# ヒラメ仔稚魚の成長,生残におよぼす給餌率の影響


| | |
|---|---|
| 誌名 | 水産増殖 = The aquiculture |
| ISSN | 03714217 |
| 巻/号 | 492 |
| 掲載ページ | p. 207-218 |
| 発行年月 | 2001年6月 |

SUISANZOSHOKU 49(2), 207−218 (2001)

# Effects of Feeding Levels on the Growth and Survival of Larval and Juvenile Japanese Flounder *Paralichthys olivaceus*

S. M. A. MOBIN, Kinya KANAI, and Kazuma YOSHIKOSHI[*1]

(Accepted April 3, 2001)

**Abstract:** Effects of feeding levels on the growth and mortality of larval and juvenile Japanese flounder *Paralichthys olivaceus* were studied in two trials. Trial 1 was conducted from 3 to 45 d posthatch while trial 2 from 3 to 36 d posthatch. In both trials, flounder eggs were hatched and larvae were reared in indoor tanks and fed enriched live feeds, *Brachionus plicatilis* and *Artemia* spp. nauplii at four different feeding levels (L1, L2, L3 and L4). The respective food densities of L1, L3, and L4 were 0.5, 2.5 and 5 times that at L2. Level L2 is the one conventionally adopted by some hatcheries for the mass production of flounder larvae and juveniles in Japan. At that level, the initial density of rotifers is 0.5 individuals (ind.)/m*l* culture water; the density gradually increases to approximately 3.5 ind./m*l* after metamorphosis. The initial density of *Artemia* spp. at L2 is 0.1 ind./m*l*, which gradually increases to 3 ind./m*l*. The larval and juveniles mean growth rates were dependent on feeding levels. In trial 1, the highest daily growth rates of 18.3% for dry body weight (BW) were observed at L4, followed by L3 (17.5%), L2 (16.5%) and L1 (16.1%). In this trial after reared for 45 d, L4 attained the highest BW of 23.9±0.8 mg (mean±SE) followed by L3 (18.1±0.8 mg), L2 (12.8±0.9 mg) and L1 (11.0±0.4 mg). In trial 1, the highest cumulative mortality (26%) was at L4 followed by L3 (16.6%), L2 (11.1%) and L1 (6.7%). At 45 d posthatch, the total length (TL) and BW differed significantly ($P<0.05$) between L1 and L3, L2 and L3, L3 and L4, L2 and L4, and L1 and L4. In trial 2, growth and mortality patterns were similar to trial 1. In both trials, the faster growth rates were accompanied by higher cumulative mortalities.

**Key words:** Japanese flounder; larvae and juveniles; feeding level; growth; mortality

## Introduction

Recent advances in rearing techniques for the mass propagation of larval and juvenile Japanese flounder *Paralichthys olivaceus* have improved stock enhancement programs in coastal waters and also land based culture in Japan[1]. In 1997, the juvenile flounder production by the governmental fish farming centers and private hatcheries was about 50 million individuals. Recently enriched rotifers *Brachionus plicatilis* and *Artemia* spp. nauplii, and micropellets have been commonly used as feed for rearing Japanese flounder larvae and juveniles, but feeding levels and live feed enrichment procedures vary from hatchery to hatchery. The conventional feeding schedule for rearing Japanese flounder larvae and juveniles is shown in Fig. 1.

In the course of Japanese flounder seed production, hatcheries have frequently suffered significant losses due to infectious and non-infectious diseases[2-4]. Besides this, different hatcheries in the western part of Japan have been occasionally encountering mass mortalities in the production of larvae and juveniles of red sea bream *Pagrus major*[5], Japanese flounder, groupers (Serranidae), devil stinger *Inimicus caledonicus* etc. from unknown causes. It is in suspect that feeding level and feed enrichment procedure might have involved in this occurrence. The objectives of this study were to find out the effects of feeding levels on the growth and mortality aimed at to draw a conclusion on the above-mentioned suspicion.

[*1] Graduate School of Science and Technology, Nagasaki University, 1-14 Bunkyo-machi, Nagasaki 852-8521, Japan.

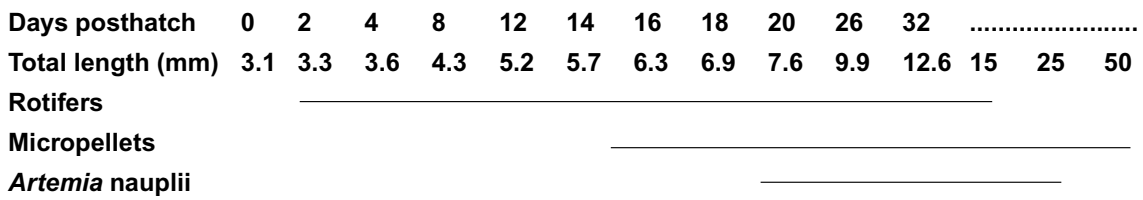


**Fig. 1.** Feeding schedule used in a conventional method for the rearing of larval and juvenile Japanese flounder.

## Materials and Methods

*Design of the experiment*

Duplicate trials, which designated as trial 1 and trial 2, were conducted in this study. They were carried out at different period of time.

*Rotifers culture*

Rotifers *Brachionus plicatilis* were cultured maintaining a density of 100-150 ind./m*l* of the culture medium. They were fed baker's yeast *Saccharomyces cerevisiae* (Kyowa Hakko Co. Ltd., Japan), three times per day (at 09:00, 13:00 and 17:00) at the rates of 1.25 g/million ind., 0.5 g/million ind. and 1.75 g/million ind., respectively. They were also fed *Nannochloropsis oculata*, the density of which was maintained at $2\times10^7$ cells/m*l* of rotifers culture water. The flounder larvae were fed enriched rotifers twice a day, morning and afternoon. To feed the larvae in the morning up to the metamorphosis period, the rotifers were enriched for 17 h with Super Capsule A-1$^{TM}$ (Chlorella Kogyo Co. Ltd., Japan) at the rate of 100 m*l*/k*l*/day. The ω-3 emulsified lipids (Chlorella Kogyo Co. Ltd., Japan) were also added to the enrichment medium at the rate of 200 m*l*/k*l*/day just 1 h before finishing the enrichment. On the other hand, to feed the larvae in the afternoon, the rotifers were enriched for 5 h with an emulsified mixture of Sujiko Oil $^{TM}$ (Nissin Sciences, Japan)—60 m*l*/k*l*/day, Feed Oil Ika $^{TM}$ (Riken Vitamin Co. Ltd., Japan)— 60 m*l*/k*l*/day and β-carotene (Tanabe Seiyaku Co. Ltd., Japan)— 0.6 g/k*l*/day. The volume of the mixture was raised to 1 *l* by tap water and stirred (and oil emulsified) for 1 min with a kitchen blender and then distributed to the tanks. To feed metamorphosed larvae and juveniles, the rotifers were enriched in the same way as described above except Super Capsule A-1$^{TM}$ was used at double of the previous rate. In both cases of enrichment, density of rotifers was maintained at 200-600 ind./m*l* of culture medium. Before adding to the fish-tanks, enriched rotifers were harvested by passing slowly the rotifer-containing enrichment medium through a mesh net (74μm), washed twice by ultraviolet (UV) irradiated sea water, and loaded into the tanks mixing with 5 *l* filtered sea water/tank. Rotifers were prepared everyday.

*Artemia culture*

*Artemia* spp. eggs (Red Jungle Brand PRO 80$^{TM}$ of U. S. A.) were incubated in UV irradiated seawater with vigorous aeration where the water temperature was kept constant at 28℃ by an electric heater. The hatched nauplii were separated after 21 h of incubation, washed twice by filter mesh and cultured in filtered seawater. The temperature was held constant at 26℃ by an electric heater. For serving nonmetamorphosed larvae, the newly hatched *Artemia* nauplii were aerated vigorously and enriched for 4 h with ω-3 emulsified lipids at a rate of 500 m*l*/k*l* of culture medium once a day where the density of nauplii was maintained at a rate of $5\times10^7$ ind./k*l*. The *Artemia* nauplii for the metamorphosed juveniles were cultured in the same procedure mentioned above, but in this case Super Capsule A-1$^{TM}$ was also added for enrichment at a rate of 100 m*l*/k*l*/day. Before introduction to the fish-tanks, enriched nauplii were washed and loaded (in the tanks) in the same method as described for rotifers. Nauplii were prepared daily and served once a day (at 13:00).

*Hatching of fish eggs*

Fertilized eggs of Japanese flounder for both trials were collected from natural spawning of a brood-stock in captivity. The diameters of eggs

of trial 1 and trial 2 were 0.91±0.05 mm (mean± SE; n=20) and 0.95±0.06 mm (mean±SE; n= 20), respectively. After temperature adjustment for 30 min, high quality eggs were collected from the surface water of the tank and disinfected for 5 min in a 30 *l* tank containing 5 mg/*l* Isojin ™ (Providone-iodine; Meiji Seika Co. Ltd., Japan). In each trial, the eggs were released in four polyethylene made 1 k*l* black-colored cylindrical indoor tanks at a rate of ten thousand eggs/tank. Water bath and heater were used to stabilize the water temperature during experiments. The system was equipped by running water system where primary filtration, UV irradiation, and secondary filtration were done to treat the water.

*Larval and juvenile rearing system*

Larval feeding was started on the third day after hatching when their functional eyes and digestive organs had developed. Before first feeding in each trial, larval densities were adjusted to 8,000 ind. per tank. Four different feeding levels (L1, L2, L3, and L4) were assigned to the four tanks for 45 d posthatch in trial 1 and 36 d posthatch in trial 2. The respective feed densities at L1, L3, and L4 were 0.5, 2.5 and 5 times that at L2 (Fig. 2A, B). Feeding level at around L2 has conventionally been adopted in some hatcheries for mass production of flounder larvae and juveniles in Japan. At this level, the initial density of rotifers is 0.5 ind./m*l* fish culture water; the density increases gradually to approximately 3.5 ind./m*l* culture water after metamorphosis. In case of *Artemia*, the initial density at L2 is 0.1 ind./m*l* fish culture water, which gradually increases to 3 ind./m*l*. In trial 1, the larvae and juveniles were fed rotifers from 3 to 42 d posthatch (3 to 36 d posthatch in trial 2); from 18 to 42 d posthatch (18 to 36 d posthatch in trial 2) they were also fed *Artemia* and from 43 to 45 d they were fed only *Artemia* (Fig. 2A, B). Everyday before adding fresh rotifers (at 09:00 and 14:00), most of the remaining rotifers in the tank were removed by re-circulating the tank water through plankton net. After the removal, expected concentration (Fig. 2A) of rotifers of each tank was adjusted by adding enriched rotifers. Before *Artemia* nauplii were added, the remaining *Artemia* were concentrated by light after terminating the blower and removed by the same method as described for rotifers. We did not use dry pellets in this study, although they are used in the conventional practice, suspecting that potential growth and mortality of larval and juvenile Japanese flounder at higher feeding levels would compromise with water quality. To meet up the deficiency of artificial diet we continued feeding rotifers along with *Artemia* to the developing flounder for a quite longer period in both trials. *N. oculata* was provided in each tank at the rate of $2\times 10^5$ cells/m*l*/day for nutrition (rotifers in tanks maintain nutritional values via continuous uptake of algae)[6], antibacterial

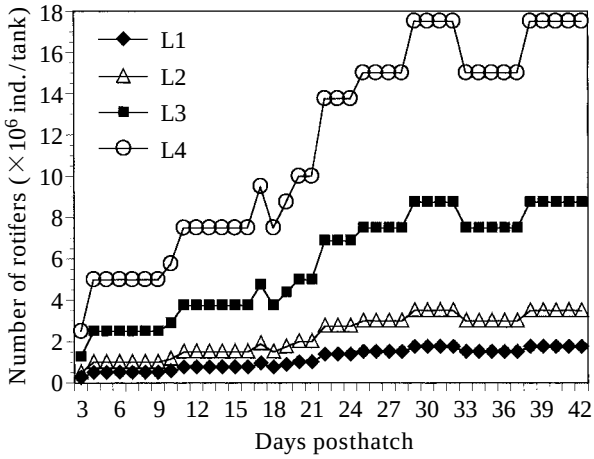


**Fig. 2A.** Number of rotifers supplied in the rearing tanks of Japanese flounder larvae and juveniles at four feeding levels in each of the trials.

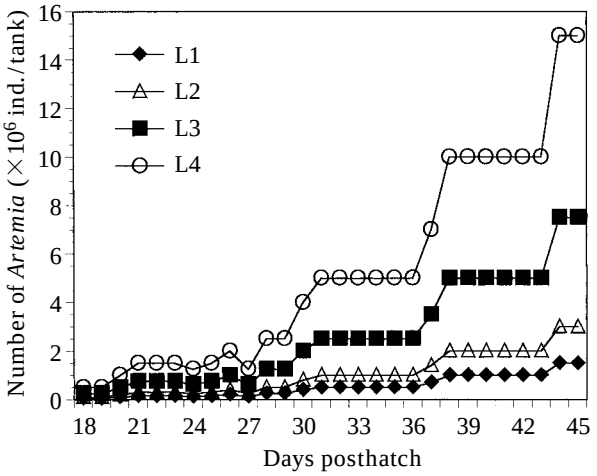


**Fig. 2B.** Number of *Artemia* nauplii supplied in the rearing tanks of Japanese flounder larvae and juveniles at four feeding levels in each of the trials.

effect[7], enhancement of feeding by increasing turbidity[8], and reduction of metabolites[9].

In this study to assess feed consumption pattern, the number of the remaining rotifers in each tank was calculated everyday from eight random samples just before adding new feed into the tanks[10]. Consumption of *Artemia* was not calculated because we could not count the number of remaining *Artemia* in the tanks as they have taxis towards illumination that makes their distribution non-uniform. At 35 d posthatch, the number of flounder in each tank was reduced to 3000 ind. in both trials.

The water exchange rate of the tanks was adjusted with the growth of the larvae and juveniles (Fig. 3). A 13h light: 11h dark period (at 06:00-19:00 light period) was established, where the light intensity at the water surface of each tank was the same but varied from 800 to 1,500 lx during the experimental period. Salinity was a constant at 35 psu throughout the experimental period. Aeration stones were used to ensure adequate supply of oxygen. Dissolved oxygen (DO) was measured everyday and remained within acceptable levels (DO$>$6 mg/*l*). Left over feed, excreta and other debris were removed from the tanks everyday by siphoning when they accumulated on the bottom of the tanks. Surface debris materials were removed manually with a net. Dead fish were removed daily and noted.

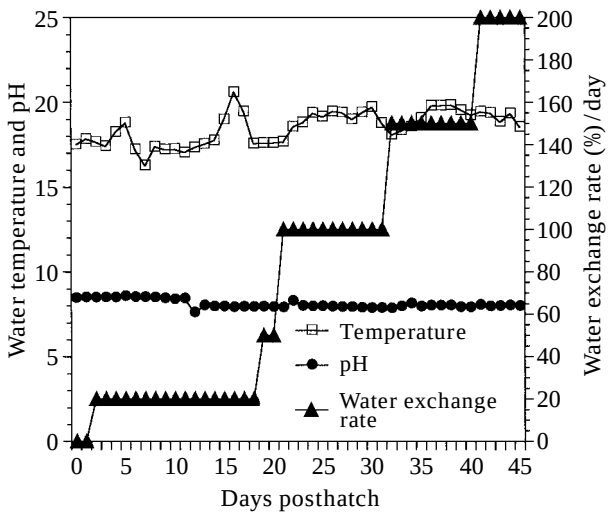


**Fig. 3.** Change of temperature, pH, and water exchange rate of the rearing water of Japanese flounder larvae and juveniles in trial 1.

*Sampling*

In trial 1 forty fish at each feeding level and sampling time were randomly sampled at every 3 d interval from 3 to 45 d posthatch. On the other hand, in trial 2 thirty fish at each feeding level and sampling time were randomly sampled at the same interval from 3 to 36 d posthatch. Total length (TL) was measured using a profile projector, Goko LP-6N (Sansei Koki Co. Ltd., Japan). The samples were preserved in 4% paraformaldehyde to determine dry body weights (BW) because of the small weights involved. Within 30 d post preservation, the fish were dried in an oven at 70℃ for 48 h, cooled in a plastic vacuum desiccator, and weighed on a digital balance (Mettler Toledo Co. Ltd., Switzerland). In trial 1 BW was measured in pooled samples up to 15 d posthatch, while in trial 2 up to 12 d posthatch. In the later samplings, individual weights were taken in both trials.

*Data analyses*

The specific growth rates for TL and BW were determined as:

$SG_L = (\ln L_t - \ln L_0)/t \times 100$

where ln = natural logarithm; $L_0$ = total length at 3 d posthatch (mm); $L_t$ = total length (mm) at 45 d posthatch for trial 1 and 33 d posthatch for trial 2.

$SG_W = (e^g - 1) \times 100$

where $g = (\ln W_t - \ln W_0)/t$, ln = natural logarithm; $W_0$ = weight (mg) at 3 d posthatch; $W_t$ = weight (mg) at 45 d posthatch for trial 1 and 33 d posthatch for trial 2.

The mortality on each day was calculated in terms of the percentage of surviving fish at the beginning of that day. As the dead fish was not visible during early larval stage, the mortality occurred between 1 and 23 d posthatch was determined by a back-calculation when the number of fish was adjusted to 3000 in each tank at 35 d posthatch. The procedure of back calculation was as follows:

Mortality occurred between 3 and 23 d posthatch = (Number of stocked fish at 3 d posthatch − number of survived fish at 35 d posthatch − loss due to sampling − number of dead fish between

24 and 35 d posthatch).

The effects of feeding levels on growth (length and weight) were investigated statistically. Bartlett test was conducted to check for homogeneity of the variances. One way analysis of variance (equal sample size) and Tukey's multiple comparison were used to determine differences among treatments and where they occurred[11]. Analyses of data (length and weight) were performed with SYSTAT for Windows[12]. The mortality between the treatments was analyzed by Chi-square test.

## Results

*Food consumption*

Rotifers consumption pattern shows that the fish of L4 in both trials consumed highest number of rotifers followed by L3, L2 and L1 (Fig. 4). In trial 1, a sharp declining trend in consumption of rotifers was observed in L4 from 26 to 42 d posthatch while in L3 the consumption reduced slightly from 27 d posthatch but it declined sharply after 32 d posthatch. In L2, consumption rate of rotifers showed an increasing trend up to 37 d posthatch but it declined abruptly after 40 d posthatch. Almost uniform increasing trend in consumption of rotifers was noted in L1 up to the end of this trial. Rotifers consumption pattern of trial 2 was similar to the pattern observed from 3 to 35 d posthatch in trial 1.

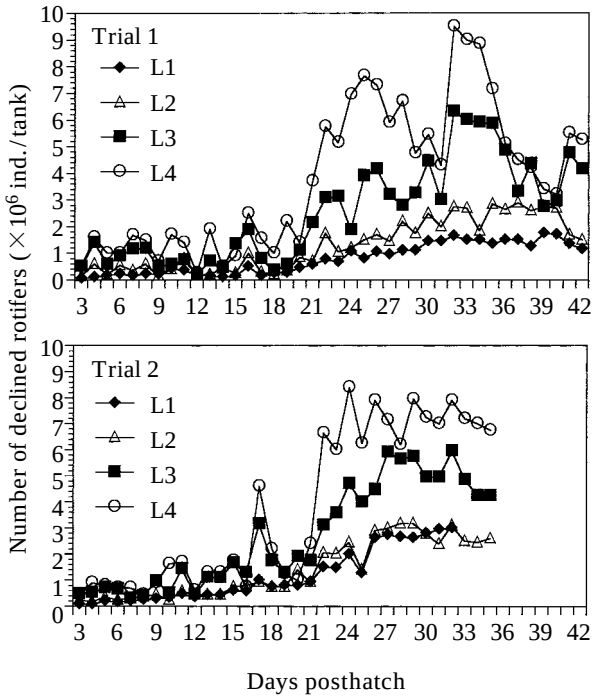


**Fig. 4.** Day-wise decline in number of rotifers at four feeding levels in the rearing tanks of Japanese flounder larvae and juveniles. This decline occurred due to consumption (by fish) and flushing out through the sieving net due to the exchange of water continuously. As the water exchange rate of all tanks was same so rotifer's concentration change rate owing to water exchange was constant in all the tanks. So, this graph is presenting the rotifer consumption pattern at four feeding levels.

*Growth rates*

In both trials, growth for TL and BW were higher in higher feeding levels (Figs. 5, 6; 7, 8). In trial 1 at 3 d posthatch, larval TL and BW of all treatments were 3.4±0.0 mm (mean±SE) and 0.02 mg, respectively. At the last day of this trial (45 d posthatch), L4 attained a TL of 23.1± 0.3 mm (mean±SE) and BW of 23.9±0.8 mg (mean±SE) followed by L3 (TL 20.9±0.5 mm, BW 18.1±0.8 mg), L2 (TL 18.2±0.4 mm, BW 12.8±0.9 mg) and L1 (TL 16.8±0.4 mm, BW 11.0±0.4 mg). Total length of Japanese flounder larvae and juveniles did not differ significantly among the treatments at 3 d posthatch but in later at all samplings they differed significantly at $P<0.001$ level. The results of pairwise comparison between treatments are indicated in Fig. 5. At 3 d posthatch larval BW in all treatments was the same but significant difference ($P<0.001$) among the four treatments was observed at 18 d posthatch and in later at all samplings. The results of pairwise comparison between treatments show (Figs. 5, 6) that from 24 to 45 d posthatch both the TL and BW were differed significantly ($P<0.05$) between feeding levels L1 and L3, L2 and L3, L3 and L4, L2 and L4, and L1 and L4. However, the TL and BW between L1 and L2 were not found significantly different throughout the experimental period. The daily growth rate for TL was highest in L4 (4.6%) followed by L3 (4.4%), L2 (4.1%) and L1 (3.8%). Similar pattern was also observed in case of body weight increment. The daily growth rate for BW was highest in L4 (18.3%), followed by L3 (17.5%), L2 (16.5%) and L1

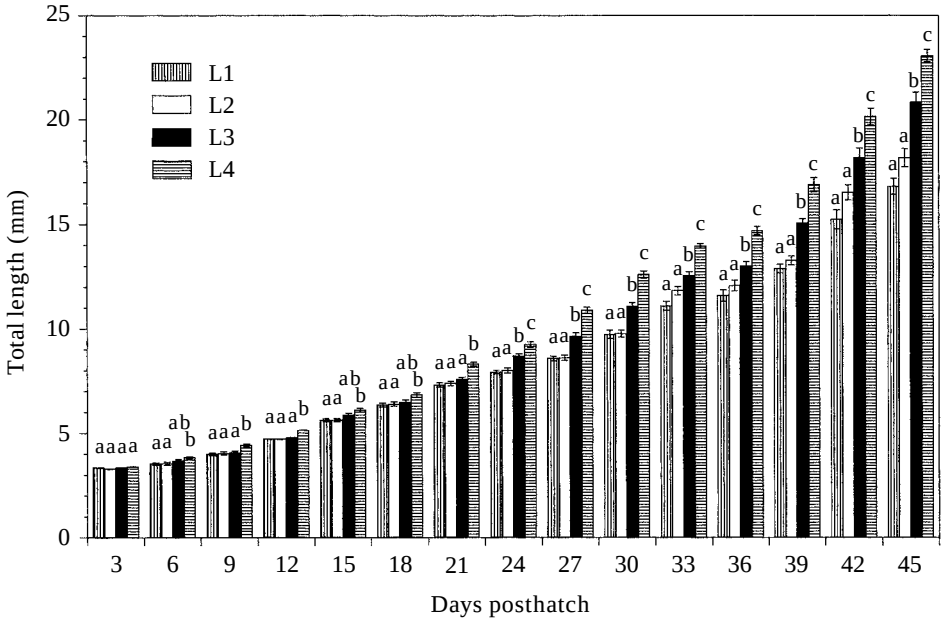


**Fig. 5.** Growth characteristics for total length of Japanese flounder larvae and juveniles (n=40) at four feeding levels in trial 1. Bars are mean±SE. Treatment combinations associated with the same letter (above the bars) are not significantly different (P>0.05).

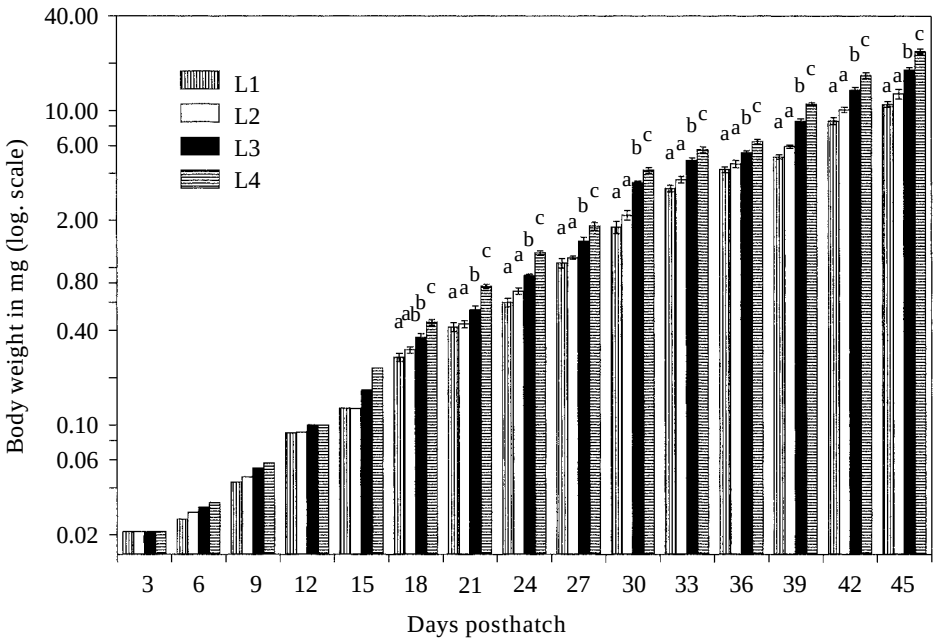


**Fig. 6.** Growth characteristics for dry weight of Japanese flounder larvae and juveniles (n=40) at four feeding levels in trial 1. Bars are mean±SE. Treatment combinations associated with the same letter (above the bars) are not significantly different (P>0.05).

(16.1%). At the end of the trial 1, L4 was one week ahead of L1 in both TL and BW.

During conducting trial 2, we decided to stop running L1 treatment from 34 d posthatch due to an occurrence of unexplained mortality, which observed only in this treatment. At 37 d posthatch all the larvae and juveniles of L4 died due to failure of electricity supply, which occurred by natural calamity that bounded us to terminate this trial.

Growth rates for TL and BW of trial 2 showed similar pattern to trial 1 (Figs. 7, 8). At 3 d posthatch, larval TL and BW of all treatments were 3.3±0.0 mm (mean±SE) and 0.03 mg, respectively. At 33 d posthatch, larval TL (mean±SE) and BW (mean±SE) of L4, L3, L2

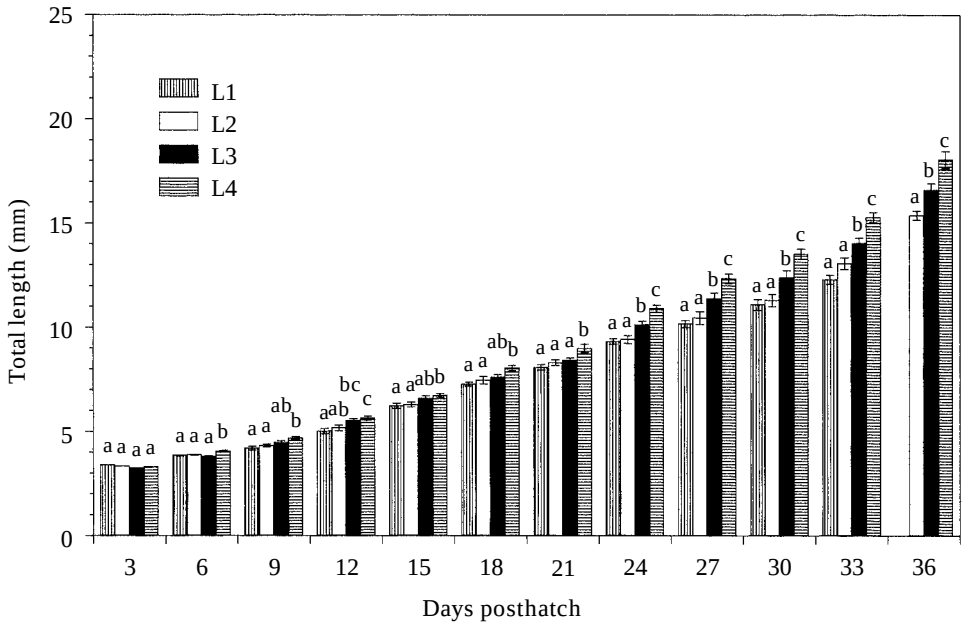


**Fig. 7.** Growth characteristics for total length of Japanese flounder larvae and juveniles (n=30) at four feeding levels in trial 2. Bars are mean±SE. Treatment combinations associated with the same letter (above the bars) are not significantly different (P>0.05).

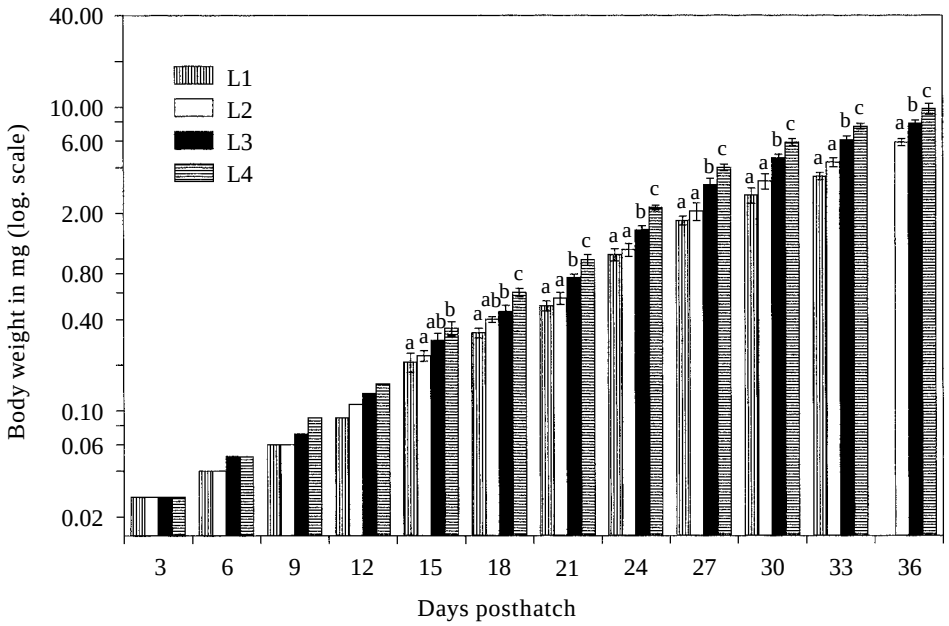


**Fig. 8.** Growth characteristics for dry weight of Japanese flounder larvae and juveniles (n=30) at four feeding levels in trial 2. Bars are mean±SE. Treatment combinations associated with the same letter (above the bars) are not significantly different (P>0.05).

and L1 reached at 15.3±0.2 mm and 7.4±0.3 mg, 14.0±0.3 mm and 6.1±0.4 mg, 13.1±0.3 mm and 4.3±0.3 mg, and 12.3±0.2 mm and 3.5±0.2 mg, respectively. At the last sampling (36 d posthatch), L4 attained TL of 18.1±0.4 mm (mean± SE) and BW of 9.7± 0.8 mg (mean±SE) followed by L3 (TL 16.6±0.3 mm, BW 7.8±0.4 mg) and L2 (TL 15.4±0.2 mm, BW 5.8± 0.3 mg), respectively. The results of the statistical analyses are indicated in Figs. 7 and 8. The highest daily growth rate (3 to 33 d posthatch) for TL and BW was observed in L4 (5.1 and 20.6%; in trial 1 4.7 and 20.5%) followed by L3 (4.9 and 19.8%; in trial 1 4.4 and 19.9%), L2 (4.5 and 18.4%; in trial 1 4.2 and 18.8%) and L1 (4.3 and 17.6%; in trial 1 4.0 and 18.2%).

*Mortality of the larvae and juveniles*

The mortality in two trials is presented in Fig. 9. In trial 1, though the mortality of L1, L3 and L4 was different throughout the experimental period, the pattern was similar. In L2, a sharp increase in mortality was observed from 40 d posthatch. At 44 d posthatch, the cumulative mortality of L4, L3, L2 and L1 was 26, 16.6, 11.1 and 6.7%, and the daily mortality from 24 to 44 d posthatch ranged from 0.28 to 2.69, 0.13 to 1.23, 0 to 1.6, and 0 to 0.3%, respectively. At 35 d posthatch the number of survivor in L4, L3, L2, and L1 treatments were 6498, 6738, 7126, and 7166 individuals; meanwhile at 45 d posthatch it was 2534, 2704, 2719, and 2830 individuals, respectively. As the dead fish was difficult to find up to 23 d posthatch, the mortality from 1 to 23 d posthatch was back calculated. The cumulative mortality at 34 d posthatch differed significantly ($P<0.001$) between feeding levels L1 and L3, L2 and L3, L3 and L4, L2 and L4, and L1 and L4. However, the mortality was not found significantly different between L1 and L2. The cumulative mortality at 44 d posthatch (from 35 to 44 d posthatch) differed significantly ($P<0.001$) between feeding levels L1 and L2, L1 and L3, L3 and L4, L2 and L4, and L1 and L4. The difference between L2 and L3 was found insignificant.

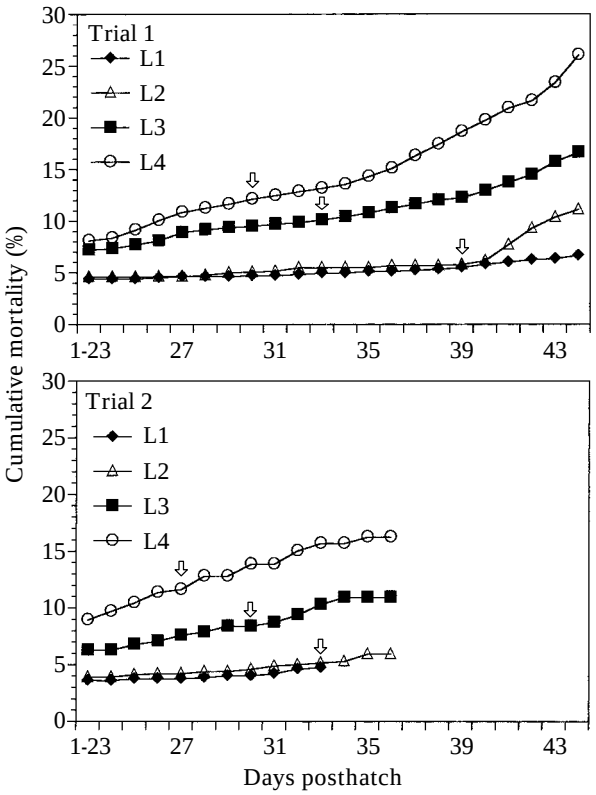


**Fig. 9.** Cumulative mortalities of Japanese flounder larvae and juveniles at four feeding levels in two trials. Arrows indicate the day of completion of metamorphosis in 50% sample. In trial 2 at L1, metamorphosis did not complete in 50% sample till 33 d posthatch while in trial 1 it completed at 39 d posthatch in both L1 and L2 levels.

The mortality pattern of trial 2 was similar to trial 1 (Fig. 9). At 33 d posthatch, cumulative mortality in L4, L3, L2 and L1 was 15.7 (13.1 in trial 1), 10.3 (10.1), 5.2 (5.5) and 4.7% (5%), respectively. Daily mortality (from 24 d to 33 d posthatch) of L4, L3, L2 and L1 ranged from 0 to 1.4% (in trial 1 from 0.3 to 1%), 0 to 1.1% (0.1 to 0.4%), 0 to 0.3% (0 to 0.4%), and 0 to 0.4% (0 to 0.1%), respectively. At 36 d posthatch, the cumulative mortality of L4, L3 and L2 rose to 16.2, 10.9, and 5.9%, respectively. At 35 d posthatch, the number of survivor at L4, L3, and L2 was 6389, 6761 and 7203 ind., respectively. At the termination day of L1 in trial 2 (33 d posthatch), the cumulative mortality was found significantly different ($P<0.001$) between feeding levels L1 and L3, L2 and L3, L3 and L4, L2 and L4, and L1 and L4. The difference between L1 and L2 was found insignificant.

*Hypomelanosis*

Pigment abnormality that was characterized by white patches or areas devoid of normal pigmentation on the ocular surface of the skin, has been observed in the juveniles at all feeding levels in trial 1. In some cases, hypomelanosis was observed in the entire surface. At the end of trial 1 (45 d posthatch), hypomelanosis was in 82.5, 60, 32.5, and 27.5% juveniles in L4, L3, L2, and L1 feeding level, respectively. At the termination day of trial 2 (36 d posthatch), it was difficult to distinguish whether there was any abnormal pigmentation in the juveniles.

## Discussion

Growth and survival rates of fish in the rearing tanks are governed by a number of factors including but not limited to water temperature, fish density, light intensity, feeding level, feed-

ing frequency, and type and nutritional quality of food[1,13-15]. In this experiment, all the above mentioned factors except feeding level were held constant during this study.

The growth rate of the Japanese flounder larvae and juveniles increased with increasing feeding level in both trials. This relationship between feeding level and growth has been observed for larval fishes of other species[13,15-19].

Consumption of rotifers was different among four tanks. The highest consumption was in L4 followed by L3, L2 and L1 (Fig. 4). This result is supported by our previous histopathological findings of these reared samples, where we detected deposition of lipid droplets in the enterocytes and accumulation of a large amount of chylomicron-like bodies in the intestinal wall at the higher feeding levels[20]. The result of the present study is correlated with another finding (our unpublished data) where we observed that gut content of larval and juvenile Japanese flounder increases with the increased feeding level. Probably, higher availability of food in higher feeding level had positively influenced the feeding habit of larvae and juveniles. As a result, flounder consumed food at a higher rate in higher feeding levels.

Laurence[21] reported that the larger winter flounder larvae *Pseudopleuronectes americanus* swim faster, search a larger volume of water, and capture the prey with greater success than the smaller one. From 6 d posthatch, larval and juvenile total length at L4 was found significantly higher than L1 and L2. The total length for L3 was significantly higher from 24 d posthatch than the lower feeding levels. These growth-rate-trend was remain unchanged up to the last their sampling, may be, due to their higher preying ability (Figs. 5, 6; Figs. 7, 8).

At metamorphosis flounders were settling down to the bottom and walls of the tanks and frequently making episodic surveys in the water column, and just after adding feeds they showed strong feeding activity moving towards the upper layer of tank water. Hence, settling and less availability of food, if any, in the bottom of the tanks do not affect feed searching capability of Japanese flounder.

Application of higher feeding levels had negative effect on the survival of flounder larval and juvenile, and higher survival rate was obtained at lower feeding level (Fig. 9). This result correlates with the findings of Mobin *et al.*[13] and Saksena and Houde[22] but partially differed from the findings of Riley[23] who reported that high feed concentration ensures better survival of plaice *Pleuroncetes platessa* during the first feeding stage but in the subsequent stage lower feed concentration ensures higher survival. The histopathological study of our reared samples revealed feeding level dependent pathological alterations in the digestive system[20]. Among the pathological alterations, adhesions among the visceral organs themselves and between those organs and peritoneal wall, blebbing and necrosis of gastric mucosal cells, vacuolar degeneration of gastric glands, necrosis of enterocytes and the intestinal wall, atrophy and necrosis of pancreatic acinar cells, reduction of glycogen in hepatocytes, and hepatic necrosis were reported to increase in severity as the feeding level increased. These pathological alterations and the observed cumulative mortality trend of this study are highly correlated. The higher incremental trend of feed consumption up to 24 d posthatch in trial 1 and 25 d posthatch in trial 2 at L4, 26 d posthatch in trial 1 and 29 d posthatch in trial 2 at L3, and 39 d posthatch in trial 1 at L2 might have attributed adverse effects on the physiology and pathology of developing flounder and is suspected to be the reason of incremental feeding level dependent mortality. Although the mortality was higher in higher feeding level, mortality was also observed among fish maintained at the lowest feeding level. This observation raised the possibility that in addition to the feeding level, the feed enrichment materials or the conventional feed enrichment procedure might also have involved in this occurrence. Besides this, relatively deteriorating environmental condition caused by heavier organic loading might have also influenced the mortality at higher feeding level. However, the physico-chemical parameters of the tanks and our observation throughout the experimental period suggest that the environ-

mental condition was not a major cause behind this occurred mortality.

The result of this experiment differed from the findings of some authors[24,25] who reported that survival of larvae increases with the prey densities.

At larval and juvenile stages of fish, if rotifers and *Artemia* nauplii are used together as live feed a high selection towards nauplii might occur. In this situation, rotifers consumption may decline. In this experiment, a declining trend in rotifers consumption was observed in L4 and L3 in both trials from 26 d posthatch and 27d posthatch, respectively. This trend was also observed in L2 in trial 1 from 40 d posthatch. The reason(s) of this declination may be the larvale and juvenils high selection towards *Artemia* nauplii or loosing appetite due to the adverse pathological effects caused by overfeeding or both of them.

Although the growth rates in L4 and L3 were found higher than L2 and L1, the survival rates were lower than the latter two feeding levels. The growth of flounder between L2 and L1 did not differ significantly in both trials and the mortality of these two treatments was found also almost the same. In trial 1, after 40 d posthatch a sharp increase in mortality was recorded in L2. From the constant result of both trials, it is suggested that a better survival can be obtained by the application of feeding level L1 or middle of L1 and L2 under the condition of this experiment. However, increment of initial stocking density of fish and use of other techniques for larval and juvenile feeding might influence the growth and survival and give different results from those reported here.

In this study, feeding level dependent hypomelanosis was observed. Hypomelanosis in flatfish have been reported by a number of authors[26-30]. Although the cause(s) responsible for hypomelanosis is not understood in this experiment, there might be some relation between the hypomelanosis and flounder's growth rate. A number of authors[31-34] suspected that inappropriate lighting, stress, nutrition, and rhodopsin deficiency might be involved with this abnormality. However, abnormal pigmentation has no pathogenic effect on fish[35].

In conclusion, daily application of higher feeding level increases the growth rate but concomitantly decreases the survival of Japanese flounder larvae and juveniles. Although higher feeding levels cause significant losses in seed production, this factor alone are apparently not the only factors responsible for mass mortality that is occurring in the seed production of Japanese flounder in Japan.

## Acknowledgements

The first author wishes to thank the Ministry of Education, Science, Sports and Culture, Japan for scholarship assistance. We are thankful to the personnel of Nagasaki Municipal Aquaculture Center for providingus research facilities and technical advise in conducting this experiment. Cordial thanks are also extended to Mr. D. Shibata for his assistance during the larval and juvenile rearing.

## References

1) Watanabe, T. (1988): Intensive marine farming in Japan, in "Intensive fish farming" (ed. by C. J. Shepherd and N.R.Bromage), BSP Professional Books, Oxford. U.K, pp. 245-265.

2) Nishioka,T., T.Furusawa, and Y.Mizuta (1997): Diseases occurring in marine fish and shellfish hatcheries in Japan (1989-1994). *Suisanzoshoku*, **45**, 285-290. (in Japanese with English abstract).

3) Nakajima, K., K. Inoue, and M. Sorimachi (1998): Viral diseases in cultured marine fish in Japan. *Fish Pathol.* **33**, 181-188.

4) Kusuda, R. and K. Kawai (1998): Bacterial diseases of cultured marine fish in Japan. *Fish Pathol.*, **33**, 221-227.

5) Mobin, S. M. A., K. Kanai, and K. Yoshikoshi (1998): Mass mortalities of red sea bream larvae accompanied with extensive viscero-peritoneal adhesion. *Fish Pathol.*, **33**, 453-454.

6) Reitan, K.I., J.R.Rainuzzo, G.Oie, and Y.Olsen (1993): Nutritional effects of algal addition in first feeding of turbot (*Scophthalmus maximus* L) larvae. *Aquaculture*, **118**, 257-275.

7) Kellam, S.J. and J.M.Walker (1989): Antibacterial activity from marine microalgae in laboratory culture. *Br. Phycol. J.*, **24**, 191-194.

8) Boehlert, G. W. and J. B. Morgan (1985): Turbidity enhances feeding abilities of larval Pacific herring, *Clupea harengus pallasi*. *Hydrobiologia*, **123**, 161-170.

9) Harada, T. (1970): The present status of marine fish cultivation research in Japan. *Helgol. Wiss. Meeresunters*., **20**, 594-601.
10) Houde, E. D. and R. C. Schekter (1981): Growth rates, rations and cohort consumption of marine fish larvae in relation to prey concentrations. *Rapp. P-V. Reun. Cons. Int. Explor. Mer*, **178**, 441-453.
11) Zar, J. H. (1984): Biostatistical analysis, second edition. Prentice Hall, Inc., Englewood Cliffs. New Jersey, USA, pp. 718.
12) Systat for Windows (1992): statistics. Version 5 edition. Evanston, IL: SYSTAT, Inc, USA.
13) Mobin, S. M. A, K. Kanai, and K. Yoshikoshi (2001): Effects of feeding levels on the pathological alterations in the digestive system of larvae and juveniles of red sea bream *Pagrus major*. *J. Aquat. Anim. Health*, **13**, (in press).
14) Hunter, J. R. (1991): Feeding ecology and predation of marine fish larvae, in "Marine fish larvae: morphology, ecology and relation to fisheries" (ed. by R. Lasker), University of Washington press, Seattle, USA, pp. 33-77.
15) Teshima, S., A. Kanazawa, and G. Kawamura (1984): Effects of several factors on growth of milkfish (*Chanos chanos* Forskal) fingerlings reared with artificial diets in aquaria. *Aquaculture*, **37**, 39-50.
16) Houde, E.D. (1977): Food concentration and stocking density effects on survival and growth of laboratory-reared larvae by bay anchovy *Anchoa mitchilli* and lined sole *Achirus lineatus*. *Mar. Biol*., **43**, 333-341.
17) Buckley, L. J., T. A. Halavik, A. S. Smigielski, and G. L. Laurence (1987): Growth and survival of the larvae of three species of temperate marine fishes reared at discrete prey densities. *Am. Fish. Soc. Symp*., **2**, 82-92.
18) Jenkins, G. P., J. W. Young, and T. L. O. Davis (1991): Density dependence of larval growth of a marine fish, the southern bluefin tuna *Thunnus maccoyii*. *Can. J. Fish. Aquat. Sci*., **48**, 1358-1363.
19) Oozeki, Y. and H. Zenitani (1996): Factors affecting the recent growth of Japanese sardine larvae (*Sardinops melanostictus*) in the Kuroshio Current, in "Survival strategies in early life stages of marine resources" (ed. by Y. Watanabe, Y. Yamashita, and Y. Oozeki), A. A. Balkema, Rotterdam, Netherlands, pp. 95-104.
20) Mobin, S. M. A., K. Kanai, and K. Yoshikoshi (2000): Histopathological alterations in the digestive system of larval and juvenile Japanese flounder *Paralichthys olivaceus* reared on four feeding levels. *J. Aquat. Anim. Health*, **12**, 196-208.
21) Laurence, G. C. (1977): A bioenergetic model for the analysis of feeding and survival potential of winter flounder *Pseudopleuronectes americanus* larvae during the period from hatching to metamorphosis. *Fish. Bull*., **75**, 529-546.
22) Saksena, V. P. and E. D. Houde (1972): Effect of food level on the growth and survival of laboratory-reared larvae of bay anchovy (*Anchoa mitchilli* Valenciennes) and scaled sardine (*Harengula pensacolae* Goode & Bean). *J. Exp. Mar. Biol.* and *Ecol*., **8**, 249-258.
23) Riley, J. D. (1966): Marine fish culture in Britain. VII. Plaice (*Pleuronectes platessa*) post-larval feeding on *Artemia salina* L. nauplii and the effects of varying feeding levels. *J. Cons. Cons. Perm. Int. Explor. Mer*, **30**, 204-221.
24) Houde, E. D. (1975): Effects of stocking density and food density on survival, growth and yield of laboratory-reared larvae of sea bream *Archosargus rhomboidalis* (L.) (Sparidae). *J. Fish Biol*., **7**, 115-127.
25) Johnson, J. H. and D. S. Dropkin (1995): Effects of prey density and short-term food deprivation on the growth and survival of American shad larvae. *J. Fish Biol*., **46**, 872-879.
26) de Veen, J. F. (1969): Abnormal pigmentation as a possible tool in the study of the populations of the plaice, *Pleuronectes platessa* L. *J. Cons. Int. Explor. Mer*, **32**, 344-383.
27) Seikai, T. (1980): Early development of squamation in relation to color anomalies in hatchery-reared flounder *Paralichthys olivaceus*. *Jpn. J. Ichthyol*., **27**, 249-255.
28) Seikai, T., J. Matsumoto, M. Shimozaki, A. Oikawa, and T. Akiyama (1987): An association of melanophores appearing at metamorphosis as vehicles of asymmetric skin color formation with pigment anomalies developed under hatchery conditions in the Japanese flounder, *Paralichthys olivaceus*. *Pigment Cell Res*., **1**, 143-151.
29) Kanazawa, A. (1991): Nutritional mechanisms causing abnormal pigmentation in cultured marbled sole larvae, *Limanda yokohamae* (Heterosomata), in "Larvi'91" (ed. by P. Lavens, P. Sorgeloos, E. Jaspers, and F. Olivier), Fish and crustacean Larviculture Symp. Gent, Belgium, Special Pub. **15**, 20-22.
30) Yamamoto, T., K. Fukusho, M. Okauchi, H. Tanaka, W. D. Nagata, T. Seikai, and T. Watanabe (1992): Effects of various foods during metamorphosis on albinism in juvenile of flounder. *Nippon Suisan Gakkaishi*, **58**, 499-508. (in Japanese with English abstract).
31) Denson, M. R. and T. I. J. Smith (1997): Diet and light intensity effects on survival, growth and pigmentation of southern flounder *Paralichthys lethostigma*. *J. World Aquacult. Soc*., **28**, 366-373.
32) Venizelos, A. and D. D. Benetti (1999): Pigment abnormalities in flatfish. *Aquaculture*, **176**, 181-188.
33) Seikai, T. (1989): Albinism of hatchery-reared flounder, *Paralichthys olivaceus* as a result of deformation of asymmetrical development of skin structure, in "The early life history of fish" (ed. by J. Blaxter, J. Gamble, and H. von Westernhagen), Proceedings of the third ICES Symposium, Vol. 191, Bergen, Norway, pp. 489.
34) Kanazawa, A. (1993): Nutritional mechanisms involved in the occurrence of abnormal pigmentation in hatchery-reared flatfish. *J. World Aquacult. Soc*. **24**, 162-166.
35) Soutar, R. (1995): The veterinary approach to turbot, in "Aquaculture for Veterinarians" (ed. by L. Brown), Pergamon, New York, p. 447.

# ヒラメ仔稚魚の成長，生残におよぼす給餌率の影響

S. M. A. Mobin・金井欣也・吉越一馬

ワムシおよびアルテミア幼生を用い，給餌率を2，3の種苗生産施設で採用されている給餌量とその0.5，2.5，5倍に設定し，2回の飼育実験を行って，ヒラメ仔稚魚の成長，生残におよぼす給餌率の影響を検討した。その結果，平均日間成長率は給餌率の高い順に18.3，17.5，16.5および16.1%（実験1）であり，日令45における累積死亡率は給餌率が高い順に26，16.6，11.1および6.7%であった。2回の実験結果はほぼ一致しており，高い給餌率は仔稚魚の速やかな成長をもたらす反面，生残率を有意に低下させることが確認され，種苗生産過程において適正な給餌率の維持が大切であることが示唆された。