クロマグロの生残および成長におよぼす微粒子配合飼料ならびにハマフエフキ孵化仔魚の日齢の影響

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Effect of Feeding Microdiet and Yolk-sac Larvae of Spangled Emperor *Lethrinus nebulosus* at Different Ages on Survival and Growth of Pacific Bluefin Tuna *Thunnus orientalis* Larvae

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Abstract: Pacific bluefin tuna (PBT) *Thunnus orientalis* larvae at 18 days post hatching (dph) (total length; 14.8±2.1 mm) were fed for 6 days on fertilized eggs, yolk-sac larvae of spangled emperor *Lethrinus nebulosus* at 1 or 3 dph, or a microdiet. After feeding, growth performance, feeding incidence and survival were evaluated. Feeding 3 dph spangled emperor yolk-sac larvae improved growth of PBT compared to the other feeds. Crude protein and essential amino acids contents in the 3 dph spangled emperor yolk-sac larvae were higher than those of the spangled emperor at 1 dph. This result suggests that yolk-sac larvae of spangled emperor at 3 dph are suitable for PBT larvae and improved growth would be due to their higher protein and essential amino acids contents. It was also observed that 40% of PBT larvae fed the microdiet, suggesting that casein peptide based microdiet is acceptable for PBT.

Key words: Bluefin tuna; Larvae; Growth; Protein

Rapid expansion of global bluefin tuna farming has led to public concern as it is perceived as a threat to the natural resource of bluefin tuna because tuna farming strongly depends on capture of wild juveniles. Dependence on wild captured tuna juveniles for bluefin tuna farming could be reduced by use of artificially raised tuna juveniles. However, a major problem in artificial juvenile production of Pacific bluefin tuna (PBT) *Thunnus orientalis* is the high mortality during the early stages. To overcome this problem, a better understanding of environmental conditions in fish tanks, favorable feeding schedule and feed materials for PBT larvae is required (Miyashita et al. 1997). In the currently-used hatchery protocol, rotifers, *Artemia* nauplii and yolk-sac larvae of marine fish are fed from 2, 10, and 13 days post hatching (dph), respectively (Miyashita et al. 1997). Because of the rapid growth of PBT during the early stages, large amounts of live food are required during prolonged rearing (Miyashita et al. 1997). Feeding live fish larvae is very important to support faster growth during the early phase of artificial production of PBT juveniles in hatcheries (Miyashita et al.

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1997). Feeding large amount of live fish larvae to PBT larvae is essential to minimize cannibalism induced by strong piscivorous nature of this species (Miyashita et al. 1997). Therefore, obtaining a continuous supply of large amounts of yolk-sac larvae of marine fish for mass production of PBT is critical for successful juvenile production. To ensure a continuous supply of yolk-sac larvae, the marine fish is also expected to spawn a large amount of eggs daily (Miyashita et al. 1997). In Amami Station, National Center for Stock Enhancement, Fisheries Research Agency of Japan, spangled emperor *Lethrinus nebulosus* has been used as the source of yolk-sac larvae for PBT, because it spawns over a comparatively longer period than the other species in the Okinawa region of Japan. Therefore, spangled emperor broodstock fish have been maintained as a source of yolk-sac larvae in Amami Station. However, yearly management of spangled emperor requires extra-labor in addition to juveniles of PBT and thus increased expenditure. In addition, it is also assumed that nutritional content in yolk-sac larvae of spangled emperor is not necessarily stable because of the rapid change in nutritional content of marine fish during the embryonic development (Fraser et al. 1988; Hilton et al. 2008). Higher requirement of docosahexaenoic acid (DHA) for PBT has been suggested (Biswas et al. 2006; Seoka et al. 2007, 2008). It is well-documented that the nutritional content in marine fish larvae is not necessarily stable, i.e., DHA since its rapid reduction during early larval stage (Fraser et al. 1988; Rainuzzo et al. 1997; Hilton et al. 2008). However, there is no study to examine the larval performance of PBT fed different age yolk-sac larvae of spangled emperor. Therefore, the present study investigates the effect of feeding yolk-sac larvae of spangled emperor at different ages on survival and growth of PBT larvae. In addition, it is considered that in order to reduce the dependence on yolk-sac larvae, production of a high quality microdiet (MD) may enable a more streamlined production system. In our previous studies, partial substitution of live food such as rotifers and *Artemia* for production of Japanese flounder *Paralichthys olivaceus* was achieved by the development of a MD formulated using casein peptide (Takeuchi et al. 2003; Wang et al. 2004). However, MD for PBT has yet to be developed. Therefore, the present study also examined the effect of feeding casein peptide-based MD on the larval performance of PBT.

Materials and Methods

Experimental fish

Fertilized eggs of PBT were obtained from Fisheries Laboratory, Kinki University, Oshima, Wakayama. They were collected on August 10, 2007, and transported to Amami Station, National Center for Stock Enhancement, Kagoshima. They were raised by feeding rotifers *Brachionus plicatilis* and *Artemia* nauplii until 17 dph. On 18 dph, they were transferred into six 200 *l* cylindrical transparent experimental tanks holding 360 larvae in each tank (Table 1). During transportation, yolk-sac larvae of spangled emperor were provided for the PBT larvae at a density of 0.2 ind./*ml* (Table 1). Water flow rate and aeration was gradually increased 100 – 500% per day and 1000 – 1800 *ml*/min, respectively (Table 1). Water temperature was maintained at 28.5 ± 0.5℃ (Table 1). Buoyant eggs of spangled emperor produced by natural spawning were manually collected and introduced in a 1000 *l*

Table 1. Rearing conditions of PBT larvae

Initial mean size of PBT (mm)[1]	14.8 ± 2.1
Initial age of PBT (days post hatching)	19
Tank volume (L)	200
Number of PBT[2]	360 (treatments 1-5) 120 (treatment 6)
Feeding frequency (times/day)	6 (live food) 48 (microdiet)
Density of live food (ind./*ml*)	0.2
Feeding amount of microdiet (g/day)	6 - 28
Experimental period (days)	6
Water temperature (℃)[3]	28.0 ± 0.5
Photoperiod	Natural
Water flow (%/day)	100 - 500
Aeration (*ml*/min)	1000 - 1800

[1] Mean ± standard deviation (n = 25).
[2] 120 fish was used for starvation.
[3] Mean ± standard deviation.

cylindrical tank designed for rearing *Artemia* (Table 1). PBT were subjected to one of six different dietary treatments: fertilized eggs of spangled emperor (egg), yolk-sac larvae of spangled emperor at 1 dph (1 dph), and 3 dph (3 dph), casein peptide based MD, limited amount of yolk-sac larvae of spangled emperor at 1 dph (Limited), and no feeding (Starvation) (Table 2). Several weaning protocols were used to facilitate changes of live food into MD for larval fish and successful weaning is crucially important for feeding experiment of MD (Kolkovski et al., 2009). The limited treatment was designed as a control for the MD treatment where the limited amount of prey fish larvae was fed to facilitate a change of feeding onto MD (Fig. 1). Spangled emperor eggs were kept in a 200 *l* tanks at natural temperature before feeding. In order to avoid hatching of spangled emperor eggs after feeding, spangled emperor eggs fed to PBT in the afternoon were kept at 22℃. MD was formulated using casein peptide produced by Taiyo Yushi Co. (Table 3). Feeding frequency of yolk-sac larvae of spangled emperor was reduced in the limited treatment, and MD treatment (Fig. 1). Yolk-sac larvae of spangled emperor were fed every two h between 6:00－18:00. Density of yolk-sac larvae of spangled emperor in fish tanks was maintained at 0.2 ind./*ml* between 6:00－14:00 and at 0.1 ind./*ml* from 16:00 onwards. Fatty acid-calcium was included in the MD as a binder (Takeuchi et al. 2003; Wang et al. 2004). Particle size of MD was 500－710 μm and 710－1000 μm. It was provided manually at 6－28 g per day per tank and amount of it was gradually increased with growth of PBT. Total length and body weight were recorded at 0 (initial), 2, 5 and 6 days after the initiation of the feeding trial. Because of difficulties in counting the exact number of survivors in the tank, survival rate was calculated by subtracting the total number of dead fish that were collected during the daily cleaning of tanks. Total length of fish was measured after being anesthetized using 0.5% ethyl 3-aminobenzoate methanesulfonate salt (Sigma Co., St. Louis, MO). Total length at 2 and 3 days after the initiation of feeding trial was measured under a universal precision measuring projector (Nikon, VB-12, Tokyo, Japan). After being anesthetized using 0.5% ethyl 3-aminobenzoate methanesulfonate salt, moisture

Table 2. Experimental treatments

1	Fertilized eggs of spangled emperor[1] (Egg)
2	Yolk-sac larvae of spangled emperor at 1 dph[1] (1 dph)
3	Yolk-sac larvae of spangled emperor at 3 dph[1] (3 dph)
4	Microdiet (MD)
5	Limited feeding (Limited)
6	Stravation

[1] *Lethrinus nebulosus.*

Spangled emperor fish eggs and embryos at the 1st or 3rd dph (treatments 1-3)

	Days after initiation of feeding trial					
Time	1	2	3	4	5	6
6:00	●	●	●	●	●	●
8:00	●	●	●	●	●	●
10:00	●	●	●	●	●	●
12:00	●	●	●	●	●	●
14:00	●	●	●	●	●	●
16:00	●	●	●	●	●	●

MD

	1	2	3	4	5	6
6:00	○	○	○	○	○	○
8:00	○●	○	○	○	○	○
10:00	○●	○●	○	○	○	○
12:00	○●	○●	○●	○	○	○
14:00	○●	○●	○●	○●	○	○
16:00	○●	○●	○●	○●	○●	○●

Limited

	1	2	3	4	5	6
6:00	-	-	-	-	-	-
8:00	●	-	-	-	-	-
10:00	●	●	-	-	-	-
12:00	●	●	●	-	-	-
14:00	●	●	●	●	-	-
16:00	●	●	●	●	●	●

Fig. 1. Changes in feeding frequency of MD, eggs or yolk-sac larvae of spangled emperor after initiation of feeding trial. Eggs or yolk-sac larvae of spangled emperor were given six times a day during the feeding trial in the egg, 1 dph or 3 dph treatments. 1dph yolk-sac larvae of spangled emperor were given five times a day on the first day but frequency of feeding eggs and yolk-sac larvae was gradually reduced over the subsequent 5 days in the MD and limited treatments. MD was fed six times a day from 1-6 days after initiation of feeding trial. ○ indicated MD given and ● did spangled emperor eggs or larvae given.

Table 3. Formulation of microdiet (%)

Emul-up[1]	49.0
C800[2]	12.2
Fatty acid-Ca[3]	23.6
L-Arginine	0.4
L-Cystine	0.9
Taurine	1.4
Mineral mixture[4]	2.0
Spirulina	1.0
DL-α-Tocopherol	0.1
Soybean lecithin	5.0
Wheat gluten	2.0
Choline chloride	0.8
Ascorbyl 2-phosphate magnesium	0.1
Vitamin mixture[5]	1.5

[1,2] Casein peptide.
[3] Prepared from fish oil.
[4] Mineral supplement supplied (mg/kg diet): Na (as NaCl) 197; Mg (as $MgSO_4 \cdot 7H_2O$) 735; Fe (as $FeC_6H_5O_7 \cdot 5H_2O$) 258; Zn (as $ZnSO_4 \cdot 7H_2O$) 40; Mn (as $MnSO_4 \cdot 5H_2O$) 18; Cu (as $CuSO_4 \cdot 5H_2O$) 3.9; Al (as $AlCl_3 \cdot 6H_2O$) 0.56; Co (as $CoCl_2 \cdot 6H_2O$) 0.15; I (as KIO_3) 0.89; α-cellulose carrier.
[5] Vitamin supplement supplied (amount/kg diet): thiamin hydrochloride, 60 mg; riboflavin, 100 mg; pyridoxine hydrochloride, 40 mg; cyanocobalamin, 0.1 mg; ascorbic acid, 5000 mg; niacin, 400 mg; calcium pantothenate, 100 mg; inositol, 2000 mg; biotin, 6 mg; folic acid, 15 mg; p-aminobenzoic acid, 50 mg; vitamin K_3, 50 mg; vitamin A acetate, 9,000 IU; vitamin D_3, 9,000 IU.

on the skin surface was removed using a paper towel, and they were weighed on an electronic balance. In order to determine feeding activity and acceptability of the MD, the number of PBT showing feeding behavior for MD was counted by visual observation for 15 min after feeding the MD. PBT having the MD in the digestive tract were regarded as fish that had accepted the MD. At 2, 4, and 6 days after initiation of feeding trial, a sample of 15 fish were taken from each tank, stomach was dissected from each fish and its content was observed under a stereo microscope (Olympus, B201, Tokyo, Japan). For chemical analysis, all fish were force starved for a half day, collected and stored at −80℃.

Chemical analysis

Frozen samples were sent to Laboratory of Fish Culture, Tokyo University of Marine Science and Technology, Shinagawa, Tokyo and subjected for proximate, amino acid and fatty acid composition analyses. Frozen spangled emperor and PBT were homogenized before chemical analysis. For fatty acid analysis, crude lipid was separated into non-polar and polar lipid fractions and used for fatty acid analysis. Moisture was determined by using 0.2−0.3 g of samples and they were heated at 110℃ for 2 h. Nitrogen content was measured by Kjeldahl's method (Matsunari et al. 2008). Crude protein content was determined by calculating the nitrogen content multiplied by 6.25. Crude lipid was extracted using a mixture of chloroform and methanol (2:1) and determined gravimetrically (Folch et al. 1957). Crude lipid was applied onto Sep-Pak silica cartridges (Waters Co., Millford, USA) and polar lipid was eluted by 20 *ml* of chloroform and 20 *ml* chloroform: methanol (49:1) mixture. Then, non-polar lipid fraction was eluted with 40 *ml* methanol. Non-polar and polar lipid contents were measured gravimetrically after evaporation of solvents. Lipid class analysis was done by Iatroscan TH-10 (Iyatron Laboratories Inc., Tokyo, Japan). For fatty acid analysis, lipid was saponified by 1 *ml* of 50% KOH in 15 *ml* ethanol and heated at 80℃ for 40 min. The saponifiable matter were esterified by 6.7% boron trifluoride in methanol and heated at 80℃ for 20 min (Morrison and Smith 1964). Fatty acid methyl esters were diluted in n-hexane and injected into the gas-liquid chromatograph (GC-14B, Shimadzu Co., Japan) equipped with a silica capillary column (30 m × 0.32 mm × 0.25 μm thickness, Supleco-Wax 10A.F, SIGMA-ALDRIDGE Co., USA). Helium was used as the carrier gas and the pressure was adjusted to 100 kPa. Temperatures in the column, injection port and detector were adjusted to 170−205 (1℃ increases per 1 min), 250 and 250℃, respectively. Fatty acid methyl esters were identified by comparing retention times against standards prepared from fish meal. For free amino acid (FAA) analysis, sample was homogenized in 2% sulfosalicylic acid and centrifuged at 3,000 rpm. Upper layer were pooled twice and injected in high performance liquid chromatography (JLC500, JEOL Ltd., Tokyo, Japan) according to Matsunari et al. (2008). Data of the constituent amino acids (CAA) were calculated by subtracting the values of FAA from that of total amino acids determined according to Shimpson et al. (1979).

Results

Growth and survival

High mortality was observed in fish fed spangled emperor eggs and also specimens subjected to starvation. Because the mortality was more than 90% in these treatments by 2 days after the initiation of the feeding trial, rearing of fish in the two tanks was terminated at 3 days after the initiation of the feeding trial. Survival rate at 6 days after initiation of feeding trial was the highest in the 3 dph treatment (Fig. 2). Survival of the MD and limited treatments was lower than that of 1 and 3 dph treatments (Fig. 2). Total length and body weight of the 3 dph treatment at 6 days were higher than those in the 1 dph treatment (Fig. 3). There was no difference in total length and body weight between the MD and the limited treatments. Difference in total length of the fish among the treatments was evident from 4 days after the initiation of feeding trial (Fig. 4). MD pellets were observed in stomach contents of 6 fish out of 15 fish in the MD treatment on the 6th days. However, there is no stomach content in fish observed at 2 and 4 days after the initiation of feeding trial.

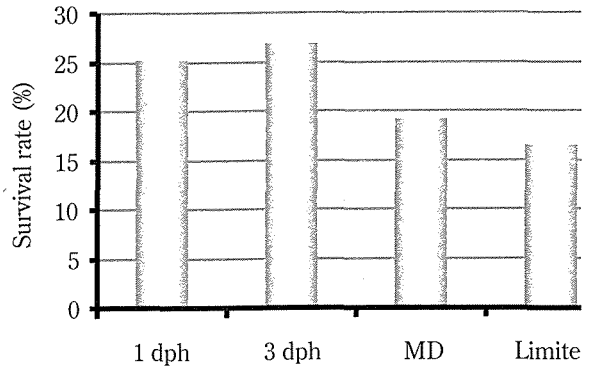

Fig. 2. Survival rate of Pacific bluefin tuna larvae fed different feed treatments for six days during the feeding trial.

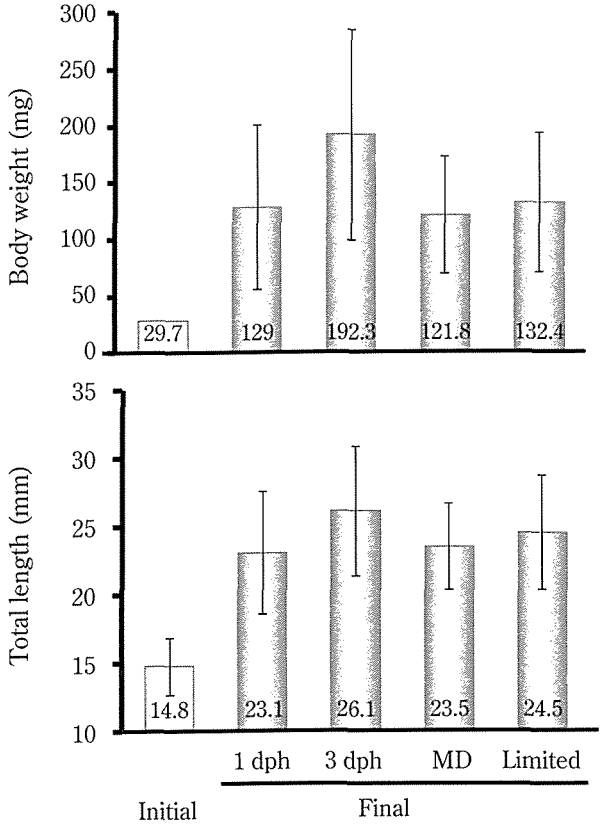

Fig. 3. Growth performance of Pacific bluefin tuna larvae fed different treatments of feed at the end of the feeding trial.

Chemical composition of live food and the MD

Crude protein content in yolk-sac larvae of spangled emperor at 3 dph was the highest among the diets (Table 4). Essential amino acid (EAA) contents in the yolk-sac larvae of spangled emperor at 3 dph was also higher than yolk-sac larvae at 1 dph and MD (Table 4). Among the EAA of live food and MD, arginine, lysine and leucine were higher than the other EAAs (Table 4). Regarding non-EAA, glycine, glutamic acid and aspartic acid were higher in yolk-sac larvae at 3 dph compared to those at 1 dph (Table 4). In contrast, crude lipid content in yolk-sac larvae of spangled emperor at 1 dph was higher than that at 3 dph (Table 4). Total FAA content was the highest in the fertilized eggs of spangled emperor (Table 5). FAA content in the MD was very low except for

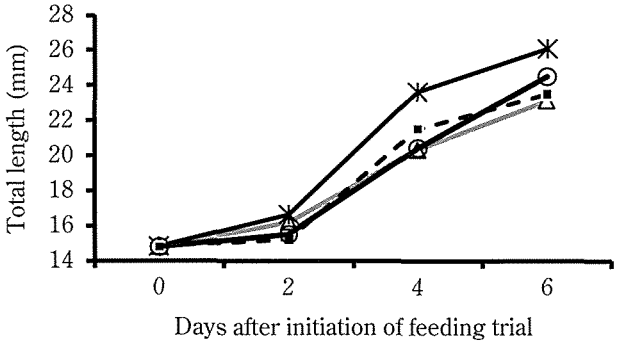

Fig. 4. Body weight and total length of Pacific bluefin tuna larvae fed different treatments of feed for 6 days. —△—, 1 dph; —✳—, 3 dph; —▪—, MD; —⊖—, Limited

arginine, taurine and cysteine that were supplemented (Table 5). Neutral lipid content was lower in yolk-sac larvae at 3 dph (5.3 g/ 100 g) than the other diets (9.5－11.2 g/ 100 g) (Table 6). Among neutral lipids, the triglyceride content in spangled emperor at 1 dph was very low (0.6) compared to the other diets (4.8－7.2 g /100 g) (Table 6). Regarding polar lipid contents, it was lower in the MD compared to the other diets. Sum of the n-3 HUFA content in both polar and non-polar lipid fractions in yolk-sac larvae at 3 dph was lower than that at 1 dph (Table 7).

Table 4. Moisture, crude protein, crude lipid and constituent amino acid content of egg, 1 dph and 3 dph spangled emperor and microdiet

	Egg	1 dph	3 dph	MD
Moisture (%)	93.7	91.5	92.9	6.8
Crude protein (%, d.b.)	68.2	68.6	78.1	59.5
Crude lipid (%,d.b.)	18.4	21.7	14.8	15.4
Essential amino acid (g/100 g, d.b.)				
Arginine	5.1	3.3	4.7	2.5
Lysine	6.6	4.0	5.1	4.3
Histidine	1.9	1.5	1.3	2.0
Phenylalanine	3.6	2.3	2.5	3.1
Leucine	9.2	4.1	5.0	5.5
Isoleucine	7.2	2.6	3.0	2.9
Methionine	2.1	1.1	1.6	1.5
Valine	5.4	2.6	3.0	3.4
Threonine	4.1	2.3	2.8	2.5
Tryptophan	ND	0.4	0.4	0.6
Non-essential amino acid (g/100 g, d.b.)				
Taurine	0.6	0.5	0.9	1.2
Alanine	6.7	3.0	3.7	1.9
Glycine	3.0	2.6	3.9	1.2
Glutamic acid	10.7	6.8	8.4	13.9
Serine	5.4	2.2	3.0	3.2
Aspartic acid	5.7	4.5	5.7	4.2
Cysteine	ND	0.3	0.3	0.8
Total amino acid	77.5	44.1	55.2	54.7

ND, not detected.

Table 5. Free amino acid compostion of spangled emperor and microdiet

	Egg	1 dph	3 dph	MD
Essential amino acid (mg/100 g, d.b.)				
Arginine	1166.5	118.5	42.1	352.1
Lysine	1361.0	124.3	61.2	33.0
Histidine	480.1	179.6	183.4	0.1
Phenylalanine	805.9	437.5	30.6	35.1
Leucine	2218.7	142.8	55.8	44.0
Isoleucine	1296.4	65.3	50.5	4.9
Methionine	748.2	214.1	16.8	5.3
Valine	1332.3	100.1	42.3	3.1
Threonine	723.1	79.9	48.7	1.6
Tryptophan	438.2	122.9	11.9	24.2
Non-essential amino acid (mg/100 g, d.b.)				
Taurine	588.0	711.4	848.7	1131.2
Alanine	1413.0	214.5	97.4	5.8
Glycine	348.0	119.2	135.7	3.3
Glutamic acid	387.0	501.5	199.4	8.6
Serine	1150.0	146.9	75.8	1.0
Aspartic acid	131.3	100.2	111.5	5.1
Cysteine	ND	ND	ND	534.3
Total amino acid	14587.7	3378.7	2011.8	2192.7

ND, not detected.

Chemical composition of PBT

Crude protein content of the 3 dph treatment was the highest among the treatments, followed by the limited treatment (Table 8). Similar crude protein content was recorded in the both 1 dph and the MD treatments (Table 8). The lowest crude lipid content was the obtained

Table 6. Lipid class content of spangled emperor and microdiet

	Spangled emperor			MD
	Egg	1 dph	3 dph	
Neutral lipid (g/100g, d.b.)				
Sterol esters	2.1	2.0	0.5	TR[1]
Triglycerides	4.8	6.3	0.6	7.2
Free fatty acids	0.1	0.3	0.4	2.6
Free sterols	0.8	2.0	0.1	0.1
Diglycerides	1.1	0.1	3.4	0.0
Monoglycerides	0.5	0.1	0.2	0.8
Polar lipid (g/100g, d.b.)				
Phosphatidic acid	0.2	0.6	0.2	0.1
Phosphatidylethanolamine	0.4	2.3	3.1	2.1
Phosphatidylserine	0.9	0.3	0.3	0.3
Phosphatidylinositol	0.1	0.1	0.2	0.0
Lysophosphatidylethanolamine	0.5	1.0	0.9	0.3
Phosphatidylcholine	5.7	5.7	3.7	1.1
Sphingomyelin	0.1	0.1	0.1	ND[2]
Lysophosphatidylcholine	1.0	TR	0.1	0.2
Total neutral lipids (g/100g, d.b.)	9.5	11.1	5.3	11.2
Total polar lipids (g/100g, d.b.)	8.9	10.6	9.5	4.2

[1] TR, trace (<0.05).
[2] ND, not detected.

Table 7. Fatty acid content of spangled emperor and microdiet (g / 100 g, dry weight basis)

	Egg		1 dph		3 dph		MD	
	NL[1]	PL[1]	NL	PL	NL	PL	NL	PL
Crude lipid	9.5	8.9	11.1	10.6	5.3	9.5	11.2	4.2
Fatty acid								
Σ Saturates	2.8	2.3	3.1	3.6	1.4	1.5	2.6	1.3
Σ Monoenes	2.6	2.8	3.6	1.5	1.1	3.6	2.1	0.7
EPA	0.4	0.5	0.4	0.5	0.3	0.3	0.6	0.0
DHA	0.7	2.0	0.8	3.1	0.4	2.5	2.5	0.2
Σ n-3 HUFA[2]	1.3	2.7	1.4	3.8	0.8	2.9	3.3	0.2

[1] NL, neutral lipid and PL, polar lipid.
[2] Σ n-3 highly unsaturated fatty acids including 20 : 4+20 : 5+22 : 5 +22 : 6.

in the MD treatment (10.7%), but the highest lipid content was detected in the 1 dph treatment (12.4%) (Table 8). Reduction of several EAA such as lysine, leucine, isoleucine and valine and non-EAA, such as glutamic acid and aspartic acid in CAA was observed in the 1 dph treatment compared to initial levels (Table 8). Very low amount of methionine was recorded in the MD treatment, compared with other treatments (Table 8). However, higher isoleucine and threonine levels were recorded in the MD treatment compared to the initial fish (Table 8). All treatments showed a lower final polar lipid content than the initial content (Table 9). Reduction of polar lipid content among treatments was mainly observed in Σ saturates and Σ monoenes (Table 9). In addition, lower neutral lipid content was recorded in the MD and limited treatments (Table 9). There is no difference in n-3 HUFA content in all treatments, except low Σ n-3 HUFA in polar lipid fraction of the limited treatment (Table 9).

Table 8. Moisture, crude protein, crude lipid, and constitutive amino acid composition in the whole body of larval bluefin tuna

Experimental lots	Initial	Final			
		Spangled emperor		MD	Limited
		1 dph	3 dph		
Moisture (%)	83.3	80.8	81.5	80.6	83.3
Crude protein (%, d.b.)	76.4	77.4	83.3	77.5	80.2
Crude lipid (%,d.b.)	14.8	13.4	12.4	10.9	11.4
Essential amino acids (g/100 g, d.b.)					
Arginine	4.2	3.8	4.0	4.3	4.3
Lysine	5.4	4.7	5.1	5.4	5.5
Histidine	1.9	2.2	2.2	2.2	2.4
Phenylalanine	2.7	2.3	2.5	2.7	2.7
Leucine	4.9	4.2	4.6	5.0	5.1
Isoleucine	2.5	2.1	2.5	3.1	3.0
Methionine	1.9	1.7	1.8	0.8	1.9
Valine	3.0	2.5	2.8	3.4	3.3
Threonine	3.0	2.7	2.9	3.4	3.1
Tryptophan	0.4	0.6	0.6	0.4	0.7
Non-essential amino acids (g/100 g, d.b.)					
Taurine	1.4	1.2	1.4	1.3	1.2
Alanine	3.9	3.7	3.7	3.8	4.0
Glycine	3.7	3.9	3.7	3.9	4.1
Glutamic acid	9.5	8.5	9.1	9.5	9.7
Serine	3.2	2.8	2.9	2.8	3.1
Aspartic acid	6.1	5.5	5.8	6.0	6.2
Total amino acids	57.7	52.4	55.6	58.0	60.3

Discussion

The present study demonstrated that feeding yolk-sac larvae at 3 dph improved larval growth of PBT. A higher content of crude protein but lower content of crude lipid was observed in yolk-sac larvae at 3 dph compared to those at 1 dph. Growth improvement of the 3 dph treatment might be due to the live food with a higher crude protein content. In addition, amino acid analysis revealed that several CAAs are higher in the 3 dph treatment. Biswas et al. (2009) suggested that PBT larvae require 62% of crude protein in the diet and a higher dietary protein inclusion induced growth retardation. The present study revealed that yolk-sac larvae of spangled emperor at 1 and 3 dph contained 63 and 73% of crude protein, respectively. However, improved growth was observed in PBT in the 3 dph treatment compared to that of the 1 dph treatment. This inconsistent result could be due to the different types of the feeds; Biswas et al. (2009) used a compound food,

Table 9. Fatty acid content in the whole body of larval bluefin tuna (g / 100 g, dry weight basis)

Experimental lots	Initial		Spangled emperor				MD		Limited	
			1 dph		3 dph					
	NL[1]	PL[1]	NL	PL	NL	PL	NL	PL	NL	PL
Crude lipid	5.4	9.4	5.3	8.1	5.3	7.1	3.5	7.4	4.6	6.8
Fatty acid (g/100 g, d.b.)										
Σ saturates	1.2	3.4	1.6	2.8	1.6	2.5	1.1	2.5	1.0	2.3
Σ monoenes	0.8	1.4	1.1	1.1	0.8	0.9	0.3	1.0	0.5	1.0
EPA	0.4	0.4	0.2	0.3	0.3	0.2	0.2	0.2	0.3	0.2
DHA	0.7	2.0	0.8	2.4	0.8	2.2	0.6	2.2	0.6	1.9
Σ n-3 HUFA[2]	1.2	2.6	1.1	2.8	1.1	2.4	0.8	2.6	1.0	2.2

[1] NL, neutral lipid and PL, polar lipid.
[2] Σ n-3 highly unsaturated fatty acids including 20:4+20:5+22:5+22:6.

whereas the present study used live food.

The present study demonstrated that CAA content in yolk-sac larvae of spangled emperor at 3 dph was higher than those at 1 dph. Higher EAA (arginine, lysine, leucine, methionine, and threonine) content was observed in the CAA in yolk-sac larvae at 3 dph. However, some of the EAA (arginine, lysine, phenylalanine, methionine, valine, and tryptophan) content in FAA of the yolk-sac larvae of spangled emperor at 3 dph was lower than those at 1 dph at two to ten-fold except histidine and leucine. Considering that growth retardation of PBT was probably induced by nutritional deficiency, growth retardation of PBT in the 1 dph treatment could be induced by EAA deficiency in CAA rather than that in FAA. A similar observation was made by Seoka et al. (2007) who tried to determine the essential nutrients deficient in *Artemia* when fed to PBT. They compared larval performance in PBT solely fed on enriched *Artemia* or yolk-sac larvae of Japanese parrotfish *Oplegnathus fasciatus* and found retarded growth and lower survival in fish fed *Artemia*. As pointed out by these authors, it was thought that the major cause of the poor performance of PBT fed on *Artemia* was probably induced by a deficiency of HUFA in *Artemia*. However, no difference was observed in the n-3 HUFA content in fish of all treatments. This implies that the difference in the larval performance in this experiment was not due to a deficiency of n-3 HUFA in the diet. It is well-accepted that FAA is a better energy source for fish larvae compared to protein because of its higher digestibility (Rønnestad et al. 2003; Zhang et al. 2006). It was observed that the yolk-sac larvae of Japanese parrotfish contains higher FAA than *Artemia*, suggesting that higher FAA content promoted early growth of PBT fed yolk-sac larvae of Japanese parrotfish. However, it should be pointed out that the total FAA content in both *Artemia* and Japanese parrotfish is much lower than those in CAA (less than 20 fold lower; Seoka et al. (2007). In addition, the present study also demonstrated that faster growth was observed in PBT of the 3 dph treatment, even though the total FAA content in yolk-sac larvae of spangled emperor at 3 dph was lower than that of fish at 1 dph. These results implied that CAA had a higher potential to promote early growth of PBT. Although the amino acid requirement of PBT larvae is unclear, difference in amino acid content in spangled emperor larvae at 1 and 3 dph potentially affected early growth of PBT.

In recent studies, taurine has been suggested to be one of the essential nutrients for marine fish larvae in the early stages (Kim et al. 2005; Matsunari et al. 2005). Growth promotion by taurine supplementation was documented in recent papers on red sea bream, Japanese flounder, and Pacific cod, *G. macrocephalus* (Wang et al. 2004; Kim et al. 2005; Matsunari et al. 2005, 2008). However, it was observed that there was no difference in the taurine content between spangled emperor larvae at 1 and 3 dph in the present study. Determining taurine requirement of PBT larvae is a subject for future studies.

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クロマグロの生残および成長におよぼす微粒子配合飼料ならびにハマフエフキ孵化仔魚の日齢の影響

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全長14.8 mmのクロマグロ仔魚（孵化後18日齢）にハマフエフキ浮上卵，日齢1または3の孵化仔魚，およびカゼインを主なペプチド源とする微粒子配合飼料を給餌して6日間飼育した。また，対照として絶食区を設けた。その結果，浮上卵および絶食区では3日目までにすべての魚が斃死した。一方，日齢3のハマフエフキ孵化仔魚を給餌して飼育した区では，成長が改善された。日齢3のハマフエフキ孵化仔魚の粗タンパク質含量および必須アミノ酸含量は，日齢1のものよりも多かった。以上から，日齢3のハマフエフキ孵化仔魚がクロマグロ仔魚の餌として適していることが示唆された。また，微粒子配合飼料は，約4割の個体で摂餌が確認され，カゼインペプチドを配合した微粒子飼料を摂餌することが示唆された。