Aquaculture, 90 (1990) 63–74
Elsevier Science Publishers B.V., Amsterdam

Improved larval survival at metamorphosis of Asian seabass (*Lates calcarifer*) using ω3-HUFA-enriched live food

Philippe Dhert[a,b], Patrick Lavens[b], Marietta Duray[a] and Patrick Sorgeloos[b]

[a]*Southeast Asian Fisheries Development Center, Aquaculture Department, P.O. Box 256, Iloilo City (The Philippines)*

[b]*Laboratory of Aquaculture and Artemia Reference Center, Faculty of Agricultural Sciences, State University of Ghent, Rozier 44, B-9000 Ghent (Belgium)*

(Accepted 25 January 1990)

ABSTRACT

Dhert, Ph., Lavens, P., Duray, M. and Sorgeloos, P., 1990. Improved larval survival at metamorphosis of Asian seabass (*Lates calcarifer*) using ω3-HUFA-enriched live food. *Aquaculture*, 90: 63–74.

Asian seabass (*Lates calcarifer*) larvae were fed *Brachionus* cultured on *Chlorella* and, as soon as ingestion was possible, different types of *Artemia*, i.e., nauplii of the San Francisco Bay (SFB) strain, Great Salt Lake (GSL) strain or GSL nauplii that had been bioencapsulated with an emulsion containing high levels of the ω3-HUFAs (highly unsaturated fatty acids) 20:5 and 22:6. San Francisco Bay *Artemia* with a good natural fatty acid profile and small body size could be offered earlier than the larger but HUFA-poor Great Salt Lake strain. The poor nutritional quality of the latter, however, could be corrected by enriching the nauplii with an ω3-HUFA emulsion for 24 h, after which time high levels of the ω3-HUFAs 20:5 and 22:6 were obtained. When the *Artemia* diet offered before metamorphosis included natural or supplemented essential fatty acids, no significant differences in dry weight, length or survival of the fish were noticed, as compared to fish fed the naturally deficient GSL *Artemia*. Onset of metamorphosis and physiological condition after metamorphosis, however, were influenced by the HUFA content of the ingested prey. Seabass larvae fed SFB or enriched GSL *Artemia* started metamorphosis on day 19, while those in the non-enriched series never achieved metamorphosis and died of a nutritional deficiency syndrome by day 27. An indication of the physiological condition of the larvae and the early detection of the syndrome was possible by subjecting 21- and 25-day-old larvae to a stress test: abrupt exposure of the larvae to 65-ppt saline water resulted in abundant and early mortality in HUFA-deficient fish larvae. Fry receiving ω3-HUFA-fortified *Artemia* had a superior physiological condition which was reflected by significantly lower mortality figures in the stress test.

INTRODUCTION

During recent years success in larviculture of European seabass (*Dicentrarchus labrax*) and seabream (*Sparus aurata*) has improved due to the appli-

0044-8486/90/$03.50

cation of ω3-HUFA (highly unsaturated fatty acid) enrichment of the larval diets *Brachionus* and *Artemia*.

The variability of the nutritional value of live foods for marine larval fish is well documented (Sick, 1976; Watanabe et al., 1978, 1980; Claus et al., 1979; Bottino et al., 1980; Kuhlmann et al., 1981; Léger et al., 1986). In the early 1980s it was shown that the nutritional value of *Artemia* and rotifers is mainly governed by the presence in sufficient quantities of the highly unsaturated fatty acids 20:5ω3 and 22:6ω3. In this regard, Léger et al. (1986) demonstrated that when these components are present in inadequate amounts, the HUFA content of brine shrimps and rotifers can be enhanced by allowing them to take up emulsified HUFAs. Various enrichment techniques have been elaborated for optimal enrichment of these live prey. For example, by using the self-emulsifying product SELCO (Artemia Systems NV-SA, Ghent, Belgium), the ω3-HUFA levels in *Artemia* can be increased from about 2 mg/g DW in freshly hatched nauplii to about 40 mg/g DW after 24 h of enrichment. Applying these new enrichment diets and techniques, various studies have been conducted to evaluate the beneficial effects of feeding these enriched prey to different marine fish species. Significantly improved culture success, in terms of larval growth, survival, metamorphosis and resistance to stress conditions, has been documented for European seabass and bream, and for the Pacific rabbitfish and dolphinfish (Sorgeloos et al., 1988).

Lates calcarifer, also called giant sea perch, Asian seabass or barramundi, has a high commercial value and can be cultured at different salinities; it is therefore considered to be a good candidate species for aquaculture, especially in South East Asia and the Pacific. In 1983 aquaculture production averaged over 2500 tonnes, the main producers being Thailand, Indonesia and Malaysia (SEAFDEC, 1983).

Using *Brachionus* and *Artemia* as live foods, new culturing techniques were developed in Thailand for larval production. Reported survival levels were about 40% during the hatchery and nursery phase (Maneewong et al., 1986a,b). Problems, however, persisted: kidney disease, overinflation of the swimbladder, high stress sensitivity, etc. (Danayadol, 1984; Danayadol and Boonranapanichagit, 1984; Bagarinao and Kungvankij, 1986). It was postulated that manipulation of the nutritional composition of the food could possibly overcome these problems (Rodgers and Barlow, 1987; Buranapanidgit et al., 1988).

In this regard we investigated the effects of HUFA-enriched *Artemia* diets on the larval rearing of *Lates calcarifer* at the Tigbauan Hatchery of the Aquaculture Department of SEAFDEC in the Philippines.

MATERIALS AND METHODS

Fertilized seabass eggs were obtained from broodstock reared on trash fish (5% body weight/day) in floating cages at Igang Research Station and shipped

to the laboratory site in Tigbauan, Iloilo, the Philippines for incubation and hatching.

In the first experiment, the influence of feeding ω3-HUFA-enriched *Artemia* versus non-enriched brine shrimp nauplii was evaluated. Sixteen tanks of 200 l capacity each were used. Newly hatched seabass larvae were introduced at densities of 30 larvae per liter (water temperature 26–29 °C; salinity 32–34 ppt). *Brachionus*, cultured on *Chlorella*, was added from day 2 until day 12. Freshly hatched *Artemia* (instar I) from San Francisco Bay (SFB; small, HUFA-rich strain) and Great Salt Lake (GSL; large, HUFA-deficient strain) were administered from day 10 or later, depending on the mouth size of the fish larvae (Dhert et al., 1990). HUFA-fortified GSL nauplii (GSLE), which were enriched for 24 h with the self-emulsifying lipid concentrate SELCO according to the technique described by Léger et al. (1987), were added from day 11 onwards. Four different feeding regimes with regard to *Artemia* were used. In the first two treatments fish larvae received SFB *Artemia* in the beginning and GSL or enriched GSL (GSLE) in a later stage. In the other two treatments larvae received GSL from the start and, in one case, this diet was again substituted by enriched *Artemia* in a later stage.

In order to find out from what larval stage a HUFA diet is essential, a second set of experiments was carried out in which the addition of enriched GSL nauplii was gradually delayed. The first period of the culture was analogous to the experiment described above. On day 10, the animals were transferred to smaller tanks (50 l) and maintained on a *Brachionus* and a HUFA-poor *Artemia* diet for 3 days. Starting on day 14, one container was given the fatty acids-fortified diet (treatment 1) while all other containers received newly hatched (treatment 2, 3, etc.), but HUFA-poor *Artemia*. Every successive day, a new tank was subsequently included in the enrichment programme. In both experiments the food distribution varied from 0.1 to 1.5 *Artemia* per ml depending on the requirement of the fish (Table 1). Dead fish were removed and counted daily, after which 50% of the water volume was replaced by filtered seawater.

Besides length and weight measurements, the physiological condition of the larvae was evaluated by measuring their resistance to stress conditions using a salinity-stress test (Dhert et al., in prep.). In the first experiment 10 fish larvae 21 days old were transferred from each culture tank (four tanks per treatment) to beakers and exposed to saline water (65 ppt). In the second experiment replicates were obtained by three repetitive samplings of 10 fish. Mortality of the fish was monitored at constant time intervals, after which the cumulative mortalities in each time interval were summed. One-way ANOVA and Duncan's multiple range test were used in detecting significant differences among treatments for the last time interval only. In order to avoid complex tables only the average values of the data of the stress tests are presented in Tables 3 and 8.

TABLE 1

Artemia concentration at each feeding, and number of feedings per day during the hatchery phase of seabass larvae

Culture day	*Artemia* conc. (indiv. ml^{-1})	Number of feedings per day
7	0.1	1
8	0.25	1
9–11	0.5	2
12–16	1	2
17	1.5	2
18–19	1	3
20–21	1	4
22	1.5	3

At the end of the rearing period, the animals were harvested, freeze-dried and sent for ω3-HUFA analysis to the Artemia Reference Center in Belgium. Fatty acid profiles were determined by capillary gas chromatography. The fish larvae were homogenized with an ultrasonic homogenizer (Sonifier B12). Total lipids were extracted according to the method of Bligh and Dyer (1959), and saponification and esterification was done according to the procedure described by Schauer and Simpson (1978). Fatty acid methyl esters (FAME) were injected on a capillary column (25 m fused silica; ID 0.32 mm; liquid phase SILAR 10C; film thickness 0.3 μm) installed in a Carlo Erba Fractovap 2330 gas chromatograph. Operating conditions were: solid injector; hydrogen carrier gas; flow rate 1.9 ml min^{-1}; FID; oven temperature 154°C to 200°C at 2°C min^{-1}. Peak identification and quantification were done with a calibrated plotter integrator (Hewlett-Packard 3390 A) and reference standards. The results are presented as area-percent FAME composition and as mg FAME g^{-1} dry weight.

RESULTS

Results from the first experiment (Table 2) reveal high mortalities (up to 50%) during the hatchery rearing of seabass. The biggest losses, however, were registered shortly after hatching at the transition from endogenous to exogenous feeding on *Brachionus*. During the *Artemia* feeding, only a few animals died and no significant differences were traced among the different feeding regimes at day 21. Feeding smaller *Artemia* at an earlier time, and/or enriched *Artemia*, improved neither length nor dry weight of the fish larvae.

The physiological condition of the fish larvae at day 21, measured as the resistance to a salinity stress, however, was significantly better for the fish that received a HUFA-rich diet: early mortality can indeed be noticed for non-enriched fish (see Table 3). The summed cumulative mortality for each

TABLE 2

Survival, dry weight (DW) and total length (TL) of 21-day-old seabass larvae reared on four different feeding regimes (abbreviations are defined in the text)

Treatment no.	Feeding regime	Survival (%)	DW (mg)	TL (mm)
1	SFB/GSL	45±4	2.38	9.91±0.28
2	SFB/GSLE	43±3	2.17	9.54±0.28
3	GSL/GSL	48±2	2.19	9.30±0.29
4	GSL/GSLE	44±1	2.10	9.42±0.81

TABLE 3

Summed cumulative mortality figures for 21-day-old seabass larvae exposed to a 65-ppt salinity stress test (abbreviations are defined in the text)

Treatment	Feeding regime	Time interval (min)					
		10–30	30–50	50–70	70–90	90–110	110–130
1	SFB/GSL	0	3	13	29	47	67
2	SFB/GSLE	0	1	6	17	31	48
3	GSL/GSL	0	4	16	32	52	72
4	GSL/GSLE	0	0	3	11	25	53

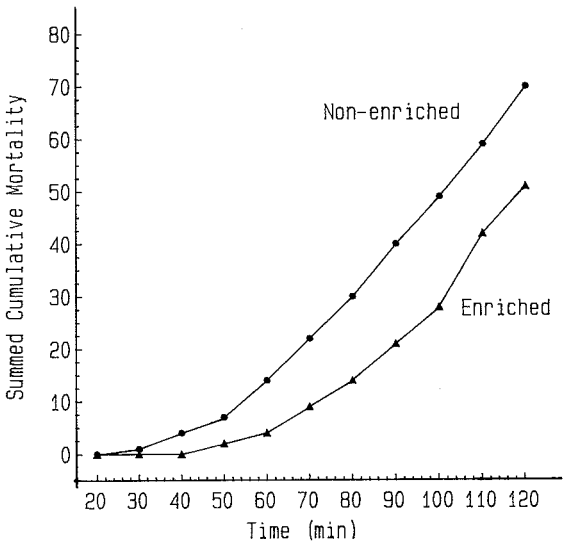

Fig. 1. Effect of enrichment diet on the physiological condition of 21-day-old seabass larvae obtained through a salinity stress test.

time interval revealed no significant difference between early-fed, small *Artemia* (SFB) and bigger *Artemia* (GSL) but highly significant differences appeared when animals with a HUFA-enriched diet were compared with animals on the control diet. The superior physiological condition of the SELCO-enriched fish (treatments 1 and 3) compared to the HUFA-non-supplemented fish (treatments 2 and 4) is illustrated in Fig. 1. Fatty acid analysis of the seabass larvae revealed that the highest levels of essential fatty acids were incorporated into body tissue of the fish fed HUFA-enriched *Artemia* (Table 4).

From day 23 onwards, significant differences in survival among the feeding trials were noticed (Table 5). Drastic mortalities, resulting in a maximum of 7% survival after metamorphosis, occurred in the tanks where no HUFA-enriched *Artemia* were administered. In contrast, seabass larvae fed the enriched GSL *Artemia* faced no difficulties with metamorphosis and continued to grow well.

The results of the second set of experiments, in which the addition of enriched GSL nauplii was gradually delayed, are schematically represented in Table 6. The shaded area on the table reflects the period in which a HUFA diet was fed. The data represent daily mortality percentages. Again, no significant mortality could be observed before day 22. From day 23 onward survival decreased fast in treatments that so far had not received HUFA enrich-

TABLE 4

Essential fatty acid composition of 21-day-old seabass larvae reared on four different feeding regimes (abbreviations are defined in the text)

Treatment no.	Feeding regime	20:5ω3 (mg/g DW)	22:6ω3 (mg/g DW)	$\Sigma\omega$3-HUFA (mg/g DW)
1	SFB/GSL	1.3	0.6	2.7
2	SFB/GSLE	4.2	3.5	9.9
3	GSL/GSL	0.8	0.4	2.0
4	GSL/GSLE	5.7	6.0	14.0

TABLE 5

Daily mortality (%) for seabass larvae on different feeding regimes

Day	SFB/GSL	SFB/GSLE	GSL/GSL	GSL/GSLE
22	1	0	4	0
23	5	0	12	0
24	12	0	48	0
25	70	0	85	0
26	91	0	96	0
27	93	0	97	0

TABLE 6

Daily mortality (%) for seabass larvae in relation to HUFA feeding (the shaded area reflects the period during which HUFA-enriched *Artemia* were fed: e.g. treatment 1 received the HUFA diet from day 14 onwards, treatment 2 from day 15 onwards, etc.)

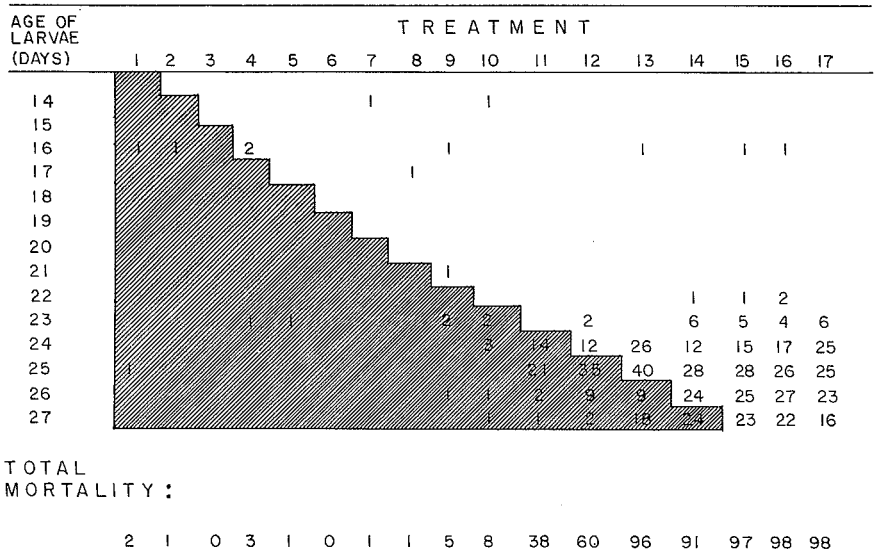

TABLE 7

Essential fatty acid composition of 22-day-old seabass larvae fed for different numbers of days on an enriched-*Artemia* diet

Number of days fed on enriched *Artemia*	20:5ω3 (mg/g DW)	22:6ω3 (mg/g DW)	Σω3-HUFA (mg/g DW)
0	0.1	0.1	0.2
1	0.5	0.2	1.4
2	4.0	0.9	7.7
3	3.2	1.5	9.1
4	5.6	3.0	12.7
5	5.0	2.2	10.4
6	3.1	2.3	7.7
7	5.3	3.0	12.0

ment. Further, lack of a HUFA diet resulted in total mortality by day 27. When SELCO-enriched *Artemia* was given shortly after the first mortality was noticed, some of the animals recovered and mortality stopped within 2 days. Maximal survival could be maintained for animals fed the HUFA diet at least 2 days before the onset of mortality, i.e., at day 20. Since only 2 days were required to achieve partial recovery, it seemed that the supply of HUFAs re-

TABLE 8

Summed cumulative mortalities of 25-day-old seabass larvae fed for different consecutive days on enriched *Artemia* when submitted to a 65-ppt salinity stress

Number of days fed on enriched *Artemia*	Time (min)										
	10	20	30	40	50	60	70	80	90	100	110
11	0	0	0	1	2	4	8	15	26	38	54
10	0	1	1	3	6	12	21	33	47	64	82
9	0	0	0	1	6	13	24	38	53	69	87
8	0	0	0	2	7	16	27	41	56	74	93
7	0	0	1	4	12	25	39	55	72	91	111
6	0	0	1	9	22	38	56	75	95	115	135
5	0	0	4	16	34	53	73	93	113	133	153
4	0	0	8	27	47	67	87	107	127	147	167
3	0	1	9	22	38	55	74	93	113	133	153
2	0	0	3	13	30	48	67	87	107	127	147
1	0	1	5	17	31	47	64	83	102	122	142
0	0	2	6	17	32	50	69	88	108	128	148

TABLE 9

Duncan's multiple range test for summed cumulative mortality figures obtained in the stress test (see Table 8)

Days on HUFA	11	10	9	8	7	6	5	4	3	2	1	0
11												
10	×											
9	×											
8	×											
7	×	×										
6	×	×	×	×								
5	×	×	×	×	×							
4	×	×	×	×	×	×						
3	×	×	×	×	×							
2	×	×	×	×	×							
1	×	×	×	×	×							
0	×	×	×	×	×							

× denotes treatments significantly different at the 0.05 level.

stored the nutritional deficiency rapidly. In this respect, the fatty acid levels in 22-day-old seabass larvae reveal interesting information (Table 7). For 20:5ω3, maximal levels were reached within 2 days but for 22:6ω3 the time required is 4 days.

When 25-day-old fingerlings were exposed to the 65-ppt stress test, significant differences in physiological condition could be detected (Table 8). Enriched larvae performed much better than non-enriched ones. Comparison

among the treatments by use of ANOVA and Duncan's multiple range test revealed that significant differences could be detected among treatments where a fortified diet was offered for more than 5 days (Table 9).

DISCUSSION

Numerous studies have demonstrated the importance of highly unsaturated fatty acids, especially of the $\omega 3$ series, as a diet component in the larviculture of marine fish (Sorgeloos et al., 1988). Our experiments confirm this for the Asian seabass, although the effects are not similar to those detected for the European seabass and bream species (Franicevic et al., 1987; Lisac et al., 1986). Whereas for the latter species a correlation exists between survival and/or growth during the total larval rearing period and the HUFA content in the diet, the *Lates* larvae show visible effects only during metamorphosis, and after this stage, differences in physiological condition. Feeding of diets fortified with essential fatty acids (EFA) is essential to overcome the critical fourth week, at which time metamorphosis occurs; otherwise mass mortality will occur by day 29. Since mortality coincides with metamorphosis and since metamorphosis is accompanied by morphological and physiological reorganization in the larvae, it is very likely that such reorganization demands higher requirements for EFA. When these are not satisfied through the food, the larvae start to display uncoordinated swimming behaviour and eventually die within a few hours. Noteworthy also is the fact that mortality was not related to the size (or growth rate) of the larval fish, but that it started invariably around day 22. The rate of metamorphosis, however, was accelerated when HUFA-rich diets had been offered before day 20: i.e., 90% instead of 55% metamorphosis was achieved by day 30 among larvae fed SELCO-enriched *Artemia.*

Although HUFA enrichment of *Artemia* nauplii does not significantly improve dry weight, length or survival of seabass before day 21, it does give them a significantly higher resistance to stress conditions, reflecting their superior physiological condition. Moreover, when the values of the stress test in experiment 2 are plotted in a linear regression (Fig. 2), the intersection between the two lines indicates a required enrichment period of about 4.7 days. This corresponds to 20-day-old larvae, and is the last day to improve the dietary value of the food to prevent mortality on day 22 or later! Every extra HUFA administration before day 20 may reduce the final mortality score and may thus be beneficial to the quality of the fry. This will effectively improve survival during stress conditions, as might occur during transportation of the fry to the grow-out ponds. In addition, it is possible that the animals will perform better in grow-out conditions where they will probably be more resistant to starvation and temperature or oxygen stresses, and consequently less susceptible to diseases, eventually resulting in higher yields. In conclusion, an

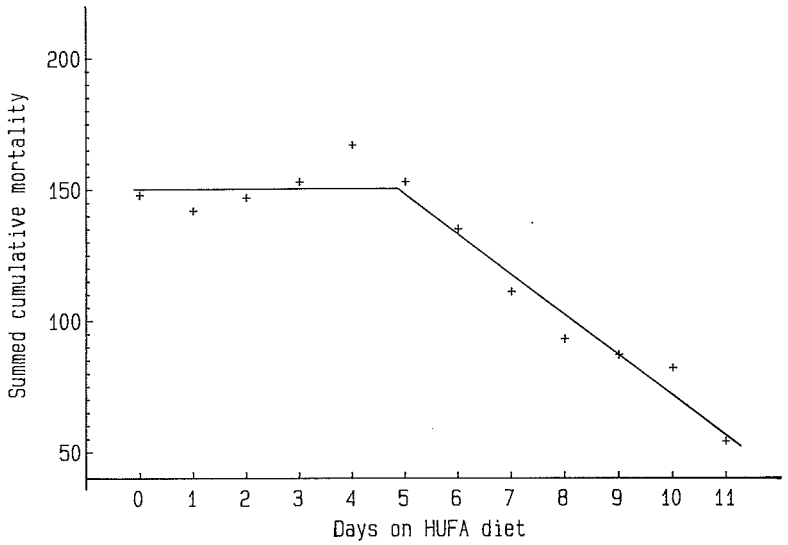

Fig. 2. Effect of the administration, for 11 consecutive days, of a HUFA diet on the physiological condition of seabass larvae, obtained through a salinity stress test.

optimal feeding strategy for the Asian seabass should include the feeding of HUFA-enriched prey in their larval development at least from day 17 onwards, and possibly from day 14 onwards with enriched *Artemia.*

Finally, it is suggested that the cumulative index derived from the salinity stress test could be used for quality control of hatchery-reared or wild seabass larvae. Such a technique is simple, can easily be applied by the farmer without any need for sophisticated equipment and will allow him to evaluate the quality of the fry before deciding to stock them in ponds or cages.

ACKNOWLEDGEMENTS

This study was supported by the Belgian Administration for Development Cooperation (ABOS), the Flemish Association for Educational Programmes Abroad (VVOB), and the Belgian National Foundation for Scientific Research (NFWO) of which P. Sorgeloos is a senior scientist. The authors wish to thank Dr. Ph. Léger for his assistance in the fatty acid analyses and Dr. E. Avila for reviewing the manuscript. The authors are particularly indebted to Mrs. B. Dhert-Van Moffaert for her technical assistance.

REFERENCES

Bagarinao, T. and Kungvankij, P., 1986. An incidence of swimbladder stress syndrome in hatchery-reared seabass (*Lates calcarifer*) larvae. Aquaculture, 51: 181–188.

Bligh, E.G. and Dyer, W.J., 1959. A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol., 37: 911–917.

Bottino, N.R., Gennity, J., Lilly, M.L., Simmons, E. and Finne, G., 1980. Seasonal and nutritional effects on the fatty acids of three species of shrimp, *Penaeus setiferus*, *Panaeus aztecus* and *Penaeus duorarum*. Aquaculture, 19: 139–148.

Buranapanidgit, J., Boonyaratpalin, M., Watanabe, T., Pechmanee, T. and Yashiro, R., 1988. Essential fatty acid requirement of juvenile seabass, *Lates calcarifer*. National Institute for Coastal Aquaculture, Technical Paper no. 3, 21 pp.

Claus, C., Benijts, F., Vandeputte, G. and Gardner, W., 1979. The biochemical composition of the larvae of two strains of *Artemia salina* (L.) reared on two different algal foods. J. Exp. Mar. Biol. Ecol., 36: 171–183.

Danayadol, Y., 1984. Study on the diseases of seabass, *Lates calcarifer*, in Southern Thailand, 1981–1983. In: Report of Thailand and Japan Joint Coastal Aquaculture Research Project, Songkla, Thailand, Apr. 1981–Mar. 1984. Japan International Cooperation Agency, Tokyo, 140–144 pp.

Danayadol, Y. and Boonranapanichagit, L., 1984. Study on the areas of occurrence and symptom of a kidney disease in cultured seabass, *Lates calcarifer*. In: Report of Thailand and Japan Joint Coastal Aquaculture Research Project, Songkla, Thailand, Apr. 1981–Mar. 1984. Japan International Cooperation Agency, Tokyo, pp. 145–155.

Dhert, Ph., Duray, M., Lavens, P. and Sorgeloos, P., 1990. Optimized feeding strategies in the larviculture of the Asian seabass, *Lates calcarifer*. In: R. Hirano and I. Hanyu (Editors), Proc. Second Asian Fisheries Forum, pp. 319–323.

Franicevic, V., Lisac, D., Buble, J., Léger, Ph. and Sorgeloos, P., 1987. Etude internationale sur l'*Artemia*. XLII. L'effet de la qualité nutritionelle de l'*Artemia* sur la croissance et la survie des larves du loup (*Dicentrarchus labrax* L.) dans une écloserie commerciale. In: B. Loix (Editor), Production in Marine Hatcheries. FAO–MEDRAP, Rovinj-Zadar, Yugoslavia, pp. 139–145.

Kuhlmann, D., Quantz, G. and Witt, U., 1981. Rearing of turbot larvae (*Scophthalmus maximus* L.) on cultured food organisms and postmetamorphosis growth on natural and artificial food. Aquaculture, 23(1/4): 183–196.

Léger, Ph., Bengtson, D.A., Simpson, K.L. and Sorgeloos, P., 1986. The use and nutritional value of *Artemia* as a food source. Oceanogr. Mar. Biol. Annu. Rev., 24: 521–623.

Léger, Ph., Naessens-Foucquaert, E. and Sorgeloos, P., 1987. International study on *Artemia*. XXXV. Techniques to manipulate the fatty acid profile in *Artemia* nauplii and the effect on its nutritional effectiveness for the marine crustacean *Mysidopsis bahia* (M.). In: P. Sorgeloos, D.A. Bengtson, W. Decleir and E. Jaspers (Editors), *Artemia* Research and its Applications. Vol. 3. Universa Press, Wetteren, Belgium, pp. 411–424.

Lisac, D., Franicevic, V., Vejmelka, Z., Buble, J., Léger, Ph. and Sorgeloos, P., 1986. International study on *Artemia*. XLIII. The effect of live food fatty acid content on growth and survival of sea bream (*Sparus aurata*) larvae. Paper presented at the Conference Ichthyopathology in Aquaculture, 21–24 October, Inter-University Center, Dubrovnik.

Maneewong, S., Ruangpanit, N., Tattanon, T. and Kraisingdecha, P., 1986a. Experiment on rearing fry of seabass, *Lates calcarifer*, from 1 to 12 days old at different densities. In: Report of Thailand and Japan Joint Coastal Aquaculture Research Project, Apr. 1984–Jan. 1986, no. 2, pp. 20–22.

Maneewong, S., Ruangpanit, N., Tattanon, T. and Kraisingdecha, P., 1986b. Experiment on rearing fry of seabass, *Lates calcarifer*, from 13 to 29 days old at different densities. In: Report of Thailand and Japan Joint Coastal Aquaculture Research Project, Apr. 1984–Jan. 1986, no. 2, pp. 23–26.

Rodgers, L.J. and Barlow, C.G., 1987. Better nutrition enhances survival of barramundi larvae. Aust. Fish., 46: 30–32.

Schauer, P.S. and Simpson, K.L., 1978. Effects of diets on the fatty acid composition of juvenile Atlantic silverside (*Menidia menidia*). In: I.W. Avault, Jr. (Editor), Proc. 9th Annu. Meet. World Maricult. Soc., Lousiana State Univ., Baton Rouge, LA, pp. 175–187.

SEAFDEC, 1983. Fishery Statistical Bulletin for South China Sea Area. Bangkok, 275 pp.

Sick, L.V., 1976. Nutritional effect of five species of marine algae on the growth, development and survival of the brine shrimp *Artemia salina.* Mar. Biol., 35: 69–78.

Sorgeloos, P., Léger, Ph. and Lavens, P., 1988. Improved larval rearing of European and Asian seabass, seabream, mahimahi, siganid and milkfish using enrichment diets for *Brachionus* and *Artemia.* World Aquacult., 19(4): 78–79.

Watanabe, T., Arahawa, T., Kitajima, C., Fukusho, K. and Fujita, S., 1978. Nutritional quality of living feed from the viewpoint of essential fatty acids for fish. Bull. Jpn. Soc. Sci. Fish., 44: 1223–1227.

Watanabe, T., Oowa, F., Kitajima, C. and Fujita, S., 1980. Relationship between dietary value of brine shrimp *Artemia salina* and their content of ω3-HUFA. Bull. Jpn. Soc. Sci. Fish., 46: 35–41.