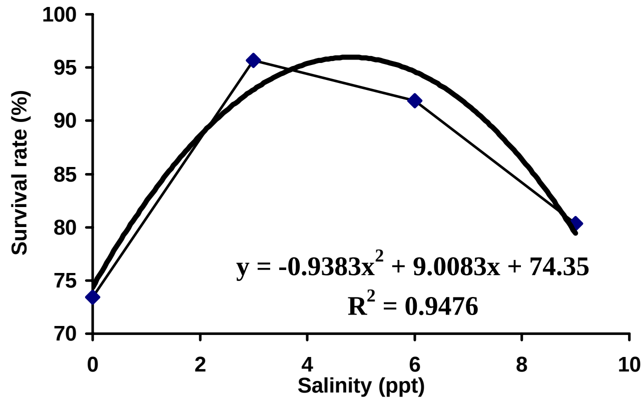

Figure 1. Survival - Salinity relationship of Nilem fish.

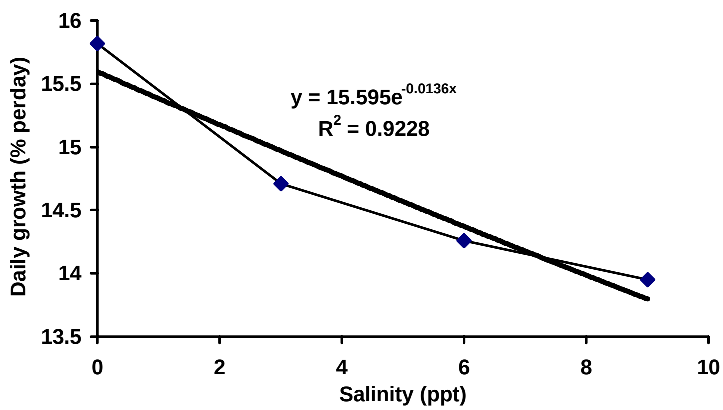

Figure 2. Daily growth rate – salinity relationship of Nilem fish.

The influence of Ca^{2+} on survival and growth rate arepresented in Table 2. The data show that addition of Ca^{2+} at 50 mg/l is the highest for survival rate (90%). Optimal survival rate is reached at a Ca^{2+} concentration of 61.11 mg/l.

The influence of dosis on larvae immersed in estradiol containing media are depicted in Figure 5 for 12 h immersion. A dose of 200 µg/l estradiol will result in 78.78% female production.

After rearing during 5 months, gonad maturity reached at the different vitamin E treatments are presented in Figure 6. Highest GMS IV are reached with vit. E treatment 400 mg/l, i.e. 75%. GSI as revealed in Figure 7 are also reached with vit. E 400 mg/l at around 21%.

Conclusion

(1). Nilem fish from Cianjur appeared to be the best for egg production.
(2). Salinity, temperature of media and addition of Ca^{2+} significantly affect survival and daily growth rate of larvae.
(3). At serx reversal, the highest female percentage is obtaineded with immersion for 12 h in medium containing 200 µg/l estradiol.
(4). For gonad maturation, the dose of vit. E supplementation is 400 mg/l to get the best GMS IV and GSI.

References

Donaldson, E.M. and T.J. Benfey (1987). In: Proc. Third Int'l Symp. Repro. Physio. Of Fish p:108-119.
Kanazawa, A. S.I. Teshima and K. Ono (1979). Comp. Biochem. Physiol. 63B:295-29298.

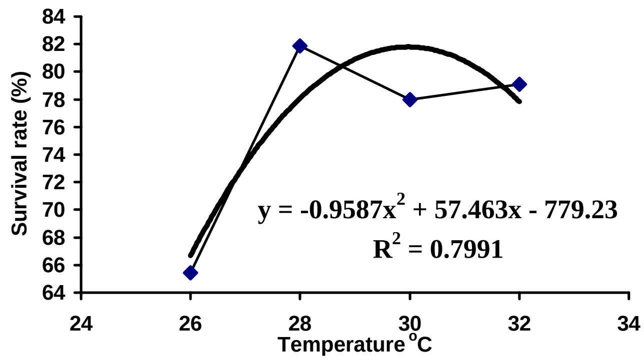

Figure 3. Relationship between survival rate of Nilem fish and temperature of media.

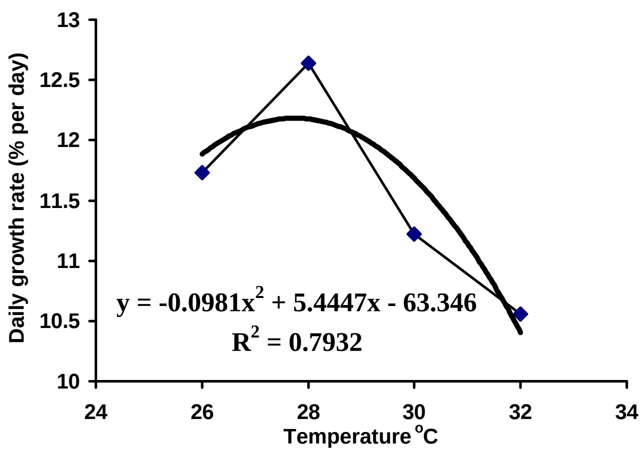

Figure 4. Relationship between daily growth rate of Nilem fish and temperature of media

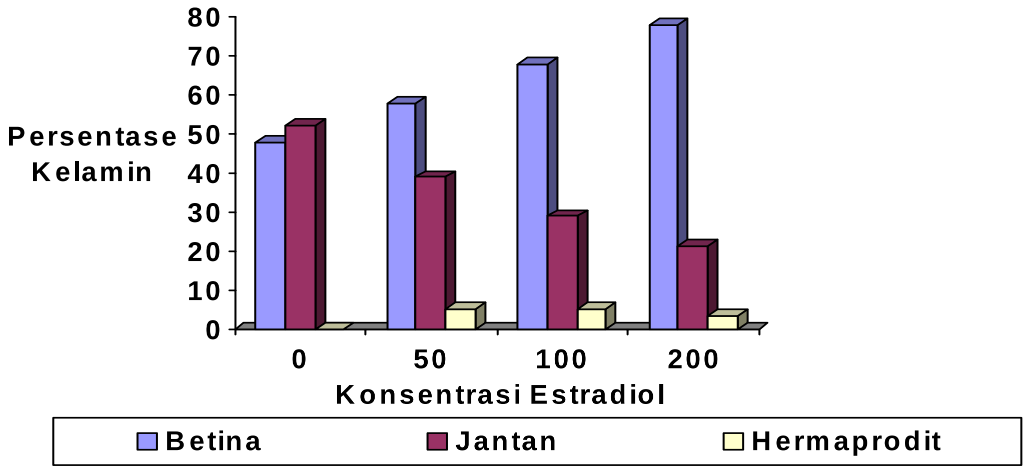

Figure 5. The influence of dose of estradiol in medium to feminisation of nilem larvae. (immersion of 12 h).

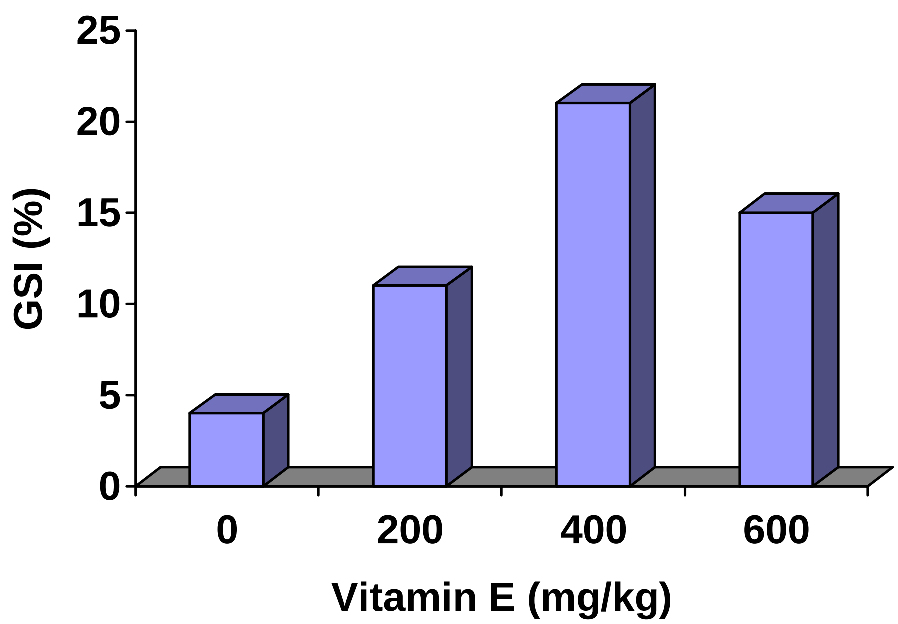

Figure 6. Gonado-somatic index of nilem fish.