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Effects of body size and temperature on the relationship between growth and ration of young Japanese flounder, based on laboratory experiments

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Abstract To derive an empirical equation for the relationship between growth and ration of young Japanese flounder, *Paralichthys olivaceus*, the effects of fish size and temperature were studied. Juvenile flounder were reared at six ration levels using squid as food in the laboratory for 14 days. Feeding experiments were conducted for six different fish sizes (7.5-337g) at 20℃ and for 70g size at two more temperature conditions (15℃ and 26℃). Significant positive linear relationships were found between growth (g/day) and daily ration (kcal/day) for all size groups and for all temperature conditions. The net conversion efficiencies (b) and the maintenance rations (c) at 20℃ could be expressed as a power function of body weight (W) as follows: $b=0.678W^{-0.095}$ and $c=0.023W^{0.746}$. The maintenance rations (c) were correlated with the three temperatures, and the relationship was expressed as $c=0.153e^{0.054T}$. The relationship between growth rate and daily ration at any size and at any temperature is described by the following equation:

$$R=0.007(e^{0.054T})W^{0.75}+1.47W^{0.10}G \quad \text{kcal/day},$$

where R=daily ration (kcal/day), T=temperature (℃), W=body weight (g) and G=growth (g/day).

Key words: *Paralichthys olivaceus*, energy model, food requirement, growth rate

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The Japanese flounder, *Paralichthys olivaceus*, is an important commercial fish in Japan and a famous traditional fisheries resource in the Seto Inland Sea. Stocking of Japanese flounder in the Seto Inland Sea has been carried out since the late 1970's and the number of released fish has increased rapidly in recent years. Success of the stock enhancement by releasing fish depends strongly on survival rate from releasing to recruitment of the wild population. The major cause of mortality in released Japanese flounder is believed to be predation in the nursery grounds, and faster growing individuals have been found to have a higher survival rate (Yamashita *et al.*, 1994). These finding suggested that one of the important factors for the effective stocking enhancement was to determine of an appropriate releasing number corresponding to food abundance in a releasing area. As the first step to know the appropriate number of releasing fish into the releasing area, food requirement of young Japanese flounder should be known. Jones and Hislop (1978) and Jones (1978) examined the relationships between growth and food intake in gadoids (*Melanogrammus aeglefinus, Merlangius merlangus, Godus morhua,* and *Pollachius virens*) to estimate the food requirement using empirical equations derived

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from laboratory experiments.

As we need to estimate how much food is required by juvenile flounder in the nursery ground, such empirical equations as reported by Jones and Hislop (1978) are valid tools. To derive the empirical equation, following data were needed: 1) the effects of fish size on the growth-ration relationship, 2) the effects of temperature on the growth-ration relationship. Therefore, the present study was undertaken to determine the effects of size and temperature on the growth-ration relationship and to derive an empirical equation for the estimation of the food requirement of young Japanese flounder in the Seto Inland Sea.

Materials and Methods

Japanese flounder were obtained from a hatchery at Atadajima, Hiroshima prefecture, in the Seto Inland Sea, Japan in 1995. The fish were kept at an ambient temperature in large rectangular tanks with flowing filtered seawater and fed on artificial food once a day until the start of the experiments.

To examine the effects of size differences on growth-ration relationships, feeding experiments were carried out for six size groups (mean initial weight±SD, 7.48±1.3g, 16.1±1.9 g, 33.3±2.2g, 69.7±5.5g, 155±11g, 337±34g) at 20℃ (mean±SD, 20.0±0.2℃). And to examine the temperature effects on growth-ration relationships, similar feeding experiments were conducted under two more temperature condition at 15℃ (mean±SD, 15.0±0.1℃) and 26℃ (mean±SD, 26.0±0.2℃) for 70g size groups, 68.4±9.1g and 69.2±5.2g, respectively. In all experiments, six ration levels (0.0, 0.5, 2.0, 4.0, 6.0 and 8.0% body weight per day) were established. The numbers of fish used at one of the six ration levels were five individuals, and then thirty fish were used at an experiment. Squid fillets were used as food.

Before the experiments, thirty fish were transferred from the reservoir tank to experimental tanks and fed on squid fillets until satiation during 10days. Each tank was divided vertically by plastic mesh partitions into several compartments where fish were placed individually. Tanks and compartment sizes were changed depending on fish size (Table 1).

The bottom of each tank was covered by a 5cm layer of sand through a 1mm mesh. The water was circulated continually through an external reservoir and filter bed and the flow rate was controlled at 8 changes per day. The water temperature was controlled in a reservoir tank using a heating-cooling unit.

At the start of the experiment, the fish were weighed while they were anesthetized with 3-aminobenzonic acid ethyl ester methanesulfonate salt (SIGMA Co. Ltd.) solution (80ppm). Fish were then placed in the compartments individually and were fed squid fillets at one of six ration levels. The squid mantles were cut into small fillets and weighed before they were fed to fish. Fish were given squid fillets once a day in the morning. Food that was not consumed

Table 1. The size of the aquaria and compartments used for the feeding experiments of young Japanese flounder

Fish size (g)	Aquaria* (size×number)	Size of compartment (width×length×depth,cm)
7.48	S×2	17×13×50
16.1	L×1	24×16×90
33.3	S×1	27×20×50
	L×1	27×20×90
69.8	L×2	31×28×90
155	L×3	40×28×90
337	L×3	40×40×90

*:Size of aquaria , S=90cm×60cm×50cm, L=180cm×90cm×90cm.

was removed from the tank in the late afternoon and weighed. The amount of food at each ration was determined at the start of the experiment based on the mean weight of all fish. Each feeding experiment lasted 14 days. At the end of each experiment, the fish were anesthetized as described above and weighed.

Daily specific growth rate was estimated by the following equation:

$$g_i = (\ln W_t - \ln W_0)/t,$$

$$G_s = (e^{g_i} - 1) \times 100,$$

where g_i=instantaneous growth rate, G_s=daily specific growth rate (% body weight), W_t=body weight at the end of the experiment, W_0=body weight at the beginning of experiment, and t=duration of the experiment (always 14 days). Since the ration need to be measured accurately, actual ration was also calculated from the mean daily food intake divided by weight at the half-way point of the experiments (day 8) using the following equation:

$$R_A = F_T/t\ /[W_0 e^{(g_i \times t_m)}] \times 100,$$

where R_A is the actual ration in percent body weight per day, F_T is the total food intake during the duration of the experiments, t is the duration of the experiment (14 days for here), W_0 is the body weight at the beginning of the experiments, g_i is the instantaneous growth rate and t_m is the mid-point of the duration of the experiments (day 8 here). Growth rate in g/day (G_g) and ration in kcal/day (R_{kcal}) were calculated as follows;

$$G_g = (W_t - W_0)/t,$$

$$R_{kcal} = F_T/t \times C_v$$

where W_t=body weight at the end of the experiment, W_0=body weight at the beginning of experiment, and t=duration of the experiment (always 14 days), F_T is the total food intake during the duration of the experiments, C_v was a mean caloric value of squid fillets. The caloric contents of the squid fillets used for food were measured with a bomb-caloric meter (Shimadzu CA-4P; Shimadzu Co. Ltd.), and the calculated mean was 1.16±0.1 kcal/g wet weight (mean ±SD, n=3).

Results

Effects of size differences Positive linear relationships between daily specific growth rates (%/day) and daily actual ration (%/day) were found in all size groups (Fig. 1).

Weight losses occurred in all fish reared at lower ration levels. The actual ration at which the fish attained maximum growth tended to decrease with increasing of fish size. Highest growth rate of 4.25%/day was obtained in 7.5g size and lowest one was 2.15%/day in 337g size. In order to standardize the measurement of ration (%/day) in terms of a common energy unit, regression analysis between the growth in g/day (G_g) and the ration in kcal/day (R_{kcal}) were carried out in accordance with Jones and Hislop (1978). Table 2 shows the results of least-squares regressions for G_g against R_{kcal} from the feeding experiments. In all cases, the relationship between daily growth in g/day and daily ration in kcal/day was fitted a linear model significantly and the equation is described as follows:

$$G_g = a + b\ R_{kcal}\ . \qquad (1)$$

If growth is zero, the equation is rewritten as follows:

$$R_{kcal} = -a/b, \qquad (2)$$

The right side (-a/b) of the equation (2) corresponds to the point where the regression line cross the food axis. If -a/b=c, the c is equivalent to daily maintenance requirements of food.

Equation (1) may also be written in terms of b and c (=-a/b) in the forms:

$$G_g = b(R_{kcal} - c), \qquad (3)$$

and then, $$b = G_g\ /(R_{kcal} - c), \qquad (4)$$

ie., b=growth/(total food-food for maintenance). Therefore the slop of regression (b) provide estimates of net efficiencies of conversion of food into growth.

Constants (a) of the regression analyses shown in Table 2 represent the rates of weight losses when the fish were not fed; values ranged from -0.053 to -0.896 (g/day). The slope values (b) in Table 2, which correspond to the net conversion efficiency, tended to be higher for smaller fish than for larger ones; values ranged from 0.536 to 0.376 (g/kcal). Since the relationship between net conversion efficiency and body

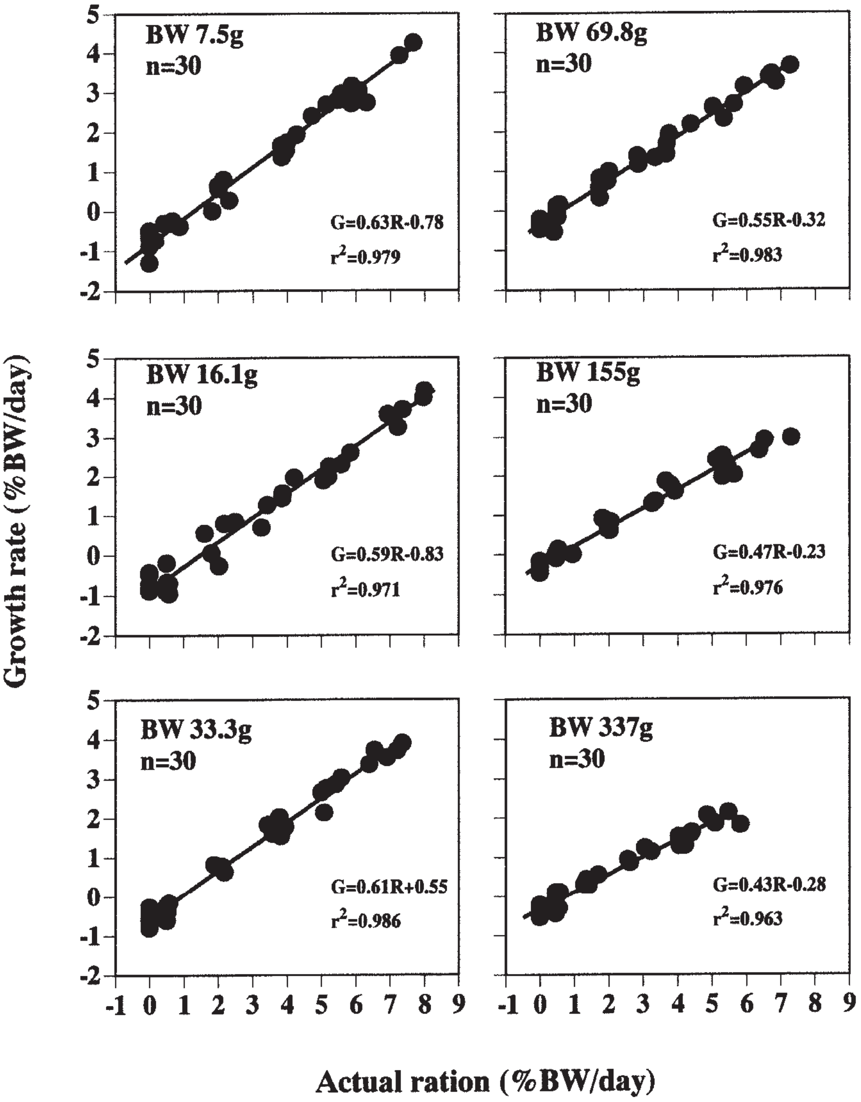

Fig. 1. Relationships between growth rates (G:%/day) and food consumption (R:%/day) for different sizes of Japanese flounder fed on squid fillets at 20℃. BW=body weight, n=number of fish.

Table 2. Slops and intercepts of regressions of growth on food consumption calculated from regression equationsof the form $G_g = a + bR_{kcal}$, where G_g = growth(g/day) and R_{kcal} = food consumption(Kcal/day)

Size (g)	N	Constant[*1] (a)	Slope[*2] (b)	Intercept on food axis[*3] (c)	Correction coefficient	p
7.48	30	-0.053	0.536	0.099	0.992	$p<0.0001$
16.1	30	-0.126	0.507	0.249	0.986	$p<0.0001$
33.3	30	-0.167	0.527	0.317	0.994	$p<0.0001$
69.7	30	-0.207	0.477	0.434	0.982	$p<0.0001$
155	30	-0.327	0.411	0.796	0.989	$p<0.0001$
337	30	-0.896	0.376	2.383	0.982	$p<0.0001$

[*1]:(a) represets the rate of weight loss when the fish is not fed.
[*2]:(b) is equivalent to the net efficiency of conversion of food into growth.
[*3]:(c) is equivalent to the food required to maintain a fish with zero growth rate.

weight can be expressed as a power function (Jones and Hislop, 1978), the values of b in Table 2 were plotted against mean initial body weight (Fig. 2-A). Both variates were plotted on logarithmic scales. The coefficient (b) tended to decrease with increasing body weight, and is described in the following equation:

$b=0.678W^{-0.095}$ (g/kcal) ($r^2=0.869$),

where b=net conversion efficiency, W=body weight.

Maintenance rations were shown in Table 2 as the intercept on the daily ration axis (c). These rations increased with size, ranging from 0.099 kcal/day for fish of 7.48g to 2.38kcal/day for fish of 337g. Since the maintenance ration is a power function of body weight (Jones and Hislop, 1978), the maintenance rations (c) were plotted against mean initial body weight on a logarithmic scale (Fig. 2-B). The resulting equation is given as follows:

$c=0.023W^{0.746}$ (kcal/day) ($r^2=0.952$),

where c=the maintenance ration, and W=body weight.

Effects of temperature Since maintenance rations increase with increasing temperature in various fish species, two feeding experiments at different temperatures (26℃ and 15℃) were conducted with fish weighing approximately 70g. Growth rates (g/day) were linearly related to ration levels (kcal/day) for fish reared at 15 and 26℃. Results of the regression analysis are shown in Table 3 together with the results of the experiment at 20℃ for the same 70g sized fish group. Maintenance rations, determined from the intercept of the daily ration axis (c), ranged from 0.349kcal/day to 0.626 kcal/day and increased with increasing temperature. The c value, which was converted into a natural logarithm, was linearly related to temperature (Fig. 3). The following equation describes the maintenance ration (c: kcal/day) as a function of water temperature (T=℃):

$c=0.153e^{0.054T}$ ($r^2=0.992$).

General equation for growth and ration According to Jones and Hislop (1978), the relationship between growth and ration at a constant temperature can be expressed as the following empirical equation:

$R=c+G/b$ (5),

where R=daily ration (kcal/day), G=growth (g/day), c=maintenance energy (kcal/day) and b=net conversion efficiency. Since c and b are both functions of body weight, they must be described as functional forms of body weight. Considering the previously calculated functional forms for maintenance energy (c) and conversion efficiency (b), equation (5) can be rewritten as follows:

$R=0.023W^{0.75}+G/0.68W^{-0.10}$ (6),

where W=body weight.

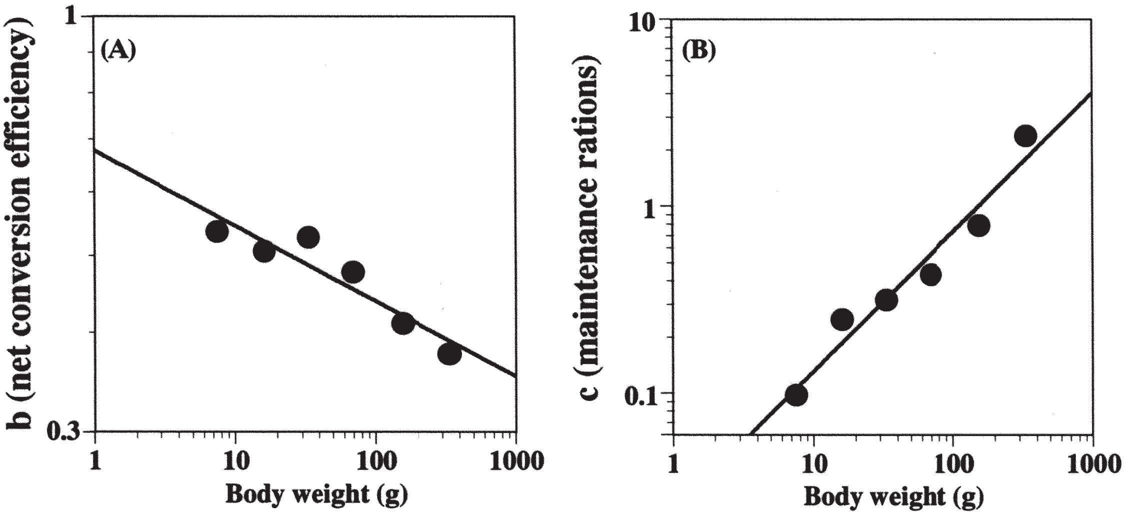

Fig. 2. Relationships between (A) the net conversion efficiency and (B) the maintenance rations, and body weight at 20℃.

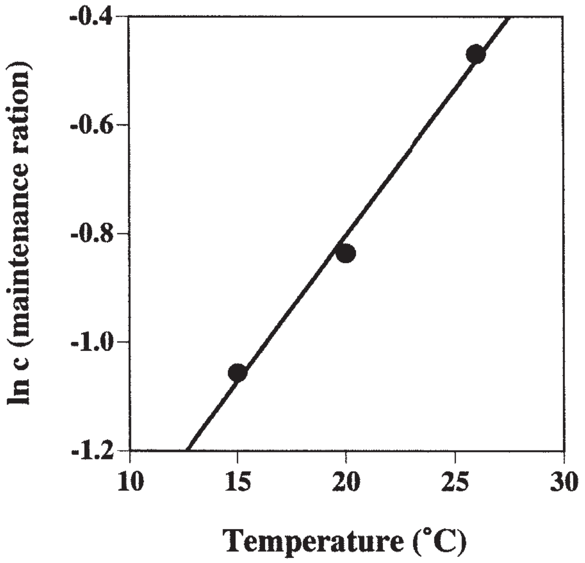

Fig. 3. Relationships between the maintenance rations and the water temperature

Maintenance energy (c) was found to be a function of temperature, with c expressed as an exponential model ($c=0.153e^{0.054T}$). Since most experiments were conducted at 20℃ in this study, temperature correction terms were based on measurements at 20℃. To convert to any temperature T℃, the temperature correction term of the maintenance energy could be written as:

$$0.023\ [e^{0.054(T-20)}]W^{0.75}.$$

The general equation derived from equation (6) with the temperature correction terms is:

$$R=0.023[e^{0.054(T-20)}]W^{0.75}+G/(0.68W^{-0.10}).$$

Therefore,

$$R=0.007(e^{0.054T})W^{0.75}+1.47W^{0.10}G \quad \text{kcal/day}, \quad (7)$$

where R=daily ration (kcal/day), T=temperature (℃), W=body weight (g) and G=growth (g/day).

Discussion

The growth rates at 20℃ obtained at maximum actual ration of Japanese flounder in this study ranged between 2.2 to 4.3 percent body weight per day. Iwata et al. (1994) reported that the daily growth rates of similar size fish groups (4g-176g: four size groups) at 20℃ of Japanese flounder fed on commercial pellets until satiation were from 1.4 to 5.4 percent per day. Koshiishi and Yasunaga (1980) also obtained the growth rates of Japanese flounder ranging from 1.6% to 4.2% in 8.8g to 88.2g body size groups. These values reported previously were very similar to our results.

In order to test a general equation derived in this study for its validity, we calculated total calories using the equation with the data reported by Iwata et al. (1994) at 20℃ (Table 4). Initial body weights and terminal body weights were described for 20 days feeding experiments with four size groups (4g, 16g, 88g and 176g). The growth rates were recalculated using the equations in this study. Total food consumed by fish for 20 days were calculated from the values reading on the figure of Iwata's paper and using the equation for the daily feeding rate in that study. The caloric values of the total food consumed by the fish in that study were calculated from the data on a catalog of the commercial pellet producing company (Higashimaru Foods inc.). The caloric value of commercial pellets is 3.4kcal/g. Estimated total calories using our general equation calculated as follows:

Table 3. Slopes and intercepts of growth on food consumption calculated from regression equalations determined from three different temperature experiments

Water temperature(℃)	Size (g)	N	Constant^{*1} (a)	Slope^{*2} (b)	Intercept on food axis^{*3} (c)	Correction coefficient	p
26	69.2	30	-0.264	0.422	0.626	0.992	p<0.0001
20	69.7	30	-0.207	0.477	0.434	0.992	p<0.0001
15	68.4	30	-0.182	0.522	0.349	0.980	p<0.0001

^{*1}:(a) represets the rate of weight loss when the fish is not fed.
^{*2}:(b) is equivalent to the net efficiency of conversion of food into growth.
^{*3}:(c) is equivalent to the food required to maintain a fish with zero growth rate.

Table 4. Testing of the general equation derived in this study for its validity using data reported by Iwata *et al.*(1994)

Size (g)	Initial body weight[*1] (g)	Terminal body weight[*1] (g)	Growth rates[*2] (%/day)	Growth rates[*2] (g/day)	Daily feeding rates[*3] (%)	Total food consumed[*4] (g)	Caluculated caiories[*5] (kcal)	Estimated calories using the equation in this study (kcal)
16	17.3	39.3	4.2	1.1	2.6	14.7	50.0	49.5
88	88.5	119.7	1.5	1.6	1.1	22.9	77.9	86.1
176	182	239	1.4	2.9	1.0	46.3	157.4	165.4

[*1]:Initial and final weight were cited from Iwata *et al.* (1994). Original data were mean of 10 to 20 individuals.

[*2]:Growth rates were recalculated using equations in this study

[*3]:Daily feeding rate was reading value from the Fig. 2 of the Iwata *et al.* (1994)

[*4]:Total food in each size group was calculated from the the following equation described in the text of Iwata *et al.* (1994)
Total food consumed=Daily feeding rate×t×$(w_t+w_0)/2\times 1/100$ were w_t=terminal body weight, w_0=initial body weight, t=duration of experiment.

[*5]:Calories were caiculated as follows: Calories=Total food consumed×3.4
where 3.4 was a caloric value of commercial pelleted diet for flounder (kcal/g) described on a cataiog of Higashimaru Food inc..

$$\text{Total calories}=\Sigma\, 0.007(e^{0.054T})[W_0(1+G_s/100)^n]^{0.75} + 1.47[W_0(1+G_s/100)^n]^{0.10}G_g, \quad (8)$$

where T=water temperature (20℃ is here), W_0=initial body weight (g), G_s=growth rate (%/day), G_g=growth rate (g/day), n=n_{th} day. The results of the total calories for three size groups (16g, 88g and 176g) are showed in Table 4. The estimated total caloric values calculated from our general equation were slightly higher with the exception of 16g size group than the calculated values from the Iwata's data. The differences between them were within 10%. Therefore the equation derived from this study was thought to be applicable to field estimation.

Growth and life history of wild Japanese flounder in the Seto Inland Sea has been investigated previously by Maehara (1992), who reported that larval flounder settled on sandy beaches in late May or early June, grew to about 100mm total length (TL) by early August, and reached about 250mm TL by early December. As weight-length and length-day relationships have been reported (Maehara, 1992), a preliminary estimation of the food requirements of wild fish in the Seto Inland Sea is possible. According to the length-day and weight length relationships determined by Maehara (1992), the mean length and weight of young flounder on August 1 are 98mm and 7.2g, respectively. After 40 days the fish grow to 144 mm and 24.7g. Therefore, the estimated daily specific growth rate during this period is 3.13% and daily growth in g is 0.44g/day. Substituting 3.13%/day for G_s, 0.44g/day for G_g, 7.2g for W_0 and 23.3℃, which is the mean temperature during August to September in the western part of the Seto Inland Sea (M. Fukuda: Natl. Res. Inst. Fish. Sci., unpublished data), for T in equation (8) of this study, the estimated value of the daily ration in kcal for young Japanese flounder is a total of 40.3kcal for 40 days. Converting this total caloric value to the weight of squid meat (1.16kcal/g), total food consumption reached 34.8g. The growth efficiency calculated from increment of body weight (24.7g−7.2g=17.5g) and total food intake (34.8g) of these population is 0.50. Since water content of squid fillets used in this study was 78%, total food intake in dry weight basis was equivalent to 7.7g. Therefore the growth efficiency in wet weight of fish per dry weight of food was 2.3. Koshiishi and Yasunaga (1980) reported that the food conversion efficiency (wet/dry basis) of Japanese flounder in 8.8g to 88g size group ranged from 0.92 to 1.24 in rearing experiments. The value of 2.3 was about two times higher than these values. Seikai *et al.* (1997) showed food conversion efficiencies were always higher in Japanese flounder juveniles fed on live mysids than fed on formula food under rearing conditions. The authors obtained the maximum efficiency of 3.00 (wet/dry basis) at 22℃ in 250mg size fish and mentioned live misids are high quality as food for juvenile flounder. This implies the food conversion efficiencies obtained from field caught fish might be higher than rearing fish. One of the another reasons caused by high conversion efficiency is that the equation estimated in the present study did not consider the energy required for swimming. Though juvenile flounder does not seem to be active swimmers like pelagic fish, they need energy for feeding and escape from predator. Thus, our estimates of daily ration are probably lower than the actual value. Further studies might be needed to estimate the ration levels of field caught Japanese flounder using such an empirical equation.

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References

Iwata N., Kikuchi K., Honda H., Kiyono M., and Kurokuwa H., 1994: Effects of temperature on the growth of Japanese flounder. *Fish. Sci.*, **60**, 527-531.

Jones R., 1978: Estimates of the food consumption of haddock (*Melanogrammus aeglefinus*) and cod (*Gadus morhua*). *J. Cons. int. Explor. Mer.,* **38**, 18-27.

Jones R. and Hislop J.R.G., 1978: Further observations on the relation between food intake and growth of gadoids in captivity. *J. Cons. int. Explor. Mer.,* **38**, 244-251.

Koshiishi Y. and Yasunaga Y., 1980: Some aspects of feeding characteristics of juvenile plaice, *Paralichtys olivaceus. Bull. Jap. Sea Reg. Fish. Lab.,* (**31**), 33-40.

Maehara T., 1992: Studies of age and growth of Japanese flounder, *Paralichthys olivaceus*, in Ehime prefectural area of Seto Inland Sea. *Bull. Ehime Pref. Fish. Exp. St.,* **5**, 13-29.

Seikai T., Takeuchi T., and Park G.W., 1997: Comparison of growth, feed efficiency, and chemical composition of Juvenile flounder fed live mysids and formura feed under laboratory condition. *Fish. Sci.,* **63**, 520-526.

Yamashita Y., Nagahora S., Yamada H., and Kitagawa D., 1994: Effects of release size on survival and growth of Japanese flounder *Paralichthys olivaceus* in coastal waters off Iwate prefecture, northeastern Japan. *Mar. Ecol. Prog. Ser.,* **105**, 269-276.