A Review of the Optimum Feeding Rates in Olive Flounder (5 g through 525 g) *Paralichthys olivaceus* Fed the Commercial Feed

Okorie Eme Okorie[1], Young Chul Kim[1], Kang-Woong Kim[2], Cheul Min An[2], Kyeong-Jun Lee[3] and Sungchul C. Bai[1]*

[1]Department of Marine Bio-materials and Aquaculture/Feeds and Foods Nutrition Research Center, Pukyong National University, Busan 608-737, Korea
[2]Aquafeed Research Center, National Fisheries Research and Development Institute, Pohang 791-802, Korea
[3]Department of Marine Life Science, Jeju National University, Jeju 690-756, Korea

Abstract

Ten feeding trials were conducted to determine and to make the guideline for the optimum feeding rates in olive flounder *Paralichthys olivaceus* fed extruded pellets at various growth stages at the optimum rearing water temperature. The entire trials were grouped into four growth stages, namely the first stage of juvenile (5 and 9 g), the second stage of juvenile (20, 30, 40 and 50 g), growing (240 and 317 g) and sub-adult (384 and 525 g) stages with 2, 4, 2 and 2 feeding trials, respectively. In the first and second experiments, results indicated that the optimum feeding rates for the first stage of juvenile olive flounder weighing 5 and 9 g could be >5.17 but <5.52% and >4.44 but <4.64% body weight (BW)/day, respectively. In the third, fourth, fifth and sixth experiments, results suggested that the optimum feeding rates for the second stage of juvenile olive flounder weighing 20, 30, 40 and 50 g could be >3.47 but <3.50, >2.85 but <3.53, >2.58 but <3.04 and >2.36 but <2.50% BW/day, respectively. In the seventh and eighth experiments, results showed the optimum feeding rates for growing olive flounder weighing 240 and 317 g to be >1.03 but <1.25 and >0.85 but <1.0 % BW/day, respectively. In the ninth and tenth experiments, the optimum feeding rates in sub-adult olive flounder weighing 384 and 525 g were shown to be >0.69 but <0.7 and >0.55 but <0.8% BW/day, respectively. We will compare these results with the previous studies done by other research groups.

Key words: Review, Feeding rates, Olive flounder, Optimum rearing temperature

Introduction

Feed costs account for the major portion of aquaculture production costs, making feed management one of the most important determinants of viability and productivity of any commercial aquaculture operation. Diet cost could represent 30-70% of the total operating cost of an aquaculture enterprise, the percentage increasing with increase intensification of the operation (Lovell, 1998; Webster et al., 2001).

Over- and underfeeding both adversely affect the growth performance and survival of fish and undermine the success of an aquaculture venture. Optimum feeding rates have been reported in different species and sizes of fish and have been found to depend on several factors such as feeding frequency, nutrient content of feed, fish species, fish size and water temperature (Collins, 1971; Andrews and Page, 1975; Minton,

1978; Hung and Lutes, 1987; Hung et al., 1989, 1993, 1995; Li and Lovell, 1992a, 1992b). Parameters employed in determination of optimum feeding rates include growth performance, feed utilization, whole-body proximate composition, serological characteristics and histological changes in fish (Cho et al., 2006, 2007; Kim et al., 2007; Okorie et al., 2013).

Olive flounder, *Paralichthys olivaceus*, has been an important species in Republic of Korea for a long time. However, the popularity of this species has grown dramatically in recent years with a corresponding increase in demand for it. Regrettably, capture production of olive flounder has been fluctuating and there is little hope that capture could grow any further to contribute any significant amounts to sustain the increasing demand for this species. Fortunately, aquaculture of olive flounder has been on the increase due to various policies by the government to boost production. The aquaculture production of this species in 1991, 2001 and 2011 were 1,815, 16,426 and 40,805 metric tons while capture in the same statistical years were 2,094, 1,707 and 4,675 metric tons, respectively (FAO, 2013). Aquaculture production accounted for approximately 90% of the 45,480 metric tons olive flounder total production in 2011 while capture production contributed a meager 10%. It is foreseen that the demand for olive flounder will continue to grow (Bai and Okorie, 2007) without any appreciable increase in capture production.

To meet the ever-growing demand for olive flounder, farmers have resorted to over-feeding fish to minimize culture time. This has the adverse effects of wasting feed, lowering feed efficiency and producing fatty fish (Minton, 1978; Andrews, 1979; Li and Lovell, 1992a, 1992b; Munsiri and Lovell, 1993). To optimize growth, cost of production and product quality, the optimum feeding rates for various sizes of this species need to be established. Furthermore, to reduce labor cost, a computer simulated automated feeding system needs to be developed using the size-specific feeding rates. Therefore, a series of feeding trials were conducted to determine the optimum feeding rates in various growth stages of olive flounder at the optimum rearing temperature in order to propose a computer simulated feeding program for this species. Here, we review results of those feeding trials along with findings by other authors in this species.

Materials and Methods

Experimental diets

The commercial feed used in all the feeding trials was supplied by Suhyup Feed Company Limited (Uiryeong, Gyeongsangnamdo, Republic of Korea). Pellet sizes with the corresponding proximate composition were selected according to the sizes of fish, as done on farm and in line with the company recommendation. Proximate composition of the experimental diets organized according to the initial fish weights is shown in Table 1.

Feed of 2.0-2.2 mm diameter was used in experiment 1 (5 g) of the first stage of juvenile olive flounder and the feed contained 60.6% crude protein, 13.3% crude lipid, 13.4% crude ash, 8.23% moisture and 17.1 MJ/kg gross energy. In experiment 2, 4-4.3 mm feed containing 56.8% crude protein, 14.4% crude lipid, 11.8% crude ash, 8.0% moisture and 17.6 MJ/kg gross energy was used.

In experiment 3 and experiment 4 in the second stage of juvenile olive flounder, 4.0-4.3 mm feed containing 56.8% crude protein, 14.4% crude lipid, 11.8% crude ash, 8.0% moisture and 17.6 MJ/kg gross energy was used. Experiment 5 and experiment 6 of this stage were done with 6.0-6.3 mm feed containing 59.5% crude protein, 12.7% crude lipid, 11.3% crude ash, 8.51% moisture and 17.3 MJ/kg gross energy.

The 9.0-9.4 mm diameter feed used in experiment 7 in the growing stage of olive flounder contained 58.6%, 14.5%, 12.7%, 8.49% and 17.5 MJ/kg crude protein, crude lipid, crude ash, moisture and gross energy, respectively. Experiment 8 was conducted using 11.0-11.4 mm diameter feed containing 56.5% crude protein, 14.6% crude lipid, 13.2% crude ash, 8.51% moisture and 17.4 MJ/kg gross energy.

In experiment 9 in the sub-adult stage of olive flounder, 13.0-13.4 mm diameter feed containing 58.1% crude protein, 13.2% crude lipid, 12.5% crude ash, 4.50% moisture and 17.3 MJ/kg gross energy was used. Experiment 10 was conducted using 15.0-15.4 mm diameter feed containing 56.0% crude protein, 14.3% crude lipid, 11.4% crude ash, 7.04% moisture and 17.6 MJ/kg gross energy.

Table 1. Proximate composition of the experimental diets (dry matter basis)

	Composition[1]						
Initial fish weight	**5 g**	**9, 20 & 30 g**	**40 & 50 g**	**240 g**	**317 g**	**384 g**	**525 g**
Moisture (%)	8.23	8.00	8.51	8.49	8.51	4.50	7.04
Crude protein (%)	60.6	56.8	59.5	58.6	56.5	58.1	56.0
Crude lipid (%)	13.3	14.4	12.7	14.5	14.6	13.2	14.3
Crude ash (%)	13.4	11.8	11.3	12.7	13.2	12.5	11.4
Gross energy (MJ/kg)	17.1	17.6	17.3	17.5	17.4	17.3	17.6
Diet size (mm)	2.0~2.2	4.0~4.3	6.0~6.3	9.0~9.4	11.0~11.4	13.0~13.4	15.0~15.4

[1]Composition of diets for the different sizes of fish as indicated in the table.

Experimental fish and feeding trials

Fish for the feeding trials in the first stage of olive flounder were collected from Hampyeong, Cheonnam while those for the rest of the trials were obtained from Jeju-do, both in the Republic of Korea. Prior to start of the experiments, fish were fed the commercial diet twice daily to apparent satiation for a week to acclimate them to the experimental diet and conditions.

Twenty and fifteen fish with initial body weights of 4.97 ± 0.11 and 9.18 ± 0.13 g (mean ± SD) in experiment 1 (5 g) and experiment 2 (9 g) of the first stage of juvenile olive flounder, respectively were randomly distributed into each of 28 aquaria. Each aquarium was then randomly assigned to one of four replicates of 7 respective feeding rates: 0, 3.0, 4.0, 4.25, 4.5, 4.75 and 5.52 (satiation)% body weight (BW)/day in experiment 1 and 0, 2.0, 3.0, 3.5, 4.0, 4.5 and 4.64 (satiation)% BW/day in experiment 2. Fish were fed three times a day (7:00, 13:00 and 19:00 h). The feeding trials were conducted by using a semi-recirculating system with twenty-eight 40-L rectangular aquaria receiving filtered seawater at the rates of 0.8 and 1.0 L/min in experiment 1 and experiment 2, respectively from the center tank.

In the second stage of juvenile olive flounder fifteen fish with initial body weights of 20.2 ± 0.54, 29.8 ± 0.52, 40.4 ± 0.69 and 50.2 ± 0.46 g (mean ± SD) in experiment 3 (20 g), experiment 4 (30 g), experiment 5 (40 g) and experiment 6 (50 g), respectively were randomly distributed into each of 18 aquaria. Each aquarium was then randomly assigned to one of three replicates of 6 respective feeding rates: 0, 1.0, 2.0, 3.0, 3.5 and 4.12 (satiation)% BW/day in experiment 3; 0, 1.0, 2.0, 2.6, 3.2 and 3.53 (satiation)% BW/day in experiment 4; 0, 1.0, 2.0, 2.4, 2.8 and 3.04 (satiation)% BW/day in experiment 5; and 0, 1.0, 1.5, 2.0, 2.5 and 2.73 (satiation)% BW/day in experiment 6. Fish were fed three times a day (8:00, 12:00 and 18:00 h). The feeding trials were conducted by using a flow-through system with eighteen 50, 100, 150 and 150 L aquaria in experiment 3, experiment 4, experiment 5 and experiment 6, respectively receiving filtered seawater from the center tank.

For the growing olive flounder, twenty fish with initial body weights of 240.4 ± 11.01 and 316.7 ± 6.18 g (mean ± SD) in experiment 7 (240 g) and experiment 8 (317 g), respectively were randomly distributed into each of 10 aquaria. Each aquarium was then randomly assigned to one of duplicates of 5 respective feeding rates: 0, 0.5, 0.75, 1.0 and 1.25 (satiation)% BW/day in experiment 7; and 0, 0.4, 0.6, 0.8 and 1.0 (satiation)% BW/day in experiment 8. Fish were fed twice a day (9:00 and 16:00). Each feeding trial was conducted by using a flow-through system with ten 1 metric ton aquaria receiving filtered seawater from the center tank.

The feeding trials in the sub-adult stage were conducted with twenty-seven and thirty fish with initial body weights of 384.2 ± 5.91 and 525.37 ± 7.12 g (mean ± SD) in experiment 9 (384 g) and experiment 10 (525 g), respectively. In each

experiment fish were randomly distributed into each of 10 aquaria. Each aquarium was then randomly assigned to one of duplicates of 5 respective feeding rates: 0, 0.3, 0.5, 0.7 and 0.9 (satiation)% BW/day in experiment 9; and 0, 0.2, 0.4, 0.6 and 0.8 (satiation)% BW/day in experiment 10. Fish were fed twice a day (9:00 and 16:00). Each feeding trial was conducted by using a flow-through system with ten 1 metric ton aquaria receiving filtered seawater from the center tank.

Each daily ration was divided into portions according to the feeding frequency in each trial. For satiation feeding, an excess amount of feed was weighed out daily and fish were fed as much as they would ingest (within 20 min) at each feeding time. Feeding was done slowly and carefully to ensure total ingestion of feed. The remaining feed was weighed and its weight subtracted from the initial weight.

Total body weight in each aquarium was determined every week and the amount of diet fed to the fish was adjusted accordingly in each trial. All the experimental aquaria were maintained at 14:10 (light:dark) and 12:12 (light:dark) in the first and second juvenile stages, respectively while the aquaria in the growing and sub-adult stages were maintained at natural light. The seawater was maintained at 20 ± 1°C by heaters in the center tank in the juvenile stages while in the growing and sub-adult stages the seawater temperature was 22 ± 2°C during the whole experimental period (Iwata et al. 1994). Supplemental aerations were provided to maintain dissolved oxygen levels near 6.5 ± 0.5 ppm and the salinity was 33 ± 1 ppt in all experiments. The duration of the experiments in the two stages of juvenile olive flounder was 2 weeks while the trials in the growing and sub-adult stages lasted for 3 weeks.

Sample collection and analysis

Weight gain (WG), specific growth rate (SGR), feed efficiency (FE), protein efficiency ratio (PER), survival and whole-body proximate composition were calculated and measured at the end of the feeding trials. Blood analyses were also carried out on fish in the second juvenile, growing and sub-adult stages, as fish in the first juvenile stage were too small for blood to be obtained easily. Hematocrit (packed cell volume; PCV), hemoglobin (Hb), glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), blood glucose and serum total protein were determined. Blood samples were obtained from the caudal vessels by using a heparinized syringe. Hematocrit was determined from three fish per aquarium using the microhematocrit method (Brown, 1980) and hemoglobin (Hb) was measured in the same three fish by the cyanmethemoglobin procedure using Drabkin's reagent. An Hb standard prepared from human blood (Sigma Chemical, St Louis, MO, USA) was used. Three fish from each aquarium were used to analyze whole-body proximate composition. Proximate composition analyses of experimental diets and fish body were performed by the standard methods of AOAC (1995). Samples of diets and fish were dried

to a constant weight at 105°C to determine moisture content. Ash was determined by incineration at 550°C; crude lipid by soxhlet extraction using Soxtec system 1046 (Foss, Hoganas, Sweden) and crude protein by Kjeldahl method (N×6.25) after acid digestion.
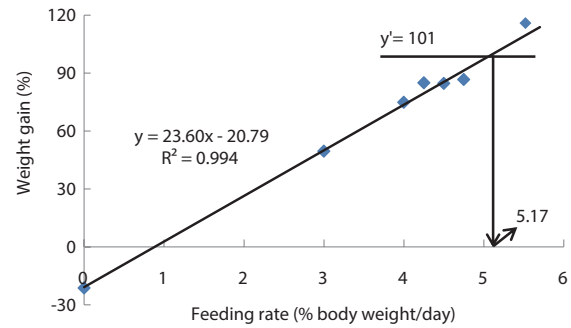
Histology

Clinical signs of fish were carefully monitored daily throughout this experiment. At the end of the feeding trial fish were autopsied and processed for histological examination. Autopsies were carried out on three randomly selected fish from each tank. All tissues and organs obtained were fixed in Bouins solution and processed for a routine histological examination.

Statistical analysis

All data were analyzed by one-way ANOVA (Statistix 3.1, Analytical Software, St. Paul, MN, USA) to test for the dietary treatments. When a significant treatment effect was observed, a least significant difference (LSD) test was used to compare means. Treatment effects were considered significant at the $P < 0.05$ level. Broken-line model was used to analyze the weight gain of olive flounder in response to feeding rate (Robbins et al., 1979).

Results

Weight gain and feed efficiency of fish in all the feeding trials are presented by the growth stages below. Growth performance (weight gain, specific growth rate, feed efficiency, protein efficiency ratio and survival), whole-body proximate

Fig. 1. Broken line analysis of weight gain of 5 g juvenile olive flounder *Paralichthys olivaceus* fed the experimental diet for 2 weeks.

composition, serological characteristics and histological observation were calculated, measured and observed in all growth stages (except for serological characteristics in the first juvenile stage) of fish. However, for clarity and conciseness only weight gain and feed efficiency are included in this review. Full results can be seen in articles on individual growth stages of fish such as in Okorie et al. (2013). Optimum feeding rates in this species in previous studies are presented in Table 2 and compared with our findings below.

Optimum feeding rates for the first stage (5 and 9 g) of juvenile olive flounder

At the end of two weeks of feeding trial in experiment 1, weight gain (WG) of fish fed at 5.52 (satiation)% body weight (BW)/day was significantly higher than those of fish fed at 0 (unfed), 3.0, 4.0, 4.25, 4.5 and 4.75% BW/day ($P < 0.05$) as shown in Table 3. There were no significant differences in WG among fish fed at 4, 4.25, 4.5 and 4.75% BW/day. Weight

Table 2. Optimum feeding rates in olive flounder *Paralichthys olivaceus* in previous studies

Initial fish weight	Optimum feeding rate	Criteria	References
3.6	2.6% BW/day	ANOVA	Kim et al. 2007
17	95% of satiation	ANOVA/regression	Cho et al. 2006
319	90% of satiation	ANOVA	Cho et al. 2007

Table 3. Effects of feeding rates on growth performance of 5 g juvenile olive flounder *Paralichthys olivaceus*[1]

|	Diets							Pooled SEM[5]
	0%	3%	4%	4.25%	4.5%	4.75%	5.52 (S[2])	
WG[3]	-21.3[d]	49.5[c]	74.8[b]	84.9[b]	84.6[b]	86.7[b]	115.9[a]	8.02
FE[4]	-	123[b]	132[ab]	141[a]	134[ab]	133[ab]	121[b]	2.16

[1] Values are means from duplicate groups of fish where the values in each row with different superscripts are significantly different ($P < 0.05$).
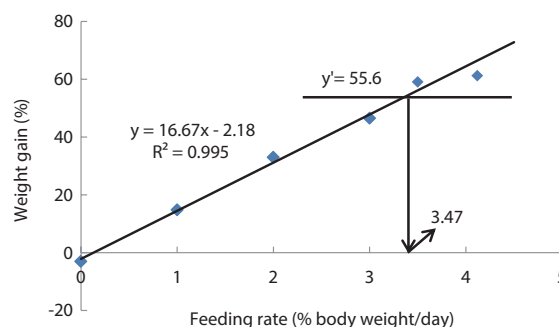[2] Satiation
[3] Weight gain (%) = (final weight - initial weight) × 100 / initial weight
[4] Feed efficiency (%) = wet weight gain (g) × 100 / dry feed intake (g)
[5] Pooled standard error of means: SD/√n
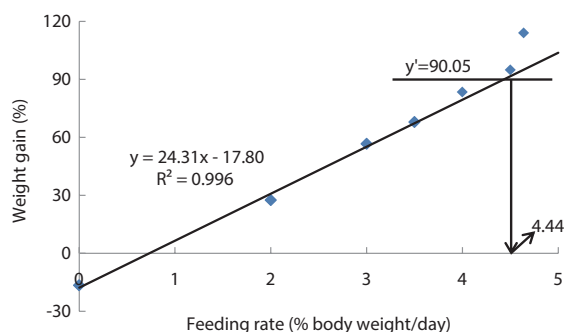
Fig. 2. Broken line analysis of weight gain of 9 g juvenile olive flounder *Paralichthys olivaceus* fed the experimental diet for 2 weeks.

Fig. 3. Broken line analysis of weight gain of 20 g juvenile olive flounder *Paralichthys olivaceus* fed the experimental diet for 2 weeks.

gain of fish fed at 3% BW/day was significantly higher than that of the unfed fish but significantly lower than those of the other treatments. The unfed fish had negative weight gain, which was significantly lower than those of fish in other treatments. Feed efficiency (FE) of fish fed at 4.25% BW/day was significantly higher than those of fish fed at 3 and 5.52% BW/day. However, there were no significant differences in FE among fish fed at 4, 4.25, 4.5 and 4.75% BW/day or among fish fed at 3, 4, 4.5, 4.75 and 5.52% BW/day. Broken line analysis of weight gain indicated that the optimum feeding rate in 5 g juvenile olive flounder could be 5.17% BW/day (Fig. 1).

In experiment 2, WG of fish fed at 4.64 (satiation)% BW/day was significantly higher than those of fish in the other treatments (Table 4). This parameter progressively increased, being significantly higher for each treatment than for the preceding one. Feed efficiency of fish fed at 2.0% BW/day was significantly lower than values for all the other fed fish. However, there were no significant differences in FE among fish fed at 3.0, 3.5, 4.0, 4.5 and 4.64% BW/day. Broken line analysis of weight gain showed that the optimum feeding rate in 9 g olive flounder could be 4.44% BW/day (Fig. 2).

Optimum feeding rates for the second stage (20, 30, 40 and 50 g) of juvenile olive flounder

Weight gain and FE of 20 g juvenile olive flounder, *P. olivaceus*, fed the experimental diet at varying feeding rates for two weeks in experiment 3 are shown in Table 5. Weight gain of fish fed at 3.5% BW/day and those fed at 4.12 (satiation)% BW/day were significantly higher than values for fish in the other treatments ($P < 0.05$). This parameter was significantly higher at each feeding rate than the preceding one for the other treatments. Feed efficiency of fish fed at 3.0, 3.5% and 4.12% BW/day were significantly higher than those of fish fed at 1.0 and 2.0% BW/day. Broken line analysis of weight gain showed the optimum feeding rate in 20 g juvenile olive flounder to be about 3.47% BW/day (Fig. 3).

Table 6 shows growth parameters of 30 g juvenile olive flounder, *P. olivaceus*, fed the experimental diet in experiment 4. After two weeks of feeding trial, WG of fish fed at 3.53 (satiation)% BW/day was significantly higher than those of fish in other treatments ($P < 0.05$). Weight gain of fish fed at 2.0, 2.6 and 3.2% BW/day were significantly higher than those of fish fed at 0 (unfed) and 1.0% BW/day. The unfed fish

Table 4. Effects of feeding rates on growth performance of 9 g juvenile olive flounder *Paralichthys olivaceus*

	Diets							Pooled SEM
	0%	2.0%	3.0%	3.5%	4.0%	4.5%	4.64%	
WG	-16.6[g]	27.5[f]	56.6[e]	67.9[d]	83.4[c]	94.9[b]	114.0[a]	7.57
FE	-	94.2[b]	130[a]	129[a]	133[a]	132[a]	137[a]	3.33

Table 5. Effects of feeding rates on growth performance of 20 g juvenile olive flounder *Paralichthys olivaceus*

	Diets						Pooled SEM
	0%	1.0%	2.0%	3.0%	3.5%	4.12%	
WG (%)	-3.0[e]	14.8[d]	33.0[c]	46.5[b]	59.1[a]	61.2[a]	5.81
FE (%)	-	89.3[c]	109[b]	128[a]	132[a]	131[a]	4.45
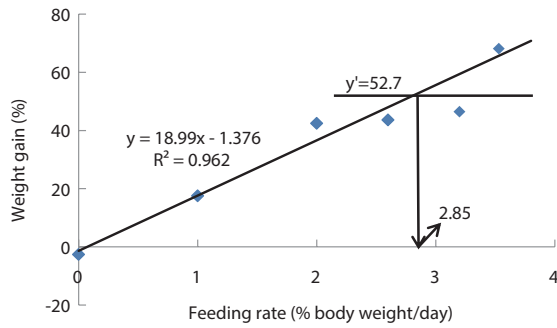
Fig. 4. Broken line analysis of weight gain of 30 g juvenile olive flounder *Paralichthys olivaceus* fed the experimental diet for 2 weeks.
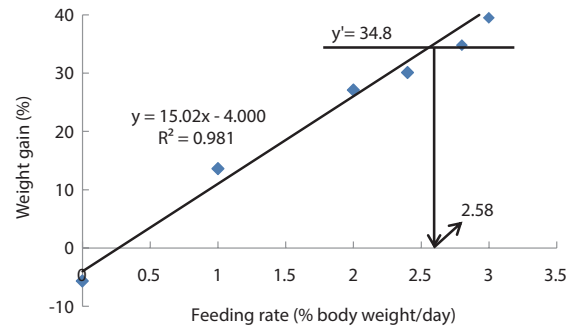
Fig. 5. Broken line analysis of weight gain of 40 g juvenile olive flounder *Paralichthys olivaceus* fed the experimental diet for 2 weeks.

had negative WG, which was significantly lower than those for the other treatments. Feed efficiency of fish fed at 2.6, 3.2 and 3.53% BW/day were significantly higher than values obtained for fish fed at 1.0 and 2.0% BW/day. Feed efficiency of fish fed at 2.0% BW/day was significantly higher than that of fish fed at 1.0% BW/day. Broken line analysis of weight gain indicated the optimum feeding rate in 30 g juvenile olive flounder, *P. olivaceus*, to be 2.85% BW/day (Fig. 4).

Growth parameters of 40 g juvenile olive flounder, *P. olivaceus*, fed the experimental diet at different feeding rates in experiment 5 are shown in Table 7. Weight gain of fish fed at 3.04 (satiation)% BW/day was significantly higher than those of fish fed at 0 (unfed), 1.0 and 2.0% BW/day ($P <$ 0.05). However, there were no significant differences in WG

among fish fed at 2.4, 2.8 and 3.04% BW/day or among fish fed at 2.0, 2.4 and 2.8% BW/day. Weight gain of the unfed fish was negative and significantly lower than values obtained for all the fed fish. Feed efficiency was significantly lower in fish fed at 1.0% BW/day than in the rest of the fish fed the experimental diet; there were no significant differences in this parameter among the other fed fish. Optimum feeding rate in 40 g juvenile olive flounder was determined to be about 2.58% BW/day based on broken line analysis of WG (Fig. 5).

In experiment 6, WG of fish fed at 2.5 and 2.73 (satiation)% BW/day were significantly higher than those of fish in the other treatments (Table 8). This parameter increased with feeding rate for other treatments, being significantly higher at each feeding rate than the preceding one. Values of FE for the fed

Table 6. Effects of feeding rates on growth performance of 30 g juvenile olive flounder *Paralichthys olivaceus*

	Diets						Pooled SEM
	0%	1.0%	2.0%	2.6%	3.2%	3.53%	
WG (%)	-2.64[d]	17.5[c]	42.4[b]	43.6[b]	46.4[b]	68.1[a]	5.57
FE (%)	-	87.1[c]	107[b]	126[a]	130[a]	129[a]	3.26

Table 7. Effects of feeding rates on growth performance of 40 g juvenile olive flounder *Paralichthys olivaceus*

	Diets						Pooled SEM
	0%	1.0%	2.0%	2.4%	2.8%	3.04%	
WG (%)	-5.68[d]	13.6[c]	27.1[b]	30.1[ab]	34.8[ab]	39.5[a]	3.47
FE (%)	-	97.0[b]	118[a]	117[a]	127[a]	121[a]	2.98

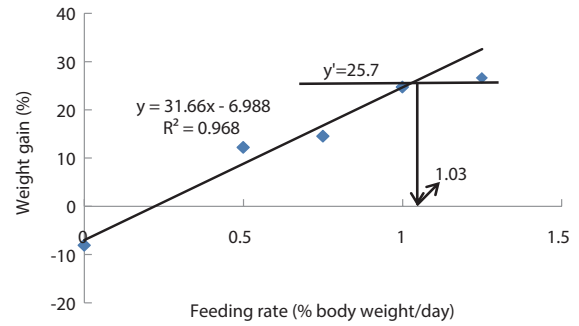**Table 8.** Effects of feeding rates on growth performance of 50 g juvenile olive flounder *Paralichthys olivaceus*

	Diets						Pooled SEM
	0%	1.0%	1.5%	2.0%	2.5%	2.73%	
WG (%)	-4.0[e]	20.5[d]	32.4[c]	43.9[b]	55.5[a]	59.0[a]	5.32
FE (%)	-	107[b]	128[a]	128[a]	137[a]	131[a]	3.38
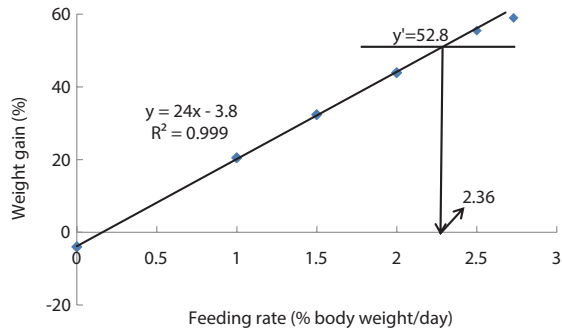
Fig. 6. Broken line analysis of weight gain of 50 g juvenile olive flounder *Paralichthys olivaceus* fed the experimental diet for 2 weeks.
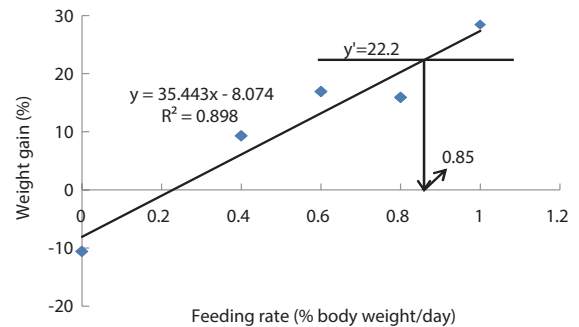

Fig. 7. Broken line analysis of weight gain of 240 g growing floun-der *Paralichthys olivaceus* fed the experimental diet for 3 weeks.

Fig. 8. Broken line analysis of weight gain of 317 g growing floun-der *Paralichthys olivaceus* fed the experimental diet for 3 weeks.

fish were not significantly different except for fish fed at 1.0% BW/day, which was significantly lower than values for other treatments. The optimum feeding rate in 50 g juvenile olive flounder was estimated to be about 2.36% BW/day based on broken line analysis of WG (Fig. 6).

Optimum feeding rates for growing (240 and 317 g) olive flounder

Weight gain and FE of 240 g growing olive flounder, *P. oli-vaceus*, fed the experimental diet at different feeding rates in experiment 7 are shown in Table 9. Weight gain of fish fed at 1.25 (satiation)% BW/day was significantly higher than those of fish fed at 0 (unfed), 0.5 and 0.75% BW/day. Weight gain of fish fed at 1.0% BW/day was significantly higher than those of fish fed at 0 and 0.5% BW/day. However, there were no significant differences in WG between fish fed at 0.5% BW/day and those fed at 0.75% BW/day, between fish fed at 0.75% BW/day and those fed at 1.0% BW/day and between fish fed at 1.0% BW/day and those fed to satiation. There were no significant differences in FE of fish fed the experimental diet at all feeding rates. The optimum feeding rate in 240 g growing

olive flounder, *P. olivaceus*, was indicated to be 1.03% BW/day based on broken line analysis of WG (Fig. 7).

Growth performance of 317 g growing olive flounder, *P. olivaceus*, fed the experimental diet at different feeding rates in experiment 8 is shown in Table 10. Weight gain of fish fed at 1.0 (satiation)% BW/day was significantly higher than those in other treatments. Weight gain was negative and significant-ly lower in the starved fish than in fish fed the experimental

Table 9. Effects of feeding rates on growth performance of 240 g growing olive flounder *Paralichthys olivaceus*

|	**Diets**					**Pooled SEM**
	0%	**0.5%**	**0.75%**	**1.0%**	**1.25%**	
WG (%)	-8.1[d]	12.2[c]	14.5[bc]	24.7[ab]	26.6[a]	5.19
FE (%)	-	94.5	90.7	136	108	10.3

Table 10. Effects of feeding rates on growth performance of 317 g growing olive flounder *Paralichthys olivaceus*

	Diets					**Pooled SEM**
	0%	**0.4%**	**0.6%**	**0.8%**	**1.0%**	
WG (%)	-10.6[c]	9.3[b]	16.9[b]	15.9[b]	28.4[a]	4.37
FE (%)	-	112.6	131.6	104.8	128.6	8.76

diet at all feeding rates. There were no significant differences in WG among fish fed at 0.4, 0.6 and 0.8% BW/day. There were no significant differences in FE of fish in all treatments. Based on broken line analysis of WG, the optimum feeding rate in 317 g growing olive flounder, *P. olivaceus*, could be 0.85% BW/day (Fig. 8).

Optimum feeding rates for sub-adult (384 and 525 g) olive flounder

At the end of the 3 weeks of feeding trial, WG of fish fed at 0.7 and 0.9 (satiation)% BW/day were significantly higher than those of fish fed at 0 (unfed), 0.3 and 0.5% BW/day in experiment 9 (Table 11). Weight gain of the unfed fish was negative and significantly lower than those of fish fed the experimental diet at all feeding rates. There were no significant differences in WG among fish fed at 0.3 and 0.5% BW/day and among those fed at 0.7% BW/day and satiation. There were no significant differences in FE of fish in all treatments. The optimum feeding rate in 384 g sub-adult olive flounder, *P. olivaceus*, was found to be 0.69% BW/day based on broken
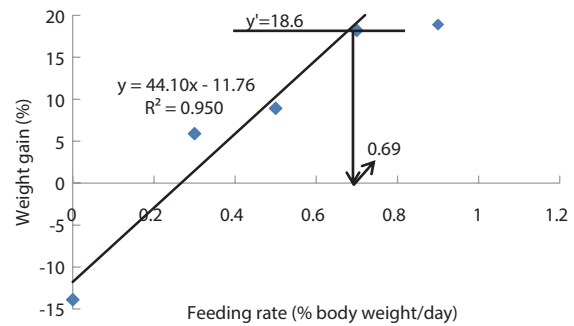
Fig. 9. Broken line analysis of weight gain of 384 g sub-adult olive flounder *Paralichthys olivaceus* fed the experimental diet for 3 weeks.

line analysis of WG as indicated in Fig. 9.

In experiment 10 (Table 12), WG of fish fed at 0.8 (satiation)% BW/day was significantly higher than those of fish fed at 0 (unfed) and 0.2% BW/day. There were no significant differences in WG among fish fed at 0.2, 0.4 and 0.6% BW/day and among those fed at 0.4, 0.6 and 0.8%

Table 11. Effects of feeding rates on growth performance of 384 g sub-adult olive flounder *Paralichthys olivaceus*

| | Diets | | | | | Pooled SEM |
	0%	0.3%	0.5%	0.7%	0.9%	
WG (%)	-13.9[c]	5.9[b]	8.9[b]	18.2[a]	18.9[a]	4.01
FE (%)	-	85.0	73.0	120	99.0	9.66

Table 12. Effects of feeding rates on growth performance of 525 g sub-adult olive flounder *Paralichthys olivaceus*

| | Diets | | | | | Pooled SEM[9] |
	0%	0.2%	0.4%	0.6%	0.8%	
WG (%)[5]	-20.7[c]	5.0[b]	10.3[ab]	12.1[ab]	17.0[a]	4.49
FE (%)[7]	-	102	113	111	112	8.92

Table 13. Optimum feeding rates in olive flounder *Paralichthys olivaceus* in the current feeding trials

| Initial fish weight	Optimum feeding rate[1]	Criteria
5	5.17~5.52	Broken line/ANOVA
9	4.44~4.64	Broken line/ANOVA
20	3.47~3.50	Broken line/ANOVA
30	2.85~3.53	Broken line/ANOVA
40	2.58~3.04	Broken line/ANOVA
50	2.36~2.50	Broken line/ANOVA
240	1.03~1.25	Broken line/ANOVA
317	0.85~1.0	Broken line/ANOVA
384	0.69~0.7	Broken line/ANOVA
525	0.55~0.8	Broken line/ANOVA

[1]Optimum feeding rate: % body weight (BW)/day
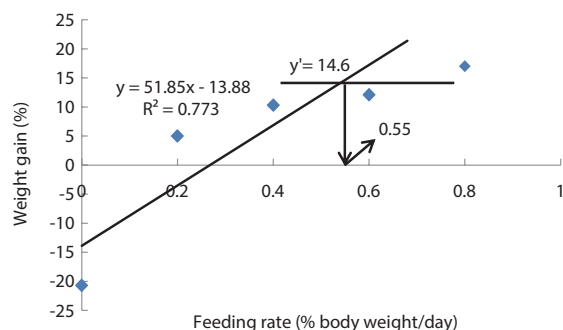
Fig. 10. Broken line analysis of weight gain of 525 g sub-adult olive flounder *Paralichthys olivaceus* fed the experimental diet for 3 weeks.

Fig. 11. Variation of feeding rates with initial weights of fish.

BW/day. Weight gain of the unfed fish was negative and significantly lower than those of fish fed the experimental diet at all feeding rates. There were no significant differences in FE of fish in all treatments. Broken line analysis of WG indicated the optimum feeding rate in 525 g sub-adult olive flounder, *P. olivaceus*, to be 0.55% BW/day (Fig. 10).

Discussion

In the above series of feeding trials we determined the optimum feeding rates at various growth stages of olive flounder at the optimum rearing temperatures using commercial feed. Representative sizes of fish were selected to cover the various growth stages, including the juvenile, growing and sub-adult stages. Proximate composition of the commercial feed used for the feeding trials was similar to the one used in a similar study by Iwata et al. (1994) in this species. Water temperatures of 20 ± 1°C for the juvenile stages and 22 ± 2°C for the growing and sub-adult stages were within the range of 20 and 25°C suggested by Iwata et al. (1994) as the optimum temperature for the growth of olive flounder.

Feeding rate affected growth performance in all the feeding trials. Similar to previous findings in other species (De Silva et al., 1986; Hung and Lutes, 1987; Xiao-Jun and Ruyung, 1992; Adebayo et al., 2000; Ng et al., 2000; Mihelakakis et al., 2002), weight gain of olive flounder improved with feeding rate in all the feeding trials in this series. Continuous increase in weight gain with feeding rate has been recorded in blackspot seabream, *Pagellus Bogaraveo* (Ozorio et al., 2009); olive flounder (Cho et al., 2007); European sea bass, *Dicentrarchus labrax* L. (Eroldogan et al., 2004); white sturgeon, *Acipenser transmontanus* (Deng et al., 2003); and gilthead sea bream, *Spurus aurata* (Mihelakakis et al., 2002) among others. However, improvement in growth performance of fish below satiation varied with fish sizes in these studies. Weight gain of 9 and 240 g fish improved more remarkably below satiation than did this parameter in other sizes of fish. Although WG continued to increase up to satiation in all
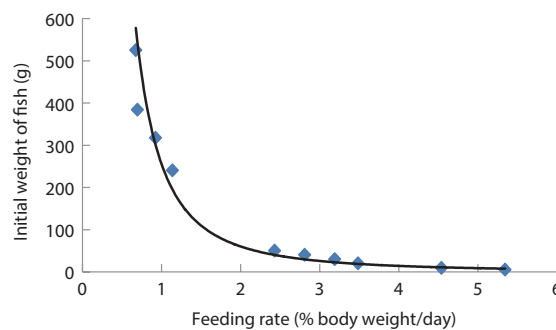
sizes of fish there was no marked change in WG of 5 g fish for instance, between the feeding rates of 4 and 4.75% body weight (BW)/day. A similar observation was recorded in this parameter among the last three treatments before satiation in 30 and 317 g fish while there were no marked improvements in growth of fish fed at the level immediately below satiation and those fed to satiation in 20, 50, and 384 g fish. But in all the experiments, growth continued to improve, at least, numerically with feeding rate up to satiation.

Feed utilization on the other hand leveled out or peaked and dropped after a certain feeding rate in almost all the feeding trials. This could be seen from the peak in feed efficiency for fish fed at 4.25% BW/day and the drop after the peak in this parameter in the 5 g fish. Maximum feed efficiency has been reported to be achieved at feeding rates below that required for maximum growth (Andrews, 1979; Brett and Groves, 1979). At low feeding rates, fish tend to optimize their digestion to extract nutrients more efficiently (Zoccarato et al., 1994; Van Ham et al., 2003). This implies an inverse relationship between feeding rate and feed efficiency after a peak. The peak in feed efficiency in the present study could be explained on the basis of greater weight gain than the incremental feed intake at feeding rates below the peak value. But beyond the peak fish could not efficiently utilize the additional food, leading to the drop in feed efficiency.

Comparing the optimum feeding rates in the different growth stages, it could be seen that the optimum feeding rates decreased with fish size (Fig. 11 and Table 13). This observation agrees with results in previous studies in various species (Page and Andrews, 1973; Skalli et al., 2004; Hatlen et al., 2005; Sweilum et al., 2005). The optimum feeding rate for 30 g white sturgeon, *Acipenser transmontanus*, was reported to be 2.0-3.0% BW/day (Hung et al., 1993) while the feeding rates for 30-100 g and 0.25-0.5 kg fish of the same species were found to be 2.0% BW/day and 1.5-2.0% BW/day, respectively (Hung and Lutes, 1987; Hung et al., 1989). Similarly, Cho et al. (2006) reported the optimum feeding rate for 17 g juvenile olive flounder, *P. olivaceus*, to be 95% of satiation while that for 319 g sub-adult fish was recorded by Cho et

al. (2007) as 90% of satiation. Growth rates of fish decrease with age. This implies increased demand for food to meet high metabolic rates in small fish; hence, the corresponding decrease in feeding rates with size.

The optimum feeding rates of >5.17 but <5.52, >4.44 but <4.64, and >3.47 but <3.50% BW/day obtained in 5, 9 and 20 g juvenile olive flounder, respectively in these feeding trials are higher than the 2.6% BW/day in 3-18 g fish in a previous study in this species (Kim et al., 2007). It is hard to explain the higher feeding rates in the current trials compared to the value in the earlier study, but by physical observation, fish were underfed at our first two feeding rates of 3 and 4% BW/day for 5 g and of 2 and 3% BW/day for 9 g fish, suggesting that the optimum feeding rates for these two sizes could be higher than the values reported by Kim et al. (2007). The optimum feeding rates of >0.85 but <1.0 % BW/day for 317 growing olive flounder as well as >0.69 but <0.7 and >0.55 but <0.8% BW/day for 384 g and 525 g sub-adult fish, respectively are lower than 1.5-2.0% BW/day for 250-500 g white sturgeon, *Acipenser transmontanus* (Hung et al., 1989). The optimum feeding rate for 319 g olive flounder was reported as percentage of satiation (90% of satiation) rather than in BW/day (Cho et al. 2007).

In conclusion, based on ANOVA and broken line analysis of WG the optimum feeding rates in 5, 9, 20, 30, 40 and 50 g juvenile olive flounder, *P. olivaceus*, could be >5.17 but <5.52, >4.44 but <4.64, >3.47 but <3.50, >2.85 but <3.53, >2.58 but <3.04 and >2.36 but <2.50% BW/day, respectively. ANOVA and broken line analysis of WG indicated the optimum feeding rates in 240 and 317 g growing olive flounder, *P. olivaceus*, to be >1.03 but <1.25 and >0.85 but <1.0 % BW/day, respectively while those in 384 g and 525 g sub-adult fish could be >0.69 but <0.7 and >0.55 but <0.8% BW/day, respectively.

Acknowledgments

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