クロマグロ仔魚の成育に悪影響を及ぼすアルテミアの栄養素

誌名	水産増殖 = The aquiculture
ISSN	03714217
著者名	瀬岡,学 倉田,道雄 畑中,康宏 Amal K. Biswas Ji Seung Chul 熊井,英水
発行元	水産増殖談話会
巻/号	55巻1号
掲載ページ	p. 55-64
発行年月	2007年3月

Aquaculture Science **55**(1), 55－64 (2007)

Possible Nutrients in *Artemia* Affecting the Larval Growth of Pacific Bluefin Tuna *Thunnus orientalis*

Manabu SEOKA[1,*], Michio KURATA[2], Yasuhiro HATANAKA[1], Amal Kumar BISWAS[1], Seung Chul JI[1] and Hidemi KUMAI[1]

Abstract: Feeding of *Artemia* enriched with commercial enrichers (enriched *Artemia*) induces the growth failure of the Pacific bluefin tuna (PBT) *Thunnus orientalis* larvae but feeding of yolk-sac larvae of marine fish promotes larval growth. However, the underlying causes of this phenomenon have never been explained. Therefore, in this study, two consecutive experiments, 1) a nutritional comparison among enriched rotifers, enriched *Artemia*, and yolk-sac larvae of Japanese knifejaw *Oplegnathus fasciatus* and 2) a preliminary rearing trial of bluefin tuna larvae by feeding either enriched *Artemia* or yolk-sac larvae, were conducted to predict the possible nutritional cause(s). No remarkable differences were found in proximate and amino acid compositions among the live feed, while phosphatidylchorine, sterol ester, and docosahexaenoic acid (DHA) levels were comparatively low in enriched *Artemia*. The carbohydrate content in enriched *Artemia* was similar to that in enriched rotifers but higher than that in yolk-sac larvae. In the rearing trial for 6 days, the growth and survival of PBT larvae fed yolk-sac larvae were superior to those fed enriched *Artemia*. The PBT larvae fed yolk-sac larvae had significantly higher levels of triacylglycerol and DHA. The results suggest that the nutritional inferiority of enriched *Artemia* to yolk-sac larvae is partly responsible for the growth failure of PBT larvae fed enriched *Artemia*.

Key words: Bluefin tuna; *Thunnus orientalis*; *Artemia*; Yolk-sac larvae; Rotifers

The Fisheries Laboratory of Kinki University (FLKU) has succeeded in mapping the life cycle of the Pacific bluefin tuna (PBT) *Thunnus orientalis* under aquaculture conditions (Sawada et al. 2005). However, PBT culture in Japan has been dependent on the wild capture of juveniles for production because the technology of artificial larviculture has not yet been established. Some serious problems have been considered as limiting factors which suppress the mass production of PBT larvae; mass mortality caused by the adhesion of larvae to rearing water surface, the serious attack of larvae on their siblings, and trauma due to collisions with the tank wall, as reported by FLKU (Miyashita 2002; Sawada et al. 2005).

However, it is also empirically known in FLKU that PBT larvae cannot be reared by sole feeding of *Artemia* enriched nutritionally with commercial enrichers (enriched *Artemia*); i.e., the sole feeding of enriched *Artemia* induces serious growth failure of PBT larvae, although larval production of many marine fish species has been carried out using enriched *Artemia* throughout the world (Sorgeloos et al. 2001). Thus, PBT larviculture at FLKU has been carried out by feeding yolk-sac larvae of marine fish such as Japanese knifejaw *Oplegnathus fasciatus* and red seabream *Pagrus major* as the main live feed after the rotifer-feeding period, although enriched *Artemia* have also been used, but mainly to suppress the size variation of PBT larvae (Miyashita 2002). However, no scientific information is available on this

Received September 22, 2006: Accepted November 29, 2006.
[1] Fisheries Laboratory, Kinki University, Nachikatsuura, Wakayama 649-5145, Japan.
[2] Fisheries Laboratory, Kinki University, Kushimoto, Wakayama 649-3633, Japan.
[*] Corresponding author: E-mail: seoka@nara.kindai.ac.jp

subject. Why is it necessary to give yolk-sac larvae to PBT larvae?

In this study, therefore, two consecutive experiments, nutritional comparison among enriched rotifers, enriched *Artemia*, and yolk-sac larvae of Japanese knifejaw (Exp. 1) and then a preliminary rearing trial of PBT larvae by sole feeding with either enriched *Artemia* or yolk-sac larvae of Japanese knifejaw (Exp. 2), were conducted. In Exp. 1, we attempted to identify possible nutrients leading to the growth failure of PBT larvae. In Exp. 2, we also attempted to evaluate the effect of the possible nutrients on the growth and nutrient composition of PBT larvae. The results appear to provide further insight into the improvement of the nutritional value of *Artemia* and the development of an artificial diet for PBT larviculture.

Materials and methods

Exp. 1

The proximate composition, amino acids, fatty acids, and lipid class of rotifers *Brachinonus rotundiformis*, *Artemia franciscana* nauplii and yolk-sac larvae of Japanese knifejaw were analyzed. The rotifers and *Artemia* (INVE Aquaculture, Belgium) were enriched with a commercial enricher (Marine Glos; Nisshin Marinetech, Japan) rich in n-3 highly unsaturated fatty acids (n-3 HUFA), mainly docosahexaenoic acid (DHA). The yolk-sac larvae were hatched from eggs spontaneously spawned by cultivated broodfish of Japanese knifejaw. Rotifers and *Artemia* enriched in a similar manner have been used for the larviculture of other Japanese marine fish such as red seabream at FLKU as well as at private and public hatcheries in Japan, as the main live feed after the rotifer-feeding period.

Crude protein and crude ash were analyzed by the Association of Official Analytical Chemists (AOAC) method (AOAC 1984). Moisture was gravimetrically determined after freeze-drying for 24 h, and lipid was extracted by the method of Folch et al. (1957) and then determined gravimetrically. Carbohydrate was analyzed by the phenol-sulfuric acid reaction according to Hodge and Hofreiter (1962) using glucose as the standard. Amino acids (AA) were analyzed after HCl hydration (108℃, 24 h) and free amino acids (FAA) were extracted with 80% ethanol and analyzed, using high performance liquid chromatography (L-6200; Hitachi, Japan) (Seoka et al. 2004). Fatty acid composition of neutral (NL) and polar lipid (PL) fractions were measured using gas chromatography (G-3000; Hitachi, Japan) after transmethylation (Yoshinaka and Satoh 1989). NL and PL fractions were prepared using Sep-Pack silica cartridge (Waters, USA) by the method of Juaneda and Rocquelin (1985). Lipid classes were separated by high performance thin layer chromatography (silica gel 60 plate; Merck, Germany) using a single dimension double-development method (Olsen and Hendserson, 1989) and then quantified by densitometry using a flying spot scanner (CS-9000; Shimazu, Japan).

Samples for chemical analysis were taken in triplicate, stored at −80℃ and later freeze-dried before processing. The chemical contents were expressed as the relative % dry weight (DW) or mg/g DW. Fatty acid and lipid class compositions were shown as % of total fatty acids and % of lipid, respectively.

Exp. 2

In Exp. 2, PBT larvae having an average body weight of 31 mg (23 days after hatching), which were from eggs spontaneously spawned by cultivated broodfish at FLKU, were randomly distributed into two 500 *l* tanks at a density of 100 fish/tank. The PBT larvae were fed on either *Artemia* or yolk-sac larvae 2 or 3 times a day at a density of approximately 200 −300 individuals/*l* for 6 days (no replication). These live feeds were the same as those in Exp. 1. At the end of the rearing trial, the PBT larvae were counted and their total length was individually measured. Additionally, average body weight was calculated using the pooled PBT larvae, and then the PBT larvae were stored at −80℃ for chemical analysis. The tanks were maintained with the following environmental conditions during the rearing trial: water temperature 27.4 − 28.1℃; salinity 32 − 33 psu; flow

rate, 1 – 2 *l*/min; a natural photoperiod regime.

In Exp. 2, fatty acid composition and lipid classes of PBT larvae were analyzed because it was notable for the remarkable differences between enriched *Artemia* and yolk-sac larvae in Exp. 1. The analytical methods were the same as those in Exp. 1, and the means of data of two repeated analyses were shown.

Statistical analysis

The Kruskal-Wallis test (Kyplot 4.0 for Windows; KyensLab Incorporated, Tokyo), a non-parametric one-way ANOVA, was used to detect significant differences between the means of data of enriched *Artemia* and yolk-sac larvae and between the means of data of PBT larvae fed enriched *Artemia* and yolk-sac larvae. In this study, the data on enriched rotifers is shown, and it is considered as a reference. Therefore, no statistical analysis of the rotifer data was carried out. Results were considered significant if $P < 0.05$.

Results

Exp. 1.

The proximate composition of the live feed is shown in Table 1. Crude protein contents were similar among the live feed, ca. 60% DW, while the lipid contents of enriched *Artemia* (ca. 20% DW) and rotifers (ca. 19% DW) were about 6% lower than that of yolk-sac larvae (ca. 25% DW), although the contents of enriched *Artemia* and yolk-sac larvae were not significantly different. The carbohydrate content of enriched *Artemia* was ca. 8%, which was significantly higher than that of yolk-sac larvae (ca. 2% DW), but similar to that of enriched rotifers (ca. 8% DW). Enriched *Artemia* showed a lower content of crude ash (ca. 9% DW) than enriched rotifers (ca. 17% DW), although no remarkable difference was observed between enriched *Artemia* and yolk-sac larvae (ca. 11% DW).

AA in live feed are shown in Tables 2. The total AA content of enriched *Artemia* (ca. 520 mg/g DW) was slightly higher than that of enriched rotifers (ca. 470 mg/g DW), but almost the same as that of yolk-sac larvae (ca. 540 mg/g DW). No remarkable differences in the contents of each essential amino acid (EAA) were observed among the live feed, although the percentage of Σ EAA in total AA was slightly lower in enriched *Artemia* than in yolk-sac larvae.

On the other hand, the total FAA content of enriched *Artemia* (ca. 25 mg/g DW) and yolk-sac larvae (ca. 30 mg/g DW) was around 2.5 times higher than that of enriched rotifers (ca. 10 mg/g DW), as shown in Table 3. The percentage

Table 1. Proximate composition (% DW) of rotifers, *Artemia*, and yolk-sac larvae of *Oplegnathus fasciatus*[*1]

	Rotifers[*2]	*Artemia*[*2]	Yolk-sac larvae
Crude protein	58.1 ± 1.2	61.8 ± 1.1	63.7 ± 1.0
Lipid	18.6 ± 0.5	19.7 ± 0.6	25.1 ± 0.6
Carbohydrate	8.3 ± 0.3	7.6 ± 0.2[a]	2.4 ± 0.1[b]
Crude ash	16.5 ± 0.5	9.4 ± 0.4	11.3 ± 0.3

[*1] Data are given as the mean ± standard deviation (n = 3). Data were statistically compared between *Artemia* and yolk-sac larvae (Kruskal-Wallis test, $P<0.05$). Means with different superscripts are significantly different between *Artemia* and yolk-sac larvae. Data of the rotifers are listed as a reference.
[*2] Rotifers and *Artemia* were nutritionally enriched with a commercial enricher (Marine Glos; Nisshin Marinetech, Japan).

Table 2. Amino acids (mg/g DW) in rotifers, *Artemia*, and yolk-sac larvae of *Oplegnathus fasciatus*[*1]

	Rotifers[*2]	*Artemia*[*2]	Yolk-sac larvae
Aspartic acid	88.8 ± 0.9	83.9 ± 1.2	71.7 ± 0.7
Glutamic acid	79.2 ± 0.9	89.5 ± 1.3	82.4 ± 0.3
Serine	26.7 ± 0.7	26.8 ± 1.4	24.4 ± 0.3
Glycine	18.3 ± 0.6	24.9 ± 0.6	23.8 ± 1.6
Histidine	11.5 ± 1.2	13.2 ± 0.8	18.1 ± 0.8
Arginine	22.5 ± 1.9	26.9 ± 1.8	28.1 ± 0.4
Threonine	18.9 ± 0.3	25.4 ± 1.3	26.4 ± 0.8
Alanine	19.8 ± 0.5	29.5 ± 0.5	34.8 ± 0.9
Proline	20.7 ± 0.7	24.6 ± 1.1	20.7 ± 0.9
Tyrosine	17.2 ± 0.2	21.3 ± 0.9	22.7 ± 0.3
Valine	22.3 ± 0.3	26.3 ± 0.3	30.9 ± 1.0
Methionine	12.5 ± 0.6	14.3 ± 0.4	18.9 ± 0.9
Isoleucine	20.8 ± 0.2	21.1 ± 1.0	23.5 ± 1.0
Leucine	32.0 ± 0.8	35.0 ± 1.5	41.6 ± 1.2
Phenylalanine	21.0 ± 0.3	21.2 ± 1.1	24.4 ± 0.3
Lysine	38.7 ± 0.6	40.6 ± 0.6	46.3 ± 0.3
Total	471.0 ± 7.3	524.7 ± 2.6	538.6 ± 8.7
Σ EAA/total AA (%)[*3]	42.5 ± 0.2	42.7 ± 0.7[b]	47.9 ± 0.1[a]

[*1] Data are the mean ± standard deviation (n = 3). Data were statistically compared between *Artemia* and yolk-sac larvae (Kruskal-Wallis test, $P<0.05$). Means with different superscripts are significantly different between *Artemia* and yolk-sac larvae. Data of the rotifers are listed as a reference.
[*2] Rotifers and *Artemia* were nutritionally enriched with a commercial enricher (Marine Glos; Nisshin Marinetech, Japan).
[*3] EAA, essential amino acids.

of Σ EAA in total FAA of enriched *Artemia* was half of those of the others. This was due to the lower content of free non-essential amino acids in enriched rotifers and the higher content of some free EAA such as valine, leucine, phenylalanine in yolk-sac larvae.

The lipid class and fatty acid composition in NL and PL fractions are listed in Tables 4. No remarkable differences in NL/PL were found among the live feed; however, phosphatidylcholine (PC, ca. 11%) and sterol ester (SE, ca. 1%) levels of enriched *Artemia* as well as enriched rotifers were low compared to those of yolk-sac larvae (PC, ca. 20%; SE, ca. 24%), although triacylglycerol (TAG) was the main lipid class, regardless of the live feed.

For the fatty acid composition, enriched *Artemia* had comparatively higher levels of lenolenic acid (18 : 3n-3, LNA) in both fractions than the other live feeds (Table 5). However, enriched *Artemia* had much lower levels of DHA (ca. 7% in NL; ca. 1% in PL) than yolk-sac larvae (ca. 11% in NL; ca. 28% in PL) and enriched rotifers (ca. 17% in NL; ca. 8% in PL), in both fractions. It was notable that the DHA levels in the PL fraction of yolk-sac larvae were about 23 times higher than those of enriched *Artemia*. There were no significant differences in eicosapentaenoic acid (20 : 5n-3) and arachidonic acid (20 : 4n-6) levels of both fractions between enriched *Artemia* and yolk-sac larvae.

Exp. 2.

The rearing performance of PBT larvae fed either enriched *Artemia* or yolk-sac larvae is shown in Table 6. The survival, total length, and body weight of the enriched *Artemia*-fed group were 12.0%, 20.2 mm, and 67 mg, respectively, which were quite inferior to those of the yolk-sac larvae-fed group, 44.0%, 27.9 mm, and 240 mg, respectively.

The lipid content and lipid class composition of the whole body of the enriched *Artemia*-fed and yolk-sac larvae-fed groups are shown in Table 7. The lipid content of the yolk-sac larvae-fed group

Table 3. Free amino acids (mg/g DW) in rotifers, *Artemia*, and yolk-sac larvae of *Oplegnathus fasciatus*[*1]

	Rotifers[*2]	*Artemia*[*2]	Yolk-sac larvae
Aspartic acid	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.1
Glutamic acid	0.8 ± 0.1	2.0 ± 0.1[a]	0.9 ± 0.1[b]
Asparagine	0.3 ± 0.1	0.5 ± 0.1	0.5 ± 0.1
Serine	0.4 ± 0.1	0.5 ± 0.1[b]	1.0 ± 0.2[a]
Glutamine	0.5 ± 0.2	2.4 ± 0.3	0.7 ± 0.2
Glycine	0.3 ± 0.0	0.7 ± 0.1	0.7 ± 0.1
Histidine	0.9 ± 0.1	1.1 ± 0.1	1.0 ± 0.1
Taurine	0.1 ± 0.0	4.2 ± 0.3	4.3 ± 0.2
Arginine	1.1 ± 0.4	1.1 ± 0.3	0.7 ± 0.3
Threonine	0.3 ± 0.1	0.4 ± 0.1[b]	1.0 ± 0.2[a]
Alanine	0.6 ± 0.1	4.2 ± 0.5	2.2 ± 0.3
Proline	0.4 ± 0.1	3.8 ± 0.5[a]	0.6 ± 0.1[b]
Tyrosine	1.2 ± 0.4	1.0 ± 0.2[b]	3.1 ± 0.1[a]
Valine	0.4 ± 0.1	0.4 ± 0.1[b]	2.7 ± 0.5[a]
Methionine	0.3 ± 0.0	0.4 ± 0.1[b]	1.3 ± 0.5[a]
Isoleucine	0.3 ± 0.1	0.4 ± 0.0[b]	1.5 ± 0.1[a]
Leucine	0.5 ± 0.2	0.7 ± 0.1[b]	2.2 ± 0.1[a]
Phenylalanine	0.6 ± 0.1	0.5 ± 0.1[b]	2.4 ± 0.2[a]
Tryptophan	0.4 ± 0.0	0.3 ± 0.0[b]	1.4 ± 0.3[a]
Lysine	0.6 ± 0.1	0.7 ± 0.2	1.5 ± 0.5
Total	9.9 ± 1.5	25.2 ± 1.1	29.8 ± 2.4
Σ EAA/total FAA (%)[*3]	53.6 ± 1.3	23.0 ± 0.8[b]	52.3 ± 3.0[a]

[*1] Data are the mean ± standard deviation (SD) (n = 3). Data were statistically compared between *Artemia* and yolk-sac larvae (Kruskal-Wallis test, $P < 0.05$). Means with different superscripts are significantly different between *Artemia* and yolk-sac larvae. Data of the rotifers are listed as a reference. The SD of 0.0 indicates SD < 0.05.
[*2] Rotifers and *Artemia* were nutritionally enriched with a commercial enricher (Marine Glos; Nisshin Marinetech, Japan).
[*3] EAA, essential amino acids.

Table 4. Lipid class composition (% lipid) of rotifers, *Artemia*, and yolk-sac larvae of *Oplegnathus fasciatus*[*1]

	Rotifers[*2]	*Artemia*[*2]	Yolk-sac larvae
Neutral lipid (NL)[*3]			
S	4.0 ± 0.2	3.0 ± 0.2	7.9 ± 0.5
FFA	8.0 ± 0.2	2.2 ± 0.3	0.8 ± 0.2
TAG	47.7 ± 3.3	56.1 ± 2.3	33.5 ± 1.9
SE	6.8 ± 0.8	1.2 ± 0.3[b]	23.6 ± 0.2[a]
Others	4.2 ± 1.9	2.3 ± 2.4	0.7 ± 0.8
Polar lipid (PL)[*3]			
PC	6.7 ± 0.2	11.6 ± 0.2[b]	19.7 ± 0.1[a]
PS	1.0 ± 0.0	2.2 ± 0.4	1.6 ± 0.2
PI	3.9 ± 0.7	4.9 ± 0.5	2.4 ± 0.2
PE	12.7 ± 0.5	13.4 ± 0.4	8.5 ± 0.2
Others	5.0 ± 0.2	3.3 ± 2.4	1.3 ± 1.0
NL/PL	2.4 ± 0.3	1.8 ± 0.0	2.0 ± 0.1

[*1] Data are the mean ± standard deviation (SD) (n = 3). Data were statistically compared between *Artemia* and yolk-sac larvae (Kruskal-Wallis test, $P < 0.05$). Means with different superscripts are significantly different between *Artemia* and yolk-sac larvae. Data of the rotifers are listed as a reference. The SD of 0.0 indicates SD < 0.05.
[*2] Rotifers and *Artemia* were nutritionally enriched with a commercial enricher (Marine Glos; Nisshin Marinetech, Japan).
[*3] Lipid class abbreviations; PC, phosphatidylcholine; PS, Phosphatidylserine; PI, phosphatidylinositol; PE, phosphatidylethanolamine; S, sterol; FFA, free fatty acids; TAG, triacylglycerol; SE, sterol ester.

Table 5. Fatty acid composition (% total fatty acid) in neutral and polar lipid fractions of rotifers, *Artemia*, and yolk-sac larvae of *Oplegnathus fasciatus*[*1]

	Rotifers[*2]	*Artemia*[*2]	Yolk-sac larvae
Neutral lipid fraction			
14:0	8.7 ± 0.7	2.6 ± 0.4	3.8 ± 0.2
16:0	17.8 ± 1.8	13.4 ± 1.4	20.5 ± 0.5
16:1	8.4 ± 0.2	5.9 ± 0.5	7.8 ± 0.3
18:0	2.5 ± 0.5	2.8 ± 0.8[b]	4.5 ± 0.1[a]
18:1n-9	7.1 ± 1.0	20.6 ± 1.8	20.0 ± 0.5
18:1n-7	2.5 ± 0.5	5.1 ± 1.0	5.2 ± 0.3
18:2n-6	4.8 ± 0.3	3.6 ± 0.1[b]	6.1 ± 0.2[a]
18:3n-6	0.1 ± 0.0	0.6 ± 0.1	0.1 ± 0.0
18:3n-3	0.6 ± 0.1	15.9 ± 0.6[a]	0.6 ± 0.2[b]
20:4n-6	1.8 ± 0.1	1.2 ± 0.3	0.8 ± 0.2
20:5n-3	7.8 ± 0.6	4.3 ± 0.3	3.6 ± 0.3
22:6n-3	17.3 ± 0.3	7.2 ± 0.3[b]	10.8 ± 0.4[a]
Polar lipid fraction			
14:0	3.7 ± 0.3	0.7 ± 0.1	0.7 ± 0.1
16:0	18.2 ± 0.8	11.1 ± 0.4	24.8 ± 0.8
16:1	5.4 ± 0.4	4.0 ± 0.1[a]	2.5 ± 0.3[b]
18:0	4.6 ± 0.3	6.9 ± 0.7	6.8 ± 0.3
18:1n-9	4.6 ± 0.3	27.6 ± 0.6	9.8 ± 0.6
18:1n-7	2.8 ± 0.2	10.7 ± 0.3	2.9 ± 0.4
18:2n-6	17.2 ± 0.3	3.5 ± 0.4	4.2 ± 0.4
18:3n-6	0.1 ± 0.0	0.7 ± 0.2	ND[*3]
18:3n-3	1.6 ± 0.2	13.5 ± 1.2[a]	0.4 ± 0.1[b]
20:4n-6	2.8 ± 0.3	2.4 ± 0.1	2.7 ± 0.3
20:5n-3	6.6 ± 0.4	8.8 ± 0.3	7.9 ± 0.4
22:6n-3	7.5 ± 0.3	1.2 ± 0.0[b]	27.8 ± 0.6[a]

[*1] Data are the mean ± standard deviation (SD) (n = 3). Data were statistically compared between *Artemia* and yolk-sac larvae (Kruskal-Wallis test, $P < 0.05$). Means with different superscripts are significantly different between *Artemia* and yolk-sac larvae. Data of the rotifers are listed as a reference. The SD of 0.0 indicates SD < 0.05.
[*2] Rotifers and *Artemia* were nutritionally enriched with a commercial enricher (Marine Glos; Nisshin Marinetech, Japan).
[*3] Not detected.

Table 6. Rearing performance of Pacific bluefin tuna *Thunnus orientalis* larvae fed *Artemia* or yolk-sac larvae of *Oplegnathus fasciatus* for 6 day

	Artemia-fed[*1]	Yolk-sac larvae-fed
Number of fish		
Initial	100	100
Final	12	44
Survival (%)	12.0	44.0
Total length (mm)[*2]		
Initial	14.7 ± 1.0 (n = 10)	14.7 ± 1.0 (n = 10)
Final	20.2 ± 2.3[b] (n = 12)	27.9 ± 3.4[a] (n = 44)
Body weight (mg/fish)[*3]		
Initial	31 (n = 10)	31 (n = 10)
Final	67 (n = 12)	240 (n = 44)

[*1] *Artemia* enriched with a commercial enricher (Marine Glos; Nisshin Marinetech, Japan) were given.
[*2] Data are the mean ± standard deviation. Means with different superscripts are significantly different between bluefin tuna larvae fed *Artemia* and yolk-sac larvae (Kruskal-Wallis test, $P < 0.05$).
[*3] Data are given as the average body weight of a pooled sample of fish.

Table 7. Lipid content (% wet) and lipid class composition (% of lipid) of Pacific bluefin tuna *Thunnus orientalis* tuna larvae fed *Artemia* or yolk-sac larvae of *Oplegnathus fasciatus*[*1]

	Initial	Final	
		Artemia-fed[*2]	Yolk-sac larvae-fed
Lipid	1.8 ± 0.1	1.6 ± 0.1	2.1 ± 0.1
Neutral lipid (NL)[*3]			
S	15.8 ± 0.5	16.5 ± 2.8	14.4 ± 1.1
FFA	6.3 ± 3.5	1.8 ± 0.1	4.0 ± 5.7
TAG	6.4 ± 2.6	6.6 ± 0.8	14.9 ± 3.7
SE	2.5 ± 1.6	2.0 ± 1.6	3.1 ± 1.6
Others	2.9 ± 1.6	1.3 ± 1.1	4.1 ± 4.9
Polar lipid (PL)[*3]			
PC	26.3 ± 0.1	28.0 ± 1.3	26.6 ± 1.8
PS	7.3 ± 1.1	8.0 ± 0.1	4.8 ± 1.1
PI	4.4 ± 0.1	3.7 ± 1.1	4.2 ± 0.4
PE	21.3 ± 0.1	23.8 ± 1.8	16.0 ± 2.5
Others	7.1 ± 0.6	8.5 ± 0.5	8.2 ± 1.8
NL/PL	0.5 ± 0.0	0.4 ± 0.1	0.7 ± 0.1

[*1] Data are the mean ± standard deviation (SD) (n = 2). No significant differences were found between initial and final data and between bluefin tuna larvae fed *Artemia* and yolk-sac larvae (Kruskal-Wallis test, $P > 0.05$). The SD of 0.0 indicates SD < 0.05.
[*2] *Artemia* enriched with a commercial enricher (Marine Glos; Nisshin Marinetech, Japan) were given.
[*3] For lipid class abbreviations, see Table 4.

increased slightly from 1.8% to 2.1% during the rearing trial, while that of the enriched *Artemia*-fed group decreased slightly from 1.8% to 1.6%. However, there were no significant differences in lipid contents between the groups. In the yolk-sac larvae-fed group, furthermore, the TAG level was ca. 15%, which was about 2 times higher than in the enriched *Artemia*-fed group and initial sample of PBT larvae, although this differences were not statistically significant. PC, the main lipid class of PBT larvae, and SE remained relatively constant at levels of around 27% and 2.5%, respectively.

Fatty acid compositions in the NL and PL fractions of the whole body of PBT larvae are shown in Table 8. In both fractions, DHA levels increased in the yolk-sac larvae-fed group during the rearing trial, although these increases were not statistically significant. At the end of the rearing trial, the yolk-sac larvae-fed group had 18% and 28% of DHA in NL and PL fractions, respectively, which were about 3 times higher than those in the enriched *Artemia*-fed group. Palmitic acid (16:0) levels in both fractions were

Table 8. Fatty acid composition (% total fatty acid) in neutral and polar lipid fractions of Pacific bluefin tuna *Thunnus orientalis* larvae fed *Artemia* or yolk-sac larvae of *Oplegnathus fasciatus*[*1]

	Initial	Final	
		Artemia-fed[*2]	Yolk-sac larvae-fed
Neutral lipid fraction			
14:0	0.8 ± 0.0	1.1 ± 0.1	1.3 ± 0.1
16:0	9.9 ± 0.7	12.5 ± 0.7	19.5 ± 0.7
16:1	5.7 ± 0.4	5.4 ± 0.6	4.7 ± 0.7
18:0	8.0 ± 0.9	4.3 ± 0.4	6.9 ± 0.6
18:1n-9	20.7 ± 1.6	20.7 ± 1.6	17.4 ± 0.6
18:1n-7	7.7 ± 1.0	7.4 ± 0.8	4.5 ± 0.7
18:2n-6	2.7 ± 0.2	3.7 ± 0.3	4.1 ± 0.1
18:3n-6	0.7 ± 0.1	0.5 ± 0.1	0.3 ± 0.1
18:3n-3	8.4 ± 0.4	8.4 ± 0.4	0.4 ± 0.1
20:4n-6	2.5 ± 0.2	3.0 ± 0.3	3.0 ± 0.3
20:5n-3	6.0 ± 0.3	5.5 ± 0.4	5.5 ± 0.4
22:6n-3	2.5 ± 0.0	5.0 ± 0.2	17.9 ± 1.0
Polar lipid fraction			
14:0	0.4 ± 0.1	0.4 ± 0.1	0.4 ± 0.1
16:0	17.2 ± 1.0	16.7 ± 0.8	21.4 ± 1.1
16:1	2.8 ± 0.3	2.4 ± 0.3	1.4 ± 0.0
18:0	13.8 ± 0.6	13.1 ± 0.6	12.1 ± 0.4
18:1n-9	16.4 ± 0.1	16.5 ± 0.3	9.7 ± 0.2
18:1n-7	6.8 ± 0.2	6.0 ± 0.3	3.3 ± 0.1
18:2n-6	2.7 ± 0.2	2.6 ± 0.1	2.1 ± 0.3
18:3n-6	0.3 ± 0.0	0.3 ± 0.1	0.3 ± 0.1
18:3n-3	5.0 ± 0.3	4.4 ± 0.2	0.5 ± 0.0
20:4n-6	4.6 ± 0.1	5.1 ± 0.1	4.3 ± 0.1
20:5n-3	8.7 ± 0.3	9.3 ± 0.1	4.9 ± 0.1
22:6n-3	9.8 ± 0.1	11.2 ± 0.3	31.6 ± 0.5

[*1] Data are the mean ± standard deviation (SD) (n = 2). No significant differences were found between initial and final data and between bluefin tuna larvae fed *Artemia* and yolk-sac larvae (Kruskal-Wallis test, $P > 0.05$). The SD of 0.0 indicates SD < 0.05.
[*2] *Artemia* enriched with a commercial enricher (Marine Glos; Nisshin Marinetech, Japan) were given.

also higher in the yolk-sac larvae-fed group, but LNA levels were remarkably higher in the enriched *Artemia*-fed group (ca. 8% in NL; 4% in PL) when compared with the yolk-sac larvae-fed group (0.4% in NL; 0.5% in PL).

Discussion

Exp. 1.

The quantitative requirements for protein and/or AA have not yet been determined in PBT, but no remarkable differences in crude protein and total AA content among the live feed suggest that protein and AA contents of enriched *Artemia* are not a limiting factor for the growth of PBT larvae. The protein requirement of carnivorous fish such as Atlantic halibut *Hippoglossus hippoglossus* (Helland and Grisdale-Helland 1998), Atlantic salmon *Salmo salar* (Grisdale-Helland and Helland 1997), yellowtail *Seriola quinqueradiata* (Takeda et al. 1975), has been considered 50 − 55%, in general. The crude protein and AA contents in enriched *Artemia* were not below this range. Likewise, the qualitative AA requirement of PBT is also unclear, but the similar AA profile among the live feed suggests that enriched *Artemia* are not inferior to other live feed in respect of AA composition.

FAA are the major substrate of energy metabolism during the early development of PBT (Takii et al. 1997), as well as other marine fish (Rønnestad et al. 1999). In addition, it seems that FAA should be included in the diet at high and balanced levels, since the fish larval gut has limited ability to digest protein (Rønnestad et al. 2003). Dietary FAA are readily absorbed and utilized for energy metabolism and protein synthesis in marine fish larvae (Rønnestad et al. 2003). Therefore, the almost identical levels of total FAA in enriched *Artemia* and yolk-sac larvae suggest that enriched *Artemia* contain an adequate level of FAA. However, the higher percentage of Σ EAA in total FAA of yolk-sac larvae may indicate a deficiency in some free EAAs in enriched *Artemia* as well as in enriched rotifers. It is also indicated that *Artemia* contain markedly lower levels of FAA compared to wild copepods, which have been considered ideal live feed for marine fish larvae in nature (Fyhn et al. 1993; Conceição et al. 1997; Helland et al. 2000; Helland et al. 2003). In this study, however, it was doubtful whether 23.0% of Σ EAA in total FAA content, corresponding to only 1.1% in total AA content, in enriched *Artemia* had a major negative impact on the growth of PBT larvae.

The high carbohydrate content in enriched *Artemia* may give a negative effect on the growth of PBT larvae because the utilization ability of dietary carbohydrate has been considered low in marine carnivorous fish (Dabrowski and Guderley 2002). The high carbohydrate content in *Artemia* has also shown by other studies in which macronutrients of *Artemia* and wild copepods were compared

(Hamre et al. 2002). In addition, amylase, a carbohydrate digestive enzyme, activity of PBT larvae decreases with growth (Miyashita et al. 1998), which suggests that the capacity of PBT larvae to utilize dietary carbohydrate declines over the larval period. In contrast, there was no significant difference in crude ash content between enriched *Artemia* and yolk-sac larvae. It is therefore unlikely that crude ash content in enriched *Artemia* is a major nutritional problem in PBT larviculture.

There were remarkable differences in PC and SE levels in lipid and DHA levels in NL and PL fractions of the live feed. High levels of PC, SE and DHA have been recognized in yolk-sac larvae as well as eggs of marine fish such as red seabream (Watanabe et al. 1991), yellowtail (Verakunpiriya et al. 1996) and sand eel *Ammodytes lancea* (Tocher and Sargent 1984). These indicate that these lipid components are well-balanced nutrients for marine fish in the early developmental period. PBT eggs and larvae are also rich in these lipid components (Seoka et al. unpublished data). In addition, marine fish larvae such as Japanese knifejaw (Kanazawa et al. 1983), red seabream (Kanazawa et al. 1983), striped jack *Pseudocaranx delicatissimus* (Takeuchi et al. 1992), sea bass *Dicentrarchus labrax* (Geurden et al. 1997), and turbot *Scophthalmus maximus* (Geurden et al. 1997) have an absolute dietary phospholipid, especially PC, requirement, while DHA plays a critical role in the maintenance of physiological processes in marine fish as an essential fatty acid (Sargent et al. 1999). Therefore, these abundant lipid components in yolk-sac larvae may be quantitatively useful for PBT larval growth. In contrast, a high level of LNA, such as that recognized in enriched *Artemia*, is not typical in copepods as well as eggs and yolk-sac larvae of marine fish (Sargent and Henderson 1986).

In summary, it could be concluded here that there were differences in carbohydrate, PC, SE, and DHA levels between enriched *Artemia* and other live feed, especially yolk-sac larvae. Feeding of enriched *Artemia* may, in part, have a negative influence on the growth of PBT larvae through such nutritional differences.

Exp. 2.

In Exp. 2, the sole feeding of enriched *Artemia* induced serious growth failure of PBT larvae. Furthermore, survival was quite low in the enriched *Artemia*-fed group. These results are not contradictory to the empirical observation in the PBT larval production at FLKU, as mentioned above. On the other hand, the survival, 44%, of the yolk-sac larvae-fed group resulted from the fact that the PBT larva feed was switched from enriched *Artemia* to yolk-sac larvae at the commencement of the feeding trial in this study. It is difficult to switch PBT larva feed compared to other marine fish larvae such as red seabream and Japanese knifjaw, which can easily be weaned from *Artemia* to an artificial diet (Miyashita 2002). Cannibalism also caused mortality during the feeding trial (Sawada et al. 2005).

The feeding of enriched *Artemia* and yolk-sac larvae also influenced the lipid component of the PBT larval body. Although TAG was the main lipid class in the live feed, as shown in Exp. 1, there was a large difference in TAG level between the dietary groups, which suggests that PBT larvae showing good growth accumulate TAG in the body. TAG has been known as the main energy storage for fish (Sheridan 1988). Therefore, a low TAG level in the body appears to be a good indicator of malnutrition and growth failure of PBT larvae. The TAG level in the body is also considered an indicator of the nutritional status of other fish larvae (Fraser 1989).

DHA in NL and PL fractions were higher in yolk-sac larvae than in enriched *Artemia*, as shown in Exp. 1, and remarkable accumulation of DHA was found in the NL and PL fractions of the yolk-sac larvae-fed group in Exp. 2. These results are in agreement with other studies where dietary fatty acid composition was largely reflected in the fatty acid composition of fish larvae fed *Artemia* or copepods (McEvoy et al. 1998; Hamre et al. 2002). Therefore, the difference in DHA levels of the PBT larval body should result from higher DHA levels

in yolk-sac larvae and lower DHA levels in enriched *Artemia*. The dietary essentiality of DHA for PBT larvae is not yet known, but these results may indicate that PBT larvae selectively retain this fatty acid in the body as an essential fatty acid, as reported in other marine larval fish (Sargent et al. 1999). In this study, DHA levels are reported as % of total fatty acids, so the relative amount of DHA (mg/g live feed) is unknown. However, the relative amount of DHA in enriched *Artemia* might be lower than that in yolk-sac larvae since the total lipid content was also lower in enriched *Artemia*. Therefore, further enrichment of DHA in *Artemia* may be needed to promote the growth of PBT larvae. In addition, MacQueen Leifson et al. (2003) showed with turbot that feeding a formulated diet containing PL rich in DHA promoted larval growth but a formulated diet containing TAG rich in DHA could not. In Exp. 1, we showed the remarkably higher level of DHA in the PL fraction of yolk-sac larvae compared to enriched *Artemia*. It is difficult to enrich DHA in PL on *Artemia* (Dhont and Stappen 2003), but it will be interesting to determine whether DHA in the PL fraction of yolk-sac larvae plays a promotion role in the excellent growth of PBT larvae.

On the other hand, there were no significant differences in PC and SE levels between the dietary groups in Exp. 2, although these lipid levels were higher in yolk-sac larvae than in enriched *Artemia*, as shown in Exp. 1. There is a possibility that the higher dietary PC and SE levels promoted larval growth in the yolk-sac larvae-fed group. PL and sterol are major biomembrane components and are important for maintaining cellular function and structure. Several fish larvae have a high dietary requirement of PL, especially PC, as mentioned above. It is considered that this requirement is, in part, due to the limiting ability of *de novo* synthesis of PL in larval fish (Teshima et al. 1987). It remains unclear whether fish larvae have absolute dietary sterol and/or SE requirements, but PBT larvae which show quite fast growth compared to other marine fish larvae (Miyashita et al. 2001; Miyashita et al. 2002; Sawada et al. 2005) seem to have a comparatively high dietary requirement of lipids involved in cellular construction.

In conclusion, the results from our experiments suggest that enriched *Artemia* are inferior to yolk-sac larvae in some nutrient levels for PBT larvae. In addition, this suggestion would have consequences regarding not only improving the nutritional value of *Artemia* but also the formulation of an artificial diet for PBT larvae. However, there may be numerous another nutritional differences between *Artemia* and yolk-sac larvae. Other factors such as the digestibility of *Artemia* for PBT larvae have been presumed to be a possible cause leading to the growth failure of PBT larvae fed *Artemia*. Additional study on the nutritional requirement as well as the digestion and absorption of nutrients in PBT larvae is needed to establish efficient rearing technology for PBT larviculture.

Acknowledgements

This work was supported by the Japan Society for the Promotion of Science Grant-in-Aid for Scientific Research (S) 14104007. This work was also supported by the 21st-Century COE program of the Ministry of Education, Culture, Sports, Science and Technology of Japan.

References

AOAC (1984) Official Methods of Analysis of Association of Official Analytical Chemists, 14th ed. Arlington, 1141p.

Conceição, L. E. C., T. van der Meeren, J. A. A. Verreth, M. S. Evjen, D. F. Houlihan and H. J. Fyhn (1997) Amino acid metabolism and protein turnover in larval turbot (*Scophthalmus maximus*) fed natural zooplankton or *Artemia*. *Mar. Biol.*, **129**, 255-265.

Dabrowski K. and H. Guderley (2002) Intermediate metabolism. In "*Fish Nutrition*" (ed. by J. E. Halver and R. W. Hardy), Academic Press, New York, pp. 309-365.

Dhont, J. and G. V. Stappen (2003) Phospholipid enrichment. In "*Live Feeds in Marine Aquaculture*" (ed. by J. G. Støttrup and L. A. McEvoy), Blackwell Science, Oxford, UK, pp. 107-108.

Folch, J., M. Lees, G. H. Sloane (1957) Simple method for isolation and purification of total lipids from animal tissues. *J. Biol. Chem.*, **226**, 497-507.

Fraser, A. J. (1989) Triacylglycerol content as a condition index for fish, bivalve, and crustacean larvae. *Can. J. Fish Aqaut. Sci.*, **46**, 1868-1873.

Fyhn, H. J., R. N. Finn, S. Helland, I. Rønnestad and E. Lømsland (1993) Nutritional value of phyto- and zooplankton as live food for marine fish larvae. In "*Fish farming Technology*" (ed. by H. Reinertsen, L. A. Dahle, L. Jørgensen, K. Tvinnerheim), Balkema, Rotterdam, pp. 121-126.

Geurden, I., P. Coutteau and P. Sorgeloos (1997) Effect of dietary phospholipid supplementation on growth and fatty acid composition of European seabass (*Dicentrarchus labrax* L.) and turbot (*Scophthalmus maximus* L.) juveniles from weaning onwards. *Fish Physiol. Biochem.*, **16**, 259-272.

Grisdale-Helland, B. and S. J. Helland (1997) Replacement of protein by fat and carbohydrate in diets for Atlantic salmon (*Salmo salar*) at the end of the freshwater stage. *Aquaculture*, **152**, 167-180.

Hamre, K., I. Opstad, M. Espe, J. Solbakken, G. -I. Hemre and K. Pittman (2002) Nutrient composition and metamorphosis success of Atlantic halibut (*Hippoglossus hippoglossus*, L.) larvae fed natural zooplankton or *Artemia*. *Aquaculture Nutr.*, **8**, 139-148.

Helland, S. J. and B. Grisdale-Helland (1998) Growth, feed utilization and body composition of juvenile Atlantic halibut (*Hippoglossus hippoglossus*) fed diets differing in the ratio between the macronutrients. *Aquaculture*, **166**, 49-56.

Helland, S., B. F. Terjesen and L. Berg (2003) Free amino acid and protein content in the planktonic copepod *Temora longicornis* compared to *Artemia franciscana*. *Aquaculture*, **215**, 213-228.

Helland, S., G. V. Triantaphyllidis, H. J. Fyhn, M. S. Evjen, P. Lavens and P. Sorgeloos (2000) Modulation of the free amino acid pool and protein content in population of the brine shrimp *Artemia* spp. *Mar. Biol.*, **137**, 1005-1016.

Hodge, J. E. and B. T. Hofreiter (1962) Determination of reducing sugars and carbohydrate. In "*Methods in Carbohydrate Chemistry*" (ed. by R. L. Whistler and M. L. Wolfrom), Academic Press, New York, pp. 388-389.

Juaneda, P. and G. Rocquelin (1985) Rapid and convenient separation of phospholipids and non-phosphorus lipids from rat heart using silica cartridges. *Lipids*, **20**, 40-41.

Kanazawa, A., S. Teshima, S. Inamori and H. Matsubara (1983) Effect of dietary phospholipids on growth of the larval red sea bream and knife jaw. *Mem. Fac. Fish., Kagoshima Univ.*, **32**, 109-114.

MacQueen Leifson, R., J. M. Homme, Ø. Lie, R. Myklebust and T. Strøm (2003) Three different lipid source in formulated start-feeds for turbot (*Scophthalmus maximus*, L.) larvae - effect on growth and mitochondrial alteration in enterocytes. *Aquaculture Nutr.*, **9**, 33-42.

McEvoy, L. A., T. Naess, J. G. Bell and Ø. Lie (1998) Lipid and fatty acid composition of normal and malpigmented Atlantic halibut (*Hippoglossus hippoglossus*) fed enriched Artemia: a comparison with fry fed wild copepods. *Aquaculture*, **163**, 237-250.

Miyashita, S., K. Kato, Y. Sawada, O. Murata, Y. Ishitani, K. Shimizu, S. Yamamoto and H. Kumai (1998) Development of digestive system and digestive enzyme activities of larval and juvenile bluefin tuna, *Thunnus thynnus*, reared in the laboratory. *Suisanzohshoku*, **46**, 111-120.

Miyashita S., Y. Sawada, T. Okada, O. Murata and H. Kumai (2001) Morphological development and growth of laboratory-reared larval and juvenile *Thunnu thynnus* (Pisces: Scombridae). *Fish. Bull.*, **99**, 601-616.

Miyashita, S. (2002) Studies on the seedling production of the Pacific bluefin tuna, *Thunnus thynnus orientalis*. *Bulletin of the Fisheries Laboratory of Kinki University*, **8**, 1-171 (in Japanese, with English abstract).

Olsen, R. E. and R. J. Henderson (1989) Rapid analysis of neutral and polar marine lipids using double-development HPTLC and scanning densitometory. *J. Exp. Mar. Biol. Ecol.*, **129**, 189-197.

Rønnestad, I., A. Thorsen and R. N. Finn (1999) Fish larval nutrition: a review of recent advances in the roles of amino acids. *Aquaculture*, **177**, 201-216.

Rønnestad, I., S. K. Tonheim, H. J. Fyhn, C. R. Rojas-García, Y. Kamisaka, W. Koven, R. N. Finn, B. F. Terjesen, Y. Barr, L. E. C. Conceição (2003) The supply of amino acids during early feeding stages of marine fish larvae: a review of recent findings. *Aquaculture*, **227**, 147-164.

Sargent, J. R. and R. J. Henderson (1986) Lipids. In "*The Biological Chemistry and Marine Copepods*" (ed. by E. D. S. Corner and S. C. M. O'Hara), Clarendon Press, Oxfard, pp. 59-108.

Sargent, J., L. McEvoy, A. Estevez, G. Bell, M. Bell, J. Henderson, D. Tocher (1999) Lipid nutrition of marine fish during early development: current status and future directions. *Aquaculture*, **179**, 217-229.

Sawada, Y. , T. Okada, S. Miyashita, O. Murata and H. Kumai (2005) Completion of the pacific bluefin tuna *Thunnus orientalis* (Temminck et Schlegel) life cycle. *Aquaculture Res.*, **36**, 413-421.

Seoka, M., S. Yamada and H. Kumai (2004) Free amino acids in Japanese eel obtained by hormonal inducement. *J. Fish Biol.*, **65**, 592-596.

Sheridan, M. A. (1988) Lipid dynamics in fish: aspect of absorption, transportation, deposition and mobilization. *Comp. Biochem. Physiol.*, **90B**, 679-690.

Sorgeloos, P., P. Dhert and P. Candreva (2001) Use of the brine shrimp, *Artemia* spp., in marine fish larviculture. *Aquaculture*, **200**, 147-159.

Takeda, M., S. Shimeno, H. Hosokawa and T. Kaisyo (1975) The effect of dietary calorie-protein ratio on the growth, feed conversion and body composition of young yellowtail. *Nippon Suisan Gakkaishi*, **41**, 443-447 (in Japanese, with English abstract).

Takeuchi, T., T. Arakawa, S. Satoh and T. Watanabe (1992) Supplemental effect of phospholipids and requirement of eicosapentaenoic acid and docosahexaenoic acid of juvenile striped jack. *Nippon Suisan Gakkaishi,* **58**, 707-713.

Takii, K., S. Miyashita, S. Seoka, Y. Tanaka, Y. Kubo and H. Kumai (1997) Changes in chemical contents and enzyme activities during embryonic development of

bluefin tuna. *Fisheries Sci*., **63**, 1014-1018.
Teshima, S., A. Kanazawa, K. Horinouchi, S. Yamashitra and H. Hirata (1987) Phospholipids of the rotifers, prawn and larval fish. *Nippon Suisan Gakkaishi*, **53**, 609-615.
Tocher, D. R. and J. R. Sargent (1984) Analyses of lipids and fatty acids in ripe roes of some northwest European marine fish. *Lipids*, **19**, 492-499.
Verakunpiriya, V., T. Watanabe, K. Mushiake, V. Kiron, S. Satoh and T. Takeuchi (1996) Effect of broodstock diets on the chemical components of milt and eggs produced by yellowtail. *Fisheries Sci*., **62**, 610-619.
Watanabe, T., M. -J. Lee, J. Mizutani, T. Yamada, S. Satoh, T. Takeuchi, N. Yoshida, T. Kitada and T. Aarakawa (1991) Effective components in cuttlefish meal and raw krill for improvement of quality of red seabream *Pagrus major* eggs. *Nippon Suisan Gakkaishi*, **57**, 681-694.
Yoshinaka, R. and M. Satoh (1989) Chemistry for Fisheries. Kouseisya-kouseikaku, Tokyo, pp. 77-78 (in Japanese).

クロマグロ仔魚の成育に悪影響を及ぼすアルテミアの栄養素

瀬岡　学・倉田道雄・畑中康宏
Amal K. Biswas・Ji Seung Chul・熊井英水

アルテミアの給与がクロマグロ仔魚の成育不良を引き起こすとされる原因の一端を推察するために，ワムシをリファレンスとし，栄養強化したアルテミアとその代替餌料であるイシダイ卵黄仔魚の栄養素を比較した。その結果，アルテミアで糖質含量が高く，ホスファチジルコリン，ステロールエステルおよび中性・極性脂質画分のドコサヘキサエン酸（DHA）レベルは低かった。さらに，アルテミアとイシダイ卵黄仔魚を与えてクロマグロ仔魚を飼育したところ，成長や生残は後者を与えたクロマグロ仔魚で優れ，全魚体の中性・極性脂質画分における DHA レベルも高かった。したがって，アルテミアにおけるこれら栄養素の過不足が，クロマグロ仔魚の成育に悪影響を及ぼしている可能性が推察された。