Red Sea Bream, *Pagrus major*

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Introduction

The red sea bream, *Madai* in Japanesex, is one of the most popular fish species in Japanese aquaculture due to its economic feasibility and traditional food habits. The aquaculture production of red sea bream (*Pagrus major*) is the second largest in Japan, following the yellowtail (*Seriola quinqueradiata*). The aquaculture production of red sea bream in 1998 was 82,500 metric tonnes (Statistical and Information Department, 2000). This quantity is 1.8 times higher than that in 1988. Furthermore, nearly 81 million juveniles of this species were produced in 1998, of which 30 million were used for the stock enhancement and 51 million for aquaculture (Statistical and Information Department, 2000). Together with many nutritional studies, a variety of diets for the species have been introduced into the market in recent years. In 1998, the feeds for red sea bream reached 180,000 metric tonnes, which constitute 60% of total diets for marine fish (Aquaculture Feed Association, Tokyo, 1999, personal communication). Although most nutritional studies have focused on the growing stage of the species, studies on larval and brood-stock nutrition have also progressed in the past few years. Studies before the 1990s had been conducted using moist pellets, which contained more than 30% moisture. However, due to the recent development of dry diets, new findings are based on the data of studies with the dry diets.

Nutrient Requirements

Proteins and amino acids

Yone (1976) reported that the optimal dietary protein level was 55% with 10% dietary lipid in moist pellets for the species. However, a study using dry-type diets (Takeuchi *et al.*, 1991) indicated that, in 2 g fish, the growth performance of the 35-day trial seemed to improve with increased dietary protein from 37% to 52%, when fed diets containing white fish-meal and casein as major protein sources. In

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8 g fish, the growth rate and feed conversion efficiency appeared to be better in fish fed the test diet containing 52% dietary protein than in fish fed the 42% dietary protein, irrespective of the dietary lipid content.

Since the quality of protein sources is very important in diet formulation as well as the determination of nutrient requirements, we conducted a study to evaluate the nutritional value of several protein sources (Koshio *et al.*, 1996). Five protein sources – casein, sardine meal, squid meal, soybean-protein isolate (SPI) and krill meal – were tested (Table 4.1). Protein (55%) and gross energy (4.5 kcal g^{-1}) contents of test diets were maintained constant in all diets. The fastest growth was found in fish fed the sardine meal-based diet, followed by squid meal, krill meal, casein and SPI diets (Fig. 4.1). Survival rates in all treatments were almost the same except the SPI-fed group, which showed significantly lower survival than other groups. The poor performance of fish fed SPI-based diet could be related in part to the lack of dietary methionine and lysine, poor palatability, high standard metabolism and lower specific dynamic action (Koshio *et al.*, 1996).

Recently, due to the high cost and limited supply of high-quality fish-meal, many studies on protein replacement have been conducted for cultured species in Japan (Koshio, 1999). Although soybean meals (SBM) have some negative effects on fish performance, the products have been widely used, due to their constant supply and economically reasonable cost. Ukawa *et al.* (1994) suggested that 14 g red sea bream could utilize commercially available SBM and the inclusion of 25% SBM in the diet did not show any negative growth performance compared with fish-meal-based diet. Moreover, we tested twin-screw extruded SBM, which has a very low level of trypsin inhibitors and antigenicities, for 3 g red sea bream

Table 4.1. Compositions of test diets (g 100 g^{-1}) for red sea bream.

	Diets				
Ingredients	1	2	3	4	5
Casein (vitamin-free)	45	–	–	–	–
Brown fish-meal	–	59	–	–	–
Squid meal	–	–	64	–	–
Soybean protein	–	–	–	54	–
Krill meal	–	–	–	–	66
Dextrin	4	4	4	4	4
Alpha-starch	4	4	4	4	4
Pollack liver oil	6	6	6	6	6
Soybean lecithin	3	3	3	3	3
Mineral mixture	4	4	4	4	4
Vitamin mixture	4	4	4	4	4
n-3 HUFA	1	1	1	1	1
Attractants	2	2	2	2	2
Activated gluten	5	5	5	5	5
Alpha-cellulose	22	8	3	13	1
Total	100	100	100	100	100

(Sumule *et al.*, 2000). The growth was not significantly different between groups fed the fish-meal-based diet and the diet containing 32% twin-screw extruded SBM.

The effects of possible combinations of protein sources on fish performances have also been investigated in recent years (Aoki *et al.*, 1997, 1998, 2000). Fish averaging 27 g and 550 g were fed diets containing soybean protein concentrate, SBM, maize-gluten meal, meat meal and poultry-feather meal with or without fish-meal (Aoki *et al.*, 2000). The growth performance of juveniles was not as good as that of fish fed a commercial diet, but feed efficiency and the haematochemical condition of fish fed the above diets together with low levels of fish-meal were similar to those of fish fed the commercial diet.

There are limited studies on amino acid, including essential amino acid (EAA), requirements of red sea bream. The lysine (Lys) requirement of red sea bream (1.7 g initial weight) was investigated by feeding semimoist diets (Forster and Ogata, 1998). When zein, fish-meal, gelatin and L-lysine hydrochloric acid (HCl) were used as ingredients, they found that the requirements were close to 3.6, 4.3 and 4.4% of dietary protein for the specific growth rate, feed efficiency and nitrogen retention, respectively. Forster and Ogata's (1998) estimated values for other EAA (% of dietary protein) based on the A/E ratio (amount of each EAA/total amount of EAA) were as follows: arginine (Arg), 3.5; histidine (His), 1.4; isoleucine (Ile), 2.2; leucine (Leu), 4.2; methionine + cystine (Met + Cys), 2.2; phenylalanine + tyrosine (Phe + Tyr), 4.1; threonine (Thr), 1.8; tryptophan (Trp), 0.6; valine (Val), 2.5%. Yone (1976) reported the requirement of the ten EAA (g 100 g^{-1} diet) according to the growth response of red sea bream as follows: Arg, 3.7; His, 1.7; Ile, 2.3; Leu, 3.4; Met, 1.1; Phe, 2.5; Thr, 1.7; Trp, 0.6; Val, 3.1; Lys, 4.3. A study on the arginine requirement of red sea-bream larvae (Lopez-Alvarado and Kanazawa, 1994) suggested that the requirement was

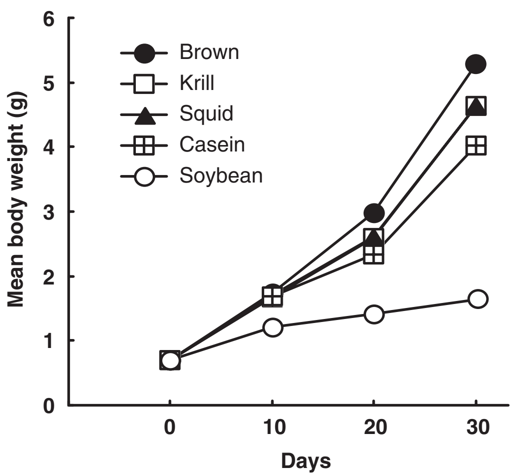

Fig. 4.1. Growth curve of red sea-bream juveniles fed diets with different protein sources. Brown, brown fish-meal.

4.2% of dietary protein (2.5% of a 60% protein diet). It is likely that larval red sea bream have a higher requirement for the amino acid than juveniles or adults for the optimal growth.

Energy

There are limited studies on the energy requirement of red sea bream. In a study on the search for suitable dietary levels of protein and lipid, four metabolizable energy (ME) levels with different dietary protein and lipid levels were tested (Takeuchi *et al.*, 1991). The study suggested that growth increased with increased dietary ME up to 3.7 kcal g^{-1} diet under the 42% protein level. With a 52% protein level, growth increased with increased dietary ME up to 4.3 kcal g^{-1} diet. The optimum calorie-to-protein ratio (kcal kg^{-1}/% protein) was 87 for the 42% protein group and 73–80 for the 52% protein group.

We conducted a study using the response surface analysis method (RSM) to determine the appropriate combination of energy-yielding nutrients for the optimal growth of red sea-bream juveniles (Koshio *et al.*, 1998). Based on the concept of the triangle graph (Toyomizu *et al.*, 1982), 24 fish-meal-based dry diets were formulated. The protein energy (PGE) intake ranged from 30 to 70%, fat energy (FGE) from 10 to 60% and carbohydrate energy (CGE) from 0 to 60%. Second-order polynomial equations were applied to construct the RSM and contour map of the growth performance. Figure 4.2 presents the results for the specific growth rate of juvenile red sea bream. The growth of the fish depended largely on the PGE. It can be concluded that red sea-bream juveniles required almost 60% of energy from protein to obtain the optimal growth. At the same time, we found that the ratio of CGE and FGE is one of the important factors that affect fish performances. As shown in Fig. 4.2, red sea bream required more energy from lipids than from carbohydrates for maximum growth and the ratio of CGE and FGE was less than 1.0 to obtain the optimal range of growth. It is also confirmed that red sea bream had a very poor ability to utilize carbohydrates since slow growth was obtained when fish took more CGE than FGE. Even when dietary CGE was not supplied, the growth did not decrease as long as enough PGE and FGE were available (Fig. 4.2).

Lipids and fatty acids

The optimal dietary lipid levels for maximum growth were examined using 30–40 g fish (Yone *et al.*, 1974; Yone, 1975). These studies suggested that the best performance was obtained in fish fed the diet containing about 10% fish-oil. They also indicated that fish fed a diet containing soybean oil showed a very poor growth rate due to the lack of eicosapentaenoic acid (EPA), 20:5n-3, and docosahexaenoic acid (DHA), 22:6n-3. Takeuchi *et al.* (1991) reported that increasing dietary energy by increasing dietary lipid resulted in improvement of the growth rate. The optimal combination seemed to be 15% dietary lipid and 52% dietary protein, together with 4 kcal g^{-1} diet of ME when red sea-bream

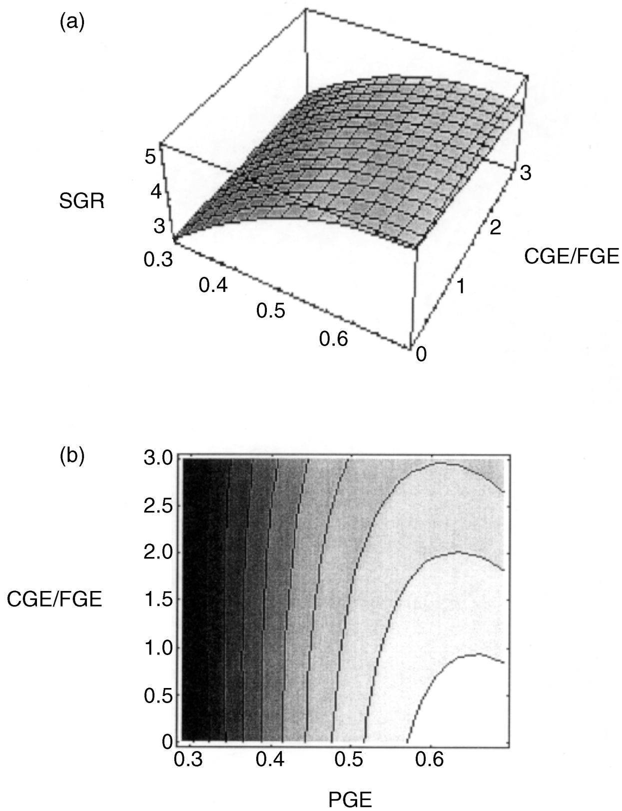

Fig. 4.2. Response surface (a) and contour map (b) for the specific growth rate (SGR) of red sea-bream juveniles fed for 30 days with 24 test diets containing a wide range of energy from protein, lipid and carbohydrate. PGE, protein energy; CGE, carbohydrate energy; FGE, fat energy.

juveniles were fed the diet containing either 1.2% or 2.2% n-3 highly unsaturated fatty acids (HUFA). A further increase of dietary lipids had no effect on the growth enhancement. They also suggested that the highest growth rate was obtained at 15% dietary lipid together with 3.7 kcal g^{-1} diet of ME. However, the study showed that the growth decreased when the ME increased up to 4.1 kcal g^{-1} diet with 20% dietary lipid. Furthermore, it was suggested that the growth performance depended on the dietary level of n-3 HUFA and that the n-3 HUFA requirement increased with increasing dietary lipid content (Takeuchi *et al.*, 1991). It was also reported that a suitable level of n-3 HUFA was approximately 20% of dietary lipid regardless of lipid levels (Takeuchi *et al.*, 1992). Among n-3 HUFA, EPA and DHA are essential fatty acids (EFA) for marine species. Dietary EPA and DHA requirements for red sea-bream juveniles were 1% and 0.5%, respectively, and the efficiency of DHA as an EFA was about twice that of EPA for 3–12 g red sea bream (Takeuchi *et al.*, 1990).

Carbohydrates

Marine fish utilize carbohydrates poorly compared with freshwater fish, and red sea bream is no exception. It was reported that the growth of 70 g red sea bream was highest in fish fed the glucose-free diet and growth indices decreased with increased dietary glucose levels (Furuichi *et al.*, 1971). The authors suggested that poor performance of fish fed the diet containing high glucose resulted from the low digestibility of carbohydrate and protein and poor utilization of carbohydrate by red sea bream. Furuichi and Yone (1980) also indicated that, when 30 g red sea bream were fed diets containing different dextrin levels (0 to 40%), the following were found in fish fed more than 30% dietary dextrin: (i) poor growth and feed efficiency; (ii) high hepatic glycogen storage; (iii) low muscle and liver lipid contents; and (iv) decreased protein and dextrin absorption with increased dietary dextrin.

In the study on the utilization of carbohydrates, Furuichi and Yone (1982) reported that the utilization of α-starch was better than that of dextrin or glucose in terms of growth and feed efficiency when fish were fed twice daily. They suggested that the utilization of low-molecule carbohydrates could be improved by multiple feeding since carbohydrates that were consumed during the successive feedings were absorbed after the activity of metabolic enzymes increased.

Vitamins and minerals

The following ten water-soluble vitamins are probably essential for red sea bream, as for other fish species: vitamins B_1, B_2, B_6, B_{12} and C, nicotinic acid, pantothenic acid, folic acid, inositol and choline. Furthermore, although there are very limited studies on the essentiality and requirement for fat-soluble vitamins namely A, D, E and K, vitamin A is probably essential.

The requirement for vitamin C (ascorbic acid (AA)) for juvenile red sea bream was reported to be less than 200 mg AA kg^{-1} diet when ascorbic acid-calcium (Ca) was used in the study (Furuichi *et al.*, 1990). However, due to the unstable characteristics of AA, derivatives have been developed in Japan. L-Ascorbyl-2-phosphate magnesium (APM) has 43% the bioavailability of AA and was tested for red sea bream (Kosutarak *et al.*, 1994). Juveniles (5 g) were fed the diets containing 0, 50, 100, 200, 500 and 1000 mg APM kg^{-1} diet for 12–13 weeks. The fish fed the diet without APM showed poor growth and survival compared with those fed the diets containing APM. Among APM-fed groups, there were no significant differences in growth and survival. On the other hand, liver AA contents increased with dietary APM and the highest accumulation of AA was found in the 1000 mg APM-fed group. Furthermore, tolerance for a rising temperature was weaker in the APM-free group than in the APM-fed groups, but there was no difference in the tolerance among APM-fed groups. It is likely that at least 50 mg APM kg^{-1} diet (equivalent to 22 mg AA kg^{-1} diet) would be required for maintaining the optimal condition of red sea-bream juveniles. Appetite loss and growth retardation are typical vitamin deficiency signs. Thus,

Table 4.2. Weight gain of red sea bream at different periods when fed vitamin-sufficient and deficient diets (modified after Yone and Fuji, 1974).

	Weight gain (g)	
Diets	Day 63	Day 102
Sufficient vitamin mixture*	51	83
B_6-deficient	22	–
Choline chloride-deficient	17	–
Pantothenic acid-deficient	37	–
B_{12}-deficient	25	–
Inositol-deficient	31	–
Nicotinic acid-deficient	31	–
B_2-deficient	31	42
B_1-deficient	43	68
C-deficient	44	59
Biotin-deficient	41	63
Folic acid-deficient	47	73
ρ-Aminobenzoic acid-deficient	45	68

* Complete vitamin mixture contained the following (mg kg^{-1} diet): B_6, 40; choline chloride, 8000; pantothenic acid, 280; B_{12}, 0.09; inositol, 4000; nicotinic acid, 800; B_2, 200; B_1, 60; C, 2000; biotin, 6; folic acid, 15; ρ-aminobenzoic acid, 400.

decreased growth occurred when each vitamin was omitted from the complete vitamin mixture (Table 4.2) (Yone and Fujii, 1974).

Recently, we examined the effect of dietary AA on the quality of artificially produced juveniles using the behaviour index (Koshio *et al.*, 1997; Sakakura *et al.*, 1998; Koshio, 2001). Since red sea bream showed the behaviour called 'tilting', which is a behavioural response to stress or shock, we examined the relationship between dietary APM and tilting frequency and duration. The study demonstrated that the frequency and duration increased with increasing dietary AA. The highest peaks of these indices were found when fish were fed the diet containing 1000 mg kg^{-1} diet, but, with longer culture periods, the fish fed the diet with less than 200 mg kg^{-1} diet also showed higher frequency and longer duration compared with fish fed an AA-free diet. Since tilting behaviour can help the fish to avoid predation after restocking in the wild, we believe that, by controlling dietary vitamin C contents, it is possible to increase the survival rates and accelerate adaptation to a wild condition after restocking.

It is believed that marine fish absorb certain amounts of minerals, such as Ca, magnesium, phosphorus (P), sodium, sulphur and other minerals, from sea water to meet part of their requirements. Studies on P and Ca have been conducted on red sea bream. Dietary Ca levels did not affect the growth and body Ca or P contents, but dietary P levels did (Sakamoto and Yone, 1973). When red sea bream were fed the P-deficient diet, growth was reduced, the ash, Ca and P contents of vertebrae decreased and body lipid increased (Sakamoto and Yone, 1973;

Sakamoto and Yone, 1978b). Since the iron level in sea water and the absorption rate of iron by fish are very low, it is necessary to give a dietary supplement of iron. It was reported that the iron requirement of red sea bream is about 150 mg kg^{-1} diet (Sakamoto and Yone, 1978a).

Practical Diets

Due to complications, including labour, facilities and the high cost of live food production, the development of microdiets for larval and early juvenile stages of red sea bream has progressed in recent years. To establish the practical use of microbound diets (MBD) in mass seed production, we conducted a feeding trial using larval red sea bream (Kanazawa *et al.*, 1989). When various protein sources were combined to approximate the amino acid pattern of larval whole-body protein, using MBD together with a small amount of live food could sustain good growth and survival in 10-day-old larvae. Information on the composition of practical microdiets, crumbs and pellets for larvae and young-stage juveniles is presented in Table 4.3. Most diets for those stages have high dietary protein and energy.

Many kinds of grow-out diets for red sea bream have been developed in recent years. Compositions of commercial feeds are given in Table 4.4. Most grow-out diets contain less protein and energy compared with larval diets. Also, since red sea bream with the typical red colour have a good market value, special diets containing more carotenoids, vitamin E and vitamin C have also been developed.

There are some variations among diets used for brood-stock (Foscarini, 1988). In most cases, fresh or frozen trash fish, shrimp and squid together with compound diets were offered to spawners. However, it was found that certain nutrients affected the quality of eggs when the diets were given shortly before or during spawning. Nutrients such as protein, vitamins A and E, squid liver oil and krill oil have been reported to increase the numbers of buoyant eggs (Watanabe *et al.*, 1984a,b, 1985a,b), which have a higher hatching rate and more normal development than sinking eggs.

Feeding Practices

A typical pattern of larval and juvenile feeding schedules of red sea bream kept at 19°C is shown in Table 4.5. Formulated diets, together with live foods, are given to larval red sea bream 20 days after hatching. Table 4.6 indicates an example of a feeding schedule for juveniles (30–60 days after hatching) using formulated diets at 18–20°C.

In the grow-out stage there are two types of feeds, moist and dry feeds, which are commercially available for red sea bream in Japan. However, due to the insufficient supply and high cost of raw materials and concern about environmental issues and cost-effective operations, dry feeds will take over the market (Nakamura, 1997). One of the most important factors in aquaculture operation is

Table 4.3. Composition of commercial microdiets, crumbs and pellets for larvae and juveniles of red sea bream.

Composition	Diet A*	Diet B†	Diet C‡	Diet D§
Moisture (%)	< 10.0	< 10.0	< 10.0	–
Crude protein (%)	> 54.0	> 55.0	> 55.0	> 56.0
Crude fat (%)	> 10.0	> 10.0	> 10.0	> 9.0
Crude ash (%)	< 13.0	< 13.0	< 17.0	< 15.0
n-3 HUFA (%)	2.9	3.2	2.8	–
Vitamin C (mg %)	150	60.0	60.0	–
Gross energy (kcal g^{-1})	5.6	–	–	–
Amino acid (mg g^{-1} of wet diet)				
Arginine	28.8	36.4	33.7	–
Lysine	39.9	43.9	40.6	–
Histidine	14.1	13.6	12.8	–
Leucine	47.7	48.0	41.1	–
Isoleucine	23.8	24.3	23.2	–
Methionine	18.6	17.5	17.0	–
Valine	27.2	28.0	26.6	–
Threonine	22.9	24.7	23.8	–
Phenylalanine	25.5	27.5	25.6	–
Tryptophan	6.2	6.8	6.8	–

* < 250 or 250–400 μm particle size.
† 250–700 μm particle size.
‡ 710 to 4000 μm particle size (crumb or pellet).
§ < 360 or 400–2700 μm particle size.
Protein sources: fish-meal, krill meal, squid meal and soybean meal; carbohydrate sources: wheat and α-starch; lipid sources: fish-oils; other ingredients: wheat gluten, yeast extracts, minerals and vitamins.

Table 4.4. Composition (%) of commercial grow-out feeds for red sea bream (10–800 g).*

Composition	Diet A	Diet B	Diet C	Diet D
Crude protein	> 45 or 46	> 46 or 48	> 48	> 44
Crude fat	> 12 or 13	> 12 or 15	> 8	> 9
Crude ash	< 13 or 14	< 17	< 16	< 15
Crude fibre	< 3	< 4	< 3	< 2
Ca	–	> 2.2	> 2.3	> 2
P	–	1.4	1.6	> 1

* Protein sources: fish-meal, squid meal and krill meal; carbohydrate sources: wheat flour, α-starch; other ingredients: soybean meal, rice bran, wheat bran, fish lipid, yeast extract, minerals, vitamins and carotenoids.

understanding the environmental conditions fully. As a result, the feeding schedule should be adjusted accordingly. To determine the optimal ration size, the following are very important points which should be considered: rearing density, mesh size of the net cage, cleaning of the net, optimal water current in the cage and health condition of the fish.

The optimal ration size of dry feeds under the different water-temperature regimes is presented in Table 4.7. Red sea bream have a passive feeding behaviour and they ignore the feeds that sink to the bottom of the cage. Therefore, longer

Table 4.5. Typical pattern of a feeding schedule used for larvae and juveniles of red sea bream at 19°C.

	Days after hatching						
Feeds or diets	0	10	20	30	40	50	60
Nannochloropsis	3===============25						
Rotifers	3======================33						
Brine shrimp			20=============40				
Formulated feeds			20=============================60				

Table 4.6. Recommended feeding practices for red sea bream, 30 to 60 days after hatching.

	Age (days after hatch)			
	30	40	50	60
Body length (mm)	12	19	26	35
Body weight (BW) (g)	0.02	0.1	0.3	0.9
Ration size (% of BW)	15	13	11	9
Feed amounts (g per 10,000 fish)	30	130	320	810
Feeding frequency (per day)	5	7	8	7

Table 4.7. Ration size (% of body weight day^{-1}) at different water temperatures for red sea bream (modified after Nakamura, 1997).

Water temp. (°C)	Body weight (g) 5–20	20–50	50–100	100–250	250–500	500–750	750–1000	> 1000
13–14	1.5–2.0	0.9–1.5	0.6–0.9	0.6–0.7	0.5–0.6	0.4–0.5	0.3–0.5	0.3–0.4
15–16	2.4–3.0	1.2–2.1	1.0–2.0	0.8–1.7	0.7–1.5	0.6–1.0	0.5–0.7	0.4–0.6
17–18	2.9–4.1	2.3–3.1	1.5–2.4	1.7–2.2	1.0–1.8	0.8–1.3	0.6–1.1	0.4–0.9
19–20	3.9–4.5	3.1–3.9	2.7–3.2	1.9–2.9	1.3–2.2	0.9–1.5	0.7–1.5	0.7–1.4
20–21	4.4–5.3	3.3–4.0	2.9–3.4	2.0–3.4	1.4–2.5	1.1–1.7	0.8–1.6	0.7–1.5
22–23	4.8–6.0	3.9–5.3	3.1–4.2	2.3–4.2	1.5–2.6	1.2–1.9	0.9–1.7	0.8–1.6
24–25	5.3–6.5	4.1–5.6	3.4–4.9	2.5–4.6	1.7–2.7	1.3–2.0	1.0–1.8	0.8–1.7
26–27	5.8–7.0	4.4–6.0	3.7–5.3	3.0–4.8	1.9–2.9	1.4–2.2	1.1–1.9	0.9–1.8
28	5.6–6.8	4.2–5.8	3.5–5.1	2.8–4.6	1.7–2.7	1.3–2.0	0.9–1.8	0.8–1.7

feeding duration and higher feeding frequency are necessary. Furthermore, fish should not be fed in only one location because stronger fish can take over the feeding, resulting in growth reduction of weaker fish.

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