Bull. Mar. Sci & Tech 12(1):61-68, 2003
Research Institute of Marine Science and Technology
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Effects of Feeding Rate and Feeding Frequency on Survival, Growth, and Body Composition of Ayu Post-Larvae *Plecoglossus altivelis*

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Abstract.—The effects of feeding rate and feeding frequency on survival, growth and body composition of ayu post-larvae (0.15 g in body weight and 3.5 cm in total length) were investigated in this study. A factorial experimental design of two feeding rates (3 and 6% of body weight of fish per meal) × five feeding frequencies (one meal in 2 d, one meal a day, two meals a day, four meals a day, and six meals a day) with three replicates was used. Survival of ayu post-larvae was significantly ($P < 0.05$) affected by feeding frequency but not by feeding rate. Survival of ayu improved linearly with feeding frequency at both feeding rates. Weight and length gains and specific growth rate (SGR) of ayu was significantly ($P < 0.05$) affected by feeding frequency but not by feeding rate, with weight and length gains and SGR linearly elevated with increasing feeding frequency at both feeding rates. The greatest weight and length gains were observed in fish receiving six meals daily at both feeding rates; however, no significant difference in weight gain was observed among two, four, and six meals a day, or in length gain between four and six meals a day. Feed efficiency ratio (FER) was significantly ($P < 0.05$) affected by both feeding rate and feeding frequency. FER linearly decreased with feeding frequency at both feeding rates or feeding rate in the same feeding frequency. When the total daily amount of feed supply was constant with various feeding frequencies at different feeding rates (one meal a day at 3% feeding rate and one meal in 2 d at 6% feeding rate, two meals a day at 3% feeding rate and one meal a day at 6% feeding rate, or four meals a day at 3% feeding rate and two meals a day at 6% feeding rate), improvement in survival, weight and length gains, and SGR was observed in fish with higher feeding frequency at lower feeding rate. Moisture, protein, and lipid content of fish were not significantly ($P > 0.05$) affected by either feeding rate or feeding frequency. However, lipid content of ayu linearly increased with feeding frequency at 6% feeding rate. The highest body lipid content was observed in fish receiving six meals daily at both feeding rates. Ash content of fish was significantly ($P < 0.05$) affected by feeding frequency but not by feeding rate. Based on performance of ayu, it can be concluded that optimum feeding rate and feeding frequency for ayu post-larvae (an initial weight of 0.15 g) were 3% per meal and four meals a day, respectively, under these experimental conditions.

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The supply of nutritionally balanced feed is very important for the growth of fish, especially during the initial growth periods (fry, larvae, and fingerling). Commercial feeds for these young fish are relatively expensive due to the high inclusion rate of several nutrients to satisfy their requirements for growth. Overfeeding these fish increases fish production cost because of the high price of their feeds and causes deterioration of water quality, which can eventually reduce growth of fish. On the other hand, feeding less than the amount to achieve optimal growth of fish is also undesirable. Therefore, determination of optimum feeding rate and feeding frequency for growth of these fish is critical from both economical and biological standpoints.

Ayu *Plecoglossus altivelis* is an anadromous fish that spawns in September or October when water temperature reaches 17–20 C (Chung 1977). Fertilized eggs are hatched at 12–20 C in 10–24 d and length of the hatched larvae is about 6 mm. Ayu larvae enter the sea immediately after hatching and remain there during winter, feeding on plankton. When water temperature reaches 13–16 C in the following March or April, they start upstream. Because of its delicious taste, ayu has received much attention in Asia for decades. Recently, ayu has also been released to enhance natural resources. Requirements for protein (Lee et al. 2002), essential fatty acids (Kanazawa et al. 1982), and the effects of dietary triglycerides (Mustafa et al. 1991) and *Chloroella*-extract (Nematipour et al. 1987) have been reported. Also, Yao et al. (1994) showed that growth of young (an initial weight of 11 g) ayu fed two meals daily to satiation was significantly higher than that of fish fed once daily, but almost the same as that of fish fed 4 meals daily.

Optimum feeding rate and feeding frequency vary depending on fish species, fish size, and rearing conditions. These factors affect availability of nutrients in feed and eventually fish performance, so that a study on proper feed allowance for growth of fish should be performed prior to other nutrition studies. Therefore, in this study, the effects of feeding rate and feeding frequency on survival, growth, and body composition of ayu post-larvae were determined.

Materials and Methods

Fish and Experimental Conditions

Eggs of ayu were collected by stripping sexually matured ayu caught in Wangpi-Chon (Kyungbook, Korea). The eggs were fertilized by the dry method and hatched at 19.5 ± 0.5 C in 12 d after fertilization. Ayu fry were fed rotifers *Brachionus plicatilis, Artemia* nauplii, and commercial ayu feed (Jeil Feed Co., Korea) in seawater as they grew. About 100-d old ayu post-larvae were used in this study. Four-hundred post-larvae (an initial weight of 0.15 ± 0.006 g and total length of 3.5 ± 0.09 cm) were randomly distributed into each of 30 300-L flow-through conical tanks (water volume, 260 L) and acclimated for 7 d before the initiation of the feeding trial. Water flow rate was 15 L/min. During the acclimation period, ayu post-larvae were fed three times daily with commercial ayu feed (0.3–0.7 mm) at the rate of 5% of body weight (BW) of fish. Dead fish were substituted with live fish during acclimation. Water temperature and photoperiod followed the natural conditions throughout the feeding trial. Water temperature and salinity during the feeding trial ranged from 10.0 to 12.0 C (Mean ± SD, 10.6 ± 0.39 C) and 32 to 33 ppt (32.5 ± 0.3 ppt), respectively. The wasted feed was daily siphon-cleaned from tanks, and dead fish were removed and counted during the feeding trial. Water quality was within acceptable ranges for growth of ayu during the feeding trial. Commercial ayu feed (a mixture of 0.3–0.7 and 0.7–1.2 mm sized feeds at the ratio of 6:4) containing 57.6% crude protein, 14.1% crude lipid, and 3.4% moisture content was fed. Since high mortality of ayu occurred after handling during the early period, the amount of feed fed was increased weekly by 10% throughout the feeding trial. Also no data on the relationship between feed allowance and early growth of ayu is available. Fish were fed 7 d a week and the feeding trial lasted for 8 wk from the middle of January to the middle of March.

Design of the Feeding Trial

A factorial design of two feeding rates (3 and 6% of BW of fish per meal) and five feeding frequencies (one meal in 2 d at 0930; one meal a day at 0930; two meals a day at 0930 and 1700; four meals a day at 0930, 1200, 1400, and 1700; and six meals a day at 0800, 0930, 1200, 1400, 1700, and 1830) with three replicates of each treatment combination was used in this study. Feeding was achieved during the daytime. All fish were hand-fed throughout the feeding trial except for fish receiving six meals daily at 0800 and 1830, which were automatically fed by Yamaha Dry Feeder (YDF 100S, Yamaha Generator Ltd., Japan).

Chemical Analysis

Two-hundred fish were randomly sampled at the initiation of the feeding trial and stored in a freezer (−40 C) for the proximate analysis. At the end of the feeding trial, all live fish from each tank were collectively weighed and sacrificed for proximate analysis of whole-body tissue. Crude protein content was determined by the Kjeldahl method using Auto Kjeldahl System (Buchi B-324/435/412, Switzerland), lipid content by ether-extraction method, moisture content by dry oven (105 C for 24 h), and ash content by muffler furnace (550 C for 4 h) for the experimental diets and fish according to standard methods (AOAC 1990). After measuring total weight of fish, total length of 30 fish randomly chosen from each tank was also measured in mm at the end of the feeding trial.

Statistical Analysis

Treatment effects were evaluated by using one-way and two-way ANOVA tests. In the case of significant treatment effects, Duncan's test (Duncan 1955) was applied to analyze the significance of the difference among the means of each treatment. Additionally, regression analysis was conducted using General Linear Model of Statistical Analysis Systems Institute, Inc. (1987) through SAS version 6.12 (SAS Institute, Cary, North Carolina, USA).

Results

Survival, weight and total length gains, specific growth rate (SGR) on weight, and feed efficiency ratio (FER) for ayu post-larvae at the end of the feeding trial are given in Table 1. Wasted feed on the bottom of the tanks receiving more than two meals daily at both feeding rates was removed daily, showing that enough feed was supplied to achieve the maximum growth of ayu post-larvae throughout the feeding trial.

Survival of ayu was significantly ($P < 0.05$) affected by feeding frequency but not by feeding rate. The highest survival was observed in ayu receiving four meals daily and the lowest in fish receiving one meal in 2 d at both feeding rates. Survival was not significantly ($P > 0.05$) different among fish receiving two, four, and six meals daily at 3% feeding rate, and between fish receiving four and six meals daily at 6% feeding rate. The highest survival was observed in fish receiving four meals daily at both feeding rates. However, survival of ayu linearly improved with feeding frequency at both feeding rates. Significant ($P < 0.05$) interaction of feeding rate and feeding frequency on survival of fish was observed.

Weight and length gains of ayu post-larvae was significantly ($P < 0.05$) affected by feeding frequency, but not by feeding rate. No significant ($P > 0.05$) difference in weight gain was observed among fish receiving two, four, and six meals a day at both feeding rates, or in length gain between four and six meals a day. However, weight and length gains of fish were linearly elevated with feeding frequency at both feeding rates. Similar response was observed for SGR. When the total daily amount of feed supply was constant with various feeding frequencies at different feeding rates (one meal a day at 3% feeding rate and one meal in 2 d at 6% feeding rate, two meals a day at 3% feeding rate and one meal a day at 6% feeding rate, or four meals a day at 3% feeding rate and two meals a day at 6% feeding rate), improvement in survival, weight and length gains, and SGR was observed in fish with higher feeding frequency at lower feeding rate.

FER was significantly ($P < 0.05$) affected by both feeding rate and feeding frequency. The highest FER was observed in fish receiving one meal daily at both feeding rates. Also FER of fish with 3% feeding rate was significantly ($P < 0.05$) higher than that of fish with 6% feeding rate except for fish receiving six meals daily. FER was not significantly different between fish receiving four and six meals daily at 3% feeding rate or among fish receiving two, four, and six meals daily at 6% feeding rate. However, FER linearly decreased with the

TABLE 1. *Survival, weight and total length gains, specific growth rate (SGR) on weight gain, and feed efficiency ratio (FER) in ayu post-larvae fed for 8 wk. Initial weight and total length of ayu post-larvae were 0.157 g, and 3.5 cm, respectively. Different superscript letters in columns are significantly different* (P < *0.05). Pooled SE for survival, wight gain, total length gain, SGR, and FER were 15.25, 0.0031, 0.040, 0.032, and 0.003, respectively. Mean values ±SE.*

Feeding rate (% BW/meal)	Feeding frequency	% BW/d	Survival (%)	Weight gain (g)	Total length gain (cm)	SGR[1]	FER[2]
3%	1 in 2 d	1.5	63.1 ±5.57 [d]	0.160 ±0.0067 [e]	1.24 ±0.123 [e]	1.30 ±0.039 [e]	0.75 ±0.043 [ab]
	1/d	3	78.9 ±2.63 [c]	0.301 ±0.0314 [cde]	1.5 ±0.114 [de]	1.97 ±0.124 [cd]	0.87 ±0.059 [a]
	2/d	6	89.8 ±3.65 [ab]	0.381 ±0.0615 [abc]	1.83 ±0.115 [bcd]	2.26 ±0.210 [abc]	0.62 ±0.086 [bc]
	4/d	12	92.3 ±2.04 [ab]	0.459 ±0.0548 [ab]	2.16 ±0.064 [abc]	2.52 ±0.172 [ab]	0.39 ±0.042 [de]
	6/d	18	89.5 ±3.38 [ab]	0.487 ±0.0735 [a]	2.45 ±0.417 [a]	2.59 ±0.214 [a]	0.27 ±0.033 [ef]
6%	1 in 2 d	3	59.5 ±4.27 [d]	0.213 ±0.0208 [de]	1.44 ±0.180 [de]	1.58 ±0.107 [de]	0.48 ±0.078 [cd]
	1/d	6	82.6 ±2.33 [bc]	0.320 ±0.0283 [bcd]	1.64 ±0.035 [cde]	2.05 ±0.112 [bcd]	0.49 ±0.056 [cd]
	2/d	12	73.6 ±3.46 [c]	0.385 ±0.0556 [abc]	1.88 ±0.121 [bcd]	2.28 ±0.185 [abc]	0.26 ±0.031 [ef]
	4/d	18	95.1 ±1.30 [a]	0.490 ±0.0609 [a]	2.36 ±0.152 [ab]	2.61 ±0.174 [a]	0.22 ±0.027 [f]
	6/d	36	90.2 ±3.07 [ab]	0.507 ±0.0458 [a]	2.47 ±0.086 [a]	2.66 ±0.125 [a]	0.14 ±0.011 [f]
Two-way ANOVA							
Feeding rate (*P*)			<0.2	<0.4	<0.3	<0.2	<0.0001
Feeding frequency (*P*)			<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Interaction (*P*)			<0.04	<0.1	<0.9	<0.9	<0.06

[1] Specific growth rate (SGR) = (Ln final weight − Ln initial weight) × 100/d.
[2] Feed efficiency ratio (FER) = Weight gain of live fish/total feed supply.

increase in feeding frequency and feeding rate. When the total daily amount of feed supply was constant with various feeding frequencies at different feeding rates, improved FER was observed in fish receiving higher feeding frequency at lower feeding rate.

Chemical composition of the whole body of ayu post-larvae at the end of the feeding trial is shown in Table 2. Moisture, protein, and lipid content of fish were not significantly ($P > 0.05$) affected by either feeding rate or feeding frequency. However, body lipid content of fish linearly increased with the increase in feeding frequency at 6% feeding rate. The highest body lipid content was observed in fish receiving six meals daily at both feeding rates. Ash content of fish was significantly ($P < 0.05$) affected by feeding frequency but not by feeding rate. The highest ash content was observed in fish fed one meal in 2 d and two meals daily for the lowest, respectively, at both feeding rates.

Discussion

Feeds are generally given in excess to fish fry and feeding rate is as high as 50% of body weight for warmwater fish, such as channel catfish *Ictalurus punctatus* (NRC 1983). Santiago et al. (1987) reported that the best growth and survival of Nile tilapia *Oreochromis niloticus* fry was obtained at 45% of BW when divided into three equal meals for 5 wk. In this study, survival, weight and length gains of ayu post-larvae

TABLE 2. *Chemical composition of ayu post-larvae at the end of the feeding trial. Different superscript letters in columns are significantly different (P < 0.05). n.s. = No significant difference (P > 0.05). Pooled SE for moisture, protein, lipid, and ash were 1.38, 0.15, 0.26, and 0.01, respectively. Mean values ± SE.*

Feeding rate (% BW/meal)	Feeding frequency	% BW/d	Moisture	Protein	Lipid	Ash
3%	1 in 2 d	1.5	81.9 ± 0.51 n.s.	13.4 ± 0.15 n.s.	2.0 ± 0.69 n.s.	2.1 ± 0.07 ^{a}
	1/d	3	81.6 ± 0.75	13.6 ± 0.57	2.0 ± 0.18	1.8 ± 0.08 ^{bc}
	2/d	6	82.0 ± 0.39	13.0 ± 0.39	2.9 ± 0.47	1.7 ± 0.06 ^{c}
	4/d	12	80.6 ± 0.02	13.2 ± 0.20	2.9 ± 0.58	1.9 ± 0.08 ^{bc}
	6/d	18	81.6 ± 0.82	12.8 ± 0.55	3.3 ± 0.69	1.8 ± 0.14 ^{bc}
6%	1 in 2 d	3	79.6 ± 2.91	13.2 ± 0.23	1.8 ± 0.09	2.0 ± 0.04 ^{ab}
	1/d	6	82.5 ± 0.02	13.5 ± 0.01	1.9 ± 0.38	1.8 ± 0.06 ^{c}
	2/d	12	82.0 ± 0.23	13.6 ± 0.35	1.4 ± 0.13	1.7 ± 0.01 ^{c}
	4/d	18	81.3 ± 0.33	13.7 ± 0.31	2.8 ± 0.63	1.8 ± 0.03 ^{c}
	6/d	36	81.2 ± 0.17	13.0 ± 0.21	2.9 ± 0.12	1.9 ± 0.07 ^{bc}
Two-way ANOVA						
Feeding rate *P*			<0.7	<0.4	<0.3	<0.2
Feeding frequency *P*			<0.6	<0.4	<0.06	<0.001
Interaction *P*			<0.5	<0.7	<0.6	<0.4

receiving four and six meals a day, which were 12 and 18% of BW per day at 3% feeding rate, and 24 and 36% of BW per day at 6% feeding rate, respectively, were significantly ($P < 0.05$) better than those of fish receiving one meal in 2 d and one meal a day, which were 1.5 and 3% of BW per day at 3% feeding rate, and 3 and 6% of BW per day at 6% feeding rate, respectively. However, weight gain of ayu was not significantly different among two, four, and six meals, and between four and six meals for length gain at either feeding rate. Feeding rate had no significant effect on survival or weight and length gains of ayu post-larvae. This indicated that the optimum feeding rate and feeding frequency for growth of ayu post-larvae seemed to be 3% of BW per meal and four meals a day, respectively. Also survival, weight and length gains, and SGR linearly improved with feeding frequency at both feeding rates. The poorest survival and growth was observed in ayu post-larvae receiving one meal in 2 d at both feeding rates, and many ayu had black-colored body probably resulting from lack of necessary nutrients for normal growth of fish due to inadequate feed allowance.

Feed allowance for growth of fish varies depending on fish species, fish size, rearing conditions and so on. Optimum feeding rate and feeding frequency generally decrease as fish grow. Yao et al. (1994) showed that growth of the initial weight of 10.9 g ayu fed a 43.4% protein diet to satiation with two meals daily was better than one meal daily, but was not improved further with four meals daily. In considering the results of Yao et al. (1994), feeding frequency may decrease from four meals to two meals daily as ayu grew from 0.15 g to 10.9 g. Recently, Lee et al. (2000b) proved that optimum feeding frequency for juvenile (initial weight of 5.7 g) rockfish *Sebastes schlegeli* was one satiation feeding daily, and gastric evacuation rate of fish supported their result. However, similar sized juvenile (initial weight of 3.5 g) flounder *Paralichthys olivaceus* grew best on a low energy diet with three meals fed daily to satiation (Lee et al. 2000a).

When the total daily amount of feed supply was constant with various feeding frequencies at different feeding rates (one meal a day at 3% of BW per meal and one meal in 2 d at 6% of BW per meal, two meals a day at 3% of BW per meal, and one meal a day at 6% of BW per meal, or four meals a day at 3% of BW per meal and two meals a day at 6% of BW per meal), improvement in survival, weight and length gains, FER, and SGR was found in ayu post-larvae with higher feeding frequency at lower feeding rate. A similar result with better survival, weight gain, and FER was observed in young red-spotted grouper *Epinephelus akaara* fed a higher feeding frequency at lower feeding rate when daily feed input was constant (Kayano et al. 1993). An increase in feeding frequency enhanced growth of various fish species (Ishiwata 1968a; Andrews and Page 1975; Kayano et al. 1993), and two or three feedings a day was sufficient for maximum growth of a number of species, such as common carp *Cyprinus carpio* (Charles et al. 1984), channel catfish (Andrews and Page 1975), yellowtail *Seriola quinqueradiata* (Ishiwata 1968b), grouper *Epinephelus tauvina* (Chua and Teng 1978), sea bass *Dicentrarchus labrax* (Tsevis et al. 1992), and rainbow trout *Oncorhynchus mykiss* (Grayton and Beamish 1977).

Because it was difficult to determine the point of satiation for ayu post-larvae and high mortality occurred after handling to measure body weight of fish, the amount of feed supply was increased weekly by 10% in this study. An increase of feeding frequency or feeding rate generally lowered FER. Although improvement in survival and growth of ayu post-larvae with feeding frequency was observed in this study, efficiency of feed decreased, agreeing with other studies (Andrews and Page 1975; Tsevis et al. 1992; Kayano et al. 1993; Harpaz et al. 1999; Lee et al. 2000a, 2000b). Improvement in FER was observed in ayu post-larvae receiving less feeding frequency at lower feeding rate, but survival and growth rate were poor. Since survival and growth rate of fish are also critical in commercial fish farming, four and six meals a day was desirable to achieve improvement in survival and growth of fish in this study.

Wasted feed and dead fish were cleaned daily in this study; however, it is difficult to daily clean in commercial fish farming. Without daily cleaning of wasted feed and dead fish, deterioration of water quality might become severe and result in reduction in survival and growth of fish with high feeding frequency at high feeding rate. Deterioration of water quality and poor performance of fish resulting from excessive feed has been reported (Li and Lovell 1992). Therefore, feeding rate and feeding frequency should be carefully monitored to minimize undesirable effects on fish performance in fish farming.

Moisture, protein, and lipid content of the whole body of ayu post-larvae were not significantly affected by either feeding rate or feeding frequency in this study. However, body lipid content of ayu linearly increased with feeding frequency, agreeing with other studies showing the increase in body lipid content with feeding frequency (Grayton and Beamish 1977; Lee et al. 2000a, 2000b). Yao et al. (1994) reported that the muscle lipid content of young ayu increased with the increase of feeding frequency from one to two meals daily, then decreased with the increase of feeding frequency from two to four meals daily. However, body lipid content of silver perch *Bidyanus bidyanus* was significantly affected by dietary energy level rather than feeding rate (Harpaz et al. 1999). The highest body lipid content was observed in ayu post-larvae receiving six meals daily at both feeding rates in this study. It might indicate that an increase in feeding frequency increased body lipid content probably because surplus feed consumed by fish was converted into body fat. Body lipid content of ayu appeared to be inversely related to body protein content of fish. When the total daily amount of feed supply was constant with various feeding frequencies at different feeding rates, body lipid content of ayu linearly increased with feeding frequency at 6% feeding rate. Kayano et al. (1993) reported that lipid contents of the liver and intraperitoneal body fat increased with the increase in feeding frequency, but muscle lipid content of red-spotted grouper decreased when daily feed supply was constant with various feeding frequencies at different feeding rates.

Based on performance of ayu post-larvae in this study, it can be concluded that the optimum feeding rate and feeding frequency for survival and growth of ayu post-larvae initially weighing 0.15 g were 3% of BW per meal and four meals a day, respectively.

Literature Cited

Andrews, J. W. and J. W. Page. 1975. The effects of frequency of feeding on culture of catfish. Transaction of American Fisheries Society 104: 317–321.

AOAC (Association of Official Analytical Chemists). 1990. Official methods of analysis. 15th edition. Association of Official Analytical Chemists, Arlington, Virginia, USA.

Charles, P. M., S. M. Sebastian, M. C. V. Raj, and M. P. Marian. 1984. Effect of feeding frequency on growth and food conversion of *Cyprinus carpio* fry. Aquaculture 40:293–300.

Chua, T. E. and S. K. Teng. 1978. Effects of feeding frequency on the growth of young estuary groupers, *Epinephelus tauvina* (Forskal), cultured in floating net cages. Aquaculture 14:31–47.

Chung, M. 1977. The Fishes of Korea. Ilgisa Publishing Co., Seoul, Korea.

Duncan, D. B. 1955. Multiple-range and multiple F tests. Biometrics 11:1–42.

Grayton, B. D. and F. W. H. Beamish. 1977. Effects of feeding frequency on food intake, growth and body composition of rainbow trout (*Salmo gairdneri*). Aquaculture 11:159–172.

Harpaz, H., D. Sklan, I. Karplus, A. Barki, and Y. Noy. 1999. Evaluation of juvenile silver perch *Bidyanus bidyanus* (Mitchell) nutritional needs using high- and low-protein diets at two feeding levels. Aquaculture Research 30:603–610.

Ishiwata, N. 1968a. Ecological studies on the feeding of fishes: VII. Frequency of feeding and satiation amount. Bulletin of the Japanese Society of Scientific Fisheries 35:979–984.

Ishiwata, N. 1968b. Ecological studies on the feeding of fishes: VIII. Frequency of feeding and growth. Bulletin of the Japanese Society of Scientific Fisheries 35:985–990.

Kanazawa, A., S. Teshima, and M. Sakamoto. 1982. Requirements of essential fatty acids for the larval ayu. Bulletin of the Japanese Society of Scientific Fisheries 48 (4):587–590.

Kayano, Y., S. Yao, S. Yamamoto, and H. Nakagawa. 1993. Effects of feeding frequency on the growth and body constituents of young red-spotted grouper, *Epinephelus akaara*. Aquaculture 110:271–278.

Lee, S. M., S. H. Cho, and D. Kim. 2000a. Effects of feeding frequency and dietary energy level on growth and body composition of juvenile olive flounder (*Paralichthys olivaceus*). Aquaculture Research 12(31):917–923.

Lee, S. M., U. K. Hwang, and S. H. Cho. 2000b. Effects of feeding frequency on growth and body composition of juvenile Korean rockfish (*Sebastes schlegeli*). Aquaculture 187:399–409.

Li, M. and R. T. Lovell. 31992. Effect of dietary protein concentration on nitrogenous waste in intensively fed catfish ponds. Journal of the World Aquaculture Society 23:122–127.

Mustafa, M. G., H. Nakagawa, S. Ohya, T. Shimizu, Y. Horikawak, and S. I. Yamamoto. 1991. Effects of various level of dietary medium chain triglycerides on growth and lipid reservation in ayu. Bulletin of the Japanese Society of Scientific Fisheries 57(12):2327–2331.

NRC (National Research Council). 1983. Nutrient requirements of warmwater fishes and shellfishes. National Academy Press, Washington, D.C., USA.

Nematipour, G. R., H. Nakagawa, K. Nanba, S. Kasahara, A. Tsujimura, and K. Akira. 1987. Effect of *Chlorella*-extract supplement to diet on lipid accumulation of ayu. Bulletin of the Japanese Society of Scientific Fisheries 53(9):1687–1692.

Santiago, C. B., M. B. Aldaba, and O. S. Reyes. 1987. Influence of feeding rate and diet form on growth and survival of Nile tilapia (*Oreochromis niloticus*) fry. Aquaculture 64:277–282.

Statistical Analysis Systems Institute Inc. (SAS). 1987. SAS users guide. SAS Institute, Cary, North Carolina, USA.

Tsevis, N., S. Klaoudatos, and A. Conides. 1992. Food conversion budget in sea bass, *Dicentrarchus labrax*, fingerlings under two different feeding frequency patterns. Aquaculture 101:293–304.

Yao, S., T. Umino, and H. Nakagawa. 1994. Effect of feeding frequency on lipid accumulation in ayu. Fisheries Science 60(6):667–671.

Journal of the World Aquaculture Society
(2003), 34(1):85-91