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Rearing of Larval Red Sea Bream and Ayu with Artificial Diets

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Abstract

This paper presents the results of attempts to rear larvae of the red sea bream (*Chrysophrys major*) and Ayu (*Plecoglossus altivelis*) with several microparticulate diets; the nylon-protein microencapsulated diet (MED), agar-polyacrylate micro-binding diet (MBD), and zein micro-coating diet (MCD). The microparticulate diets scarcely supported growth and survival of the newly hatched larvae of *C. major* and *P. altvivelis*, although the zein MCD supported growth of *P. altivelis* slightly. The nylon-protein MED promoted growth of the 10-day larvae of *C. majar* to some extent, but the survival rate was low. As for *P. altivelis*, the nylon-protein MED and zein MCD were also effective in supporting growth and survival when these diets were supplied to the 10-day larvae. The combinations of the rotifers and one of these artificial diets gave good growth and high survival of *C. major* and *P. altivelis*.

Rearing of larval fish such as the Ayu (*Plecoglossus altivelis*) and the red sea bream (*Chrysophrys major*) as a seed for artificial culture has been carried out using live food[1]. However, there are some problems on the culture of live food such as the rotifer (*Brachionus plicatilis*) and *Artemia salina*. For example, the production of live food not only needs enomous cost for the facilities and personnel expenditures, but also sometimes faces the trouble of high mortality of larval fish due to the nutritional incompleteness of live food produced[2]. In order to solve these problems, we think it desirable to develope artificial diets for larval fish as substitutes for live food. Rearing of larval fish with artificial diets will give us the knowledge on nutritional requirements and the track of clarifying the cause of malformation and other diseases in larvae.

Previously, we have succeeded in rearing the larval prawn (*Penaeus japonicus*) by using nylon-protein microencapsulated diets (nylon-protein MED) alone with good growth and survival comparable to those found in a live food, *Chaetoceros gracilis*[3,4]. In the present study, hence, we attempt to rear larvae of the red sea bream and Ayu with a similar type of the nylon-protein MED and some micro-particulate diets. This paper presents the results of these attempts.

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Materials and Methods

Preparation of micro-particulate diets In this study, 3 types of micro-particulate diets were prepared. The details of the procedures for preparation of diets were described elsewhere[4,5]. The nylon-protein MED was prepared by interfacial polymerisation procedures[4,6] using 1,6-diaminohexane, sebacoyl chloride, and the diet suspension as described previously[4]. The diet suspension was mainly composed of a whole chicken egg, squid extract, short-necked clam extract, mysid extract, powdered pollack liver oil, and carbohydrates as shown in Tables 1 and 2. The particle sizes of nylon-protein MED for the red sea bream and Ayu were 50-150 μm

Table 1. Composition of the diet suspension for the nylon-protein MED.

Ingredient	(%)
Chicken egg	53.7
Squid extract*1	5.4
Short-necked clam extract*1	10.7
Mysid extract*1	5.4
Amino acid mixture*2	5.4
Glucose	2.7
Sucrose	2.7
Dextrin	2.7
Glycogen	2.7
Powdered pollack liver oil*1	4.3
Eicosapentaenoic acid	0.52
Mineral mixture*3	2.16
Vitamin mixture*4	1.08
DNA hydrolysate	0.27
RNA hydrolysate	0.27

*1 Supplied from Riken Vitamin Co.
*2 Amino acid mixture: see Table 2.
*3 USP XII and trace elements
*4 HALVER (1957)[14]

Table 2. Composition of amino acid mixture

Amino acid*1	g	Amino acid	g
Arginine	1.6	Threonine	0.6
Histidine	0.4	Tryptophan	0.1
Isoleucine	0.6	Valine	1.0
Leucine	1.0	Cystine	0.4
Lysine	1.4	Alanine	0.6
Methionine	0.4	Na aspartate	0.4
Phenylalanine	1.3	Glycine	0.2

*1 Total amount; 10.0 g

and 100-200 μm diameters, respectively.

The micro-particulate diet with a zein-coating (zein MCD) was made as follows; the powdered diet (8.0 g) (Table 3) was mixed with zein (5.0 g) dissolved in 60% ethanol (25 ml). The elimination of ethanol followed by heating at 30-40℃, crumbling into fine particles, and sieving gave the particulate diet (size, 125-250 μm). The powdered diets with agar and sodium polyacrylate as binders (agar-polyacrylate MBD) contained the ingredients as shown in Table 4. The diet was prepared by drying at 30-40℃, powdering, sieving to give the particles (size, 125-250 μm).

Table 3. Composition of the zein MCD.

Ingredient*[1]	(%)
Casein	50
Gelatin	2
Amino acid mixture*[2]	5
Dextrin	30
Lauric acid	4
Eicosapentaenoic acid	1
Mineral mixture	4
Vitamin mixture	2
Agar	2

*[1] To the powdered diet (8.0g), zein (5.0g) was added as shown in the text.
*[2] See Table 2.

Table 4. Composition of the agar-polyacrylate MBD

Ingredient	(%)
Casein	52.0
Gelatin	11.0
Dextrin	8.0
Amino acid mixture*[1]	5.0
Pollack liver oil	9.0
Mineral mixture*[2]	8.0
Vitamin mixture*[2]	3.0
Cellulose powder	3.8
Agar	2.0
Sodium polyacrylate	0.2

*[1] See Table 2. *[2] See Table 1.

Rearing of larvae of the red sea bream and Ayu Eggs of the red sea bream and Ayu were hatched in June and in October, respectively. All feeding trials using the larval fish were conduted at the Fisheries Experimental Station of Kumamoto Prefecture. The red sea bream and Ayu larvae were grouped into lots of 15,000 and 1,000 individuals, respectively, for feeding trials. The red sea bream and Ayu larvae were reared with the several diets in a round polycarbonate tank (500 liter-capacity) as shown in Tables 5 and 6. The rotifers used as a live food were cultured with a marine type of *Chlorella*. The dietary value of diets was evaluated in terms of growth (increase in whole length and/or weight gain) and survival rate.

Results and Discussion

The feeding trials on the red sea bream were conducted to examine the dietary value of the nylon-protein MED (Table 1) and agar-polyacrylate MBD (Table 4). Fig. 1 shows the results of the feeding trials on the 3-day larvae. The red sea bream larvae fed the rotifers (group 1R) grew 3.2 to 4.0 mm whole length 10 days after hatching. The larvae fed the nylon-protein MED (group 2R) or the agar-polyacrylate MBD (group 3R) showed only a slight growth and high mortality ($<5\%$ survival). This suggests that the larval red sea bream immediately after

Table 5. Conditions of the feeding trials on larval red sea bream.

Experiment	Larvae used	Exptl. group*[1]	Diet and feeding level
I	3 day-larvae	1 R	Rotifer (500 ind./larva/day)
		2 R	Nylon-protein MED (500 particles/larva/day)
		3 R	Agar-polyacrylate MBD (1g/tank/day)
II*[2]	10 day-larvae	4 R	Rotifer (500 ind./larva/day)
		5 R	Nylon-protein MED (500 particles/larva/day)
		6 R	Rotifer (250 ind./larva/day) + Nylon-protein MED (250 particles/larva/day)
		7 R	Rotifer (250 ind./larva/day) + Agar-polyacrylate MBD (0.5g/tank/day)

*[1] In each experimental group, 15,000 larvae were reared in a 500 liter-tank and received the diet 10 times a day.
*[2] The larvae of red sea bream were cultivated with the rotifers for 10 days after hatching and then divided into the experimental groups.

Table 6. Conditions of the feeding trials on larval Ayu.

Exptl. group*[1]	Diet and feeding level: Hatching – Day 10	Diet and feeding level: Day 11 – Day 30
1 A	Rotifer (500 ind./larva/day)	Nylon-protein MED (500 particles/larva/day)
2 A	Nylon-protein MED (500 particles/larva/day)	Nylon-protein MED (500 particles/larva/day)
3 A	Rotifer (500 ind./larva/day)	Rotifer (250 ind./larva/day) + Nylon-protein MED (250 particles/larva/day)
4 A	Rotifer (500 ind./larva/day)	Rotifer (250 ind./larva/day) + Commercial diet*[2] (1.5g/tank/day)
5 A	Rotifer (500 ind./larva/day)	Zein MCD (1.5g/tank/day)
6 A	Zein MCD (1.0g/tank/day)	Zein MCD (1.5g/tank/day)
7 A	Rotifer (500 ind./larva/day)	Rotifer (250 ind./larva/day) + Zein MCD (1.5g/tank/day)

*[1] In each experimental group, 1,000 larvae were reared in a 500 liter-tank and received the diet 7 times a day.
*[2] Oriental Yeast Co., Ltd.

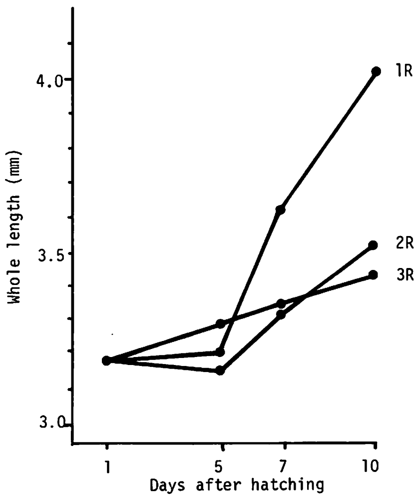

Fig. 1. Growth of the newly hatched larvae of red sea bream. The diets were given to the larvae 3 days after hatching. 1R, 2R, and 3R indicate experimental groups (see Table 5).

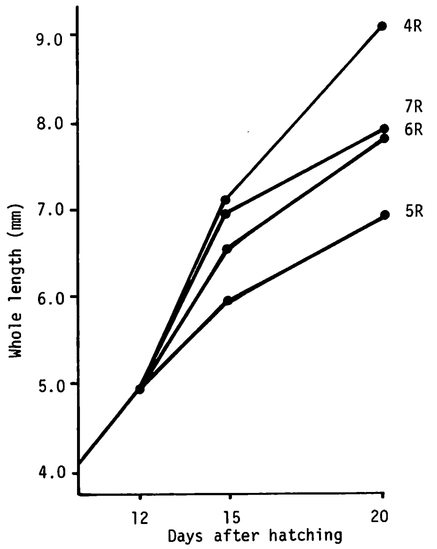

Fig. 2. Growth of the 10-day larvae of red sea bream. 4R, 5R, 6R, and 7R indicate experimental groups (see Table 5).

hatching scarcely assimilate the artificial diets examined.

Fig. 2 shows the results of the feeding trials on the 10-day larvae of red sea bream which were pre-cultured with the rotifers for 10 days after hatching. The larvae fed the nylon-protein MED (group 5R) grew from 5.0 to 7.0 mm whole length 20 days after hatching. Thus, the nylon-protein MED was effective in promoting growth of the 10-day larvae to some extent in contrast to the case with the newly hatched larvae, although the survival rate was again low (about 6% survival). However, the growth rates of the 10-day larvae fed the nylon-protein MED (group 5R) or the combinations of rotifers and micro-partculate diets (groups 6R and 7R) were inferior to that of the larvae receiving rotifers (group 4R).

Regarding the Ayu larvae, the feeding trials were carried out to examine the dietary value of the nylon-protein MED (Table 1) and the zein MCD (Table 3) by using the larvae immediately after hatching. The results are shown in Figs.3 and 4. The Ayu larvae gave low growth and survival rates when they were maintained on the nylon-protein MED (group 2A) or the zein MCD (group 6A) alone throughout the feeding period. When the Ayu larvae were fed the rotifers for 10 days and then received the nylon-protein MED (group 1A) or the zein MCD (5A) for subsequent 20 days, growth was markedly improved. The best growth and survival were observed on groups 3A, 4A, and 7A which were given both the rotifers and one of the artificial diets during the period of day 11 to day 30. The survival rate on group 5A was almost

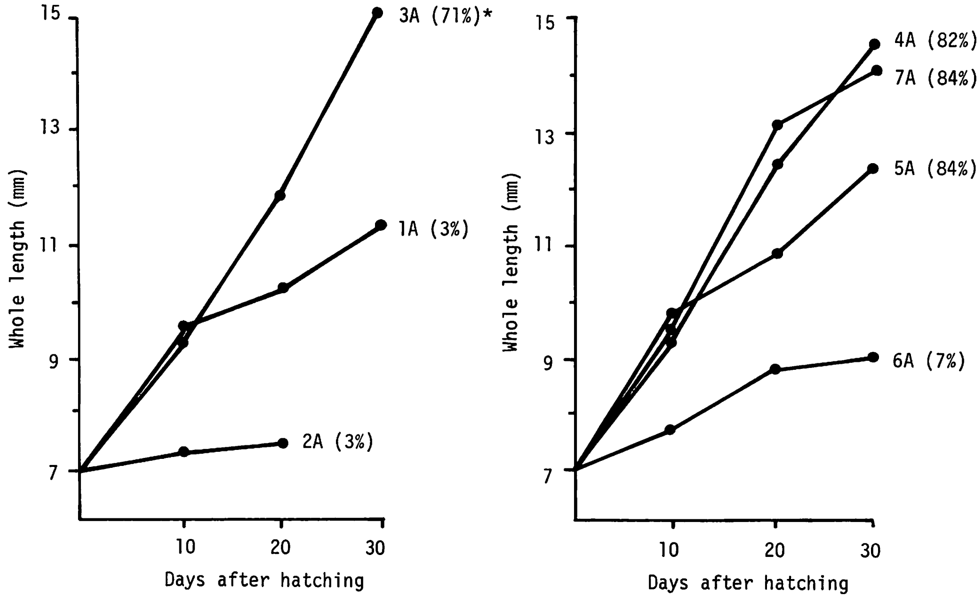

Fig. 3. Increase in the whole length of larval Ayu. Letters 1A to 7A indicate experimental groups (see Table 6). *: Survival rate

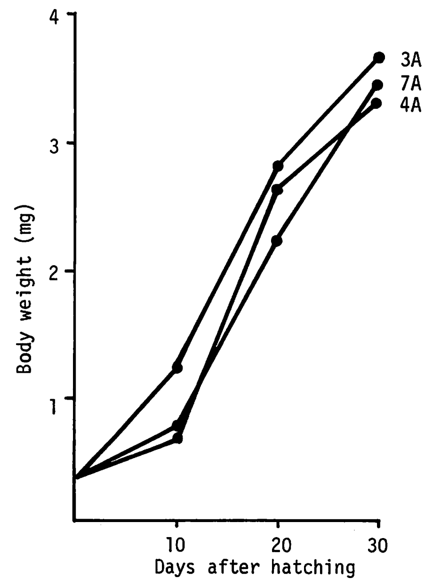

Fig. 4. Body weight gain of larval Ayu. Letters 1A to7A indicate experimental groups (see Table 6).

comparable to those of groups 4A and 7A, whereas that on group 1A was very low. These results indicate that the zein-MCD was effective in sustaining growth and survival of the newly hatched Ayu larvae to some extent. Moreover, the results of the present study show that the Ayu larvae grew well with high survival rates when they were fed the artificial diets such as the nylon-protein MED and zein MCD together with the rotifer after being reared with rotifers for 10 days after hatching.

As mentioned above, it was difficult to sustain growth and survival of the newly hatched larvae of the red sea bream and Ayu with the microparticulate diets alone, although only the zein MCD supported growth of the newly hatched Ayu slightly. However, the microparticulate diets such as the nylon-protein MED, agar-polyacrylate MBD, and zein MCD were effective in sustaining growth and survival of the slightly grown (10-day) larvae of red sea bream and/or Ayu to some extent. But, the growth of the 10-day larvae of red sea bream and Ayu maintained on the microparticulate diets alone was inferior to that of the groups receiving both the rotifers and microparticulate diets.

TANAKA *et al*[7]. have observed that the basic structucture of digestive systems of the Ayu is established immediately before a commencement of feeding at the late stage of pre-larvae (approximately 3 days after hatching), but the gastric gland does not exert its function until the transitional stage (about 135 days after hatching) from larvae to juveniles. Morphological and histochemical studies[8-13] have also shown that larval fishes assimilate dietary proteins by means of pinocytosis by the absorptive cells of posterior-gut. Considering the information, the low dietary value of the microparticulate diets for the newly hatched larvae may be attributable to the incomplete digestion of diet ingredients. In future, therefore, it may be desirable to use some digestible proteins and other nutrients in making the artificial diets for larval fishes having undeveloped digestive systems.

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