Effects of Temperature on the Growth of Japanese Flounder

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Rearing experiments were carried out to examine the effects of temperature on the growth of Japanese flounder *Paralichthys olivaceus* at 10, 15, 20, 25, and 30°C with fish of about 4, 16, 88, and 176 g initial body weight. Fish were fed to satiation twice a day with a commercial pelleted diet for 20 days.

In all body weight groups, the daily growth rates increased with temperature up to 20 or 25°C, though there was little difference in the rates between these temperatures. However, the rates decreased at 30°C. The daily feeding rates also increased with temperature up to 25°C, while the feed conversion efficiency decreased above 20°C. Therefore, the optimum temperature for the growth of Japanese flounder of the sizes tested is probably between 20 and 25°C.

The daily growth rate at 30°C decreased more in larger flounder (88 and 176 g) than in smaller ones (4 and 16 g), and the higher catabolic rate at 30°C for larger flounder appeared in the relationship between feeding rate and growth rate. Therefore, the high energy expenditure for catabolism appears to reduce the energy available for growth of larger flounder at 30°C.

Key words: Japanese flounder, temperature, daily growth rate, daily feeding rate, feed conversion efficiency, catabolic rate, energy budget

Temperature is one of the most influential environmental factors affecting the growth of fish.¹⁾ Therefore, information on the effects of temperature on the growth of fish is necessary to improve fish culture techniques. Japanese flounder *Paralichthys olivaceus* is one of the most important finfish species for mariculture in Japan. However, little effort has been made to elucidate the effects of temperature on its growth.^{2–5)} One obstacle hindering research is the difficulty of maintaining test temperatures at constant levels during experimental periods. Recently, a closed recirculating culture system for flounder has been developed which can control temperature.⁶⁾

In this study, rearing experiments were conducted utilizing the closed system with flounder of about 4, 16, 88, and 176 g initial body weight to examine the effects of temperature on their growth.

Materials and Methods

Fish

Fish of 1 to 2 g body weight were transported to our laboratory in Chiba Prefecture from the three different hatcheries shown in Table 1. They were reared in recirculating seawater aquaria of 2,000 l volume at about 20°C with a commercial pelleted diet until the start of the experiments.

Rearing Experiment

Rearing experiments were carried out with five recirculating seawater aquaria placed in an experimental room under cycles of 12 h of light and 12 h of dark with fluorescent lights. Each aquarium consisted of a fish tank, a biological filter unit, a heating-cooling unit, and an ultraviolet radiator. Total water volume in the system was about 270 l, and the water volume in the fish tank was about 180 l. The light intensity was about 800 lux at the water surface of the fish tank. The numbers of fish used in each experiment and their initial body weights are shown in Table 2. Before starting the experiments, the fish were transferred into a net cage placed in the fish tank. In order to acclimate the fish to the culture environment,

they were reared at 20°C until almost all of the fish showed active feeding behavior. These periods were 1 week for the 4 and 16 g fish groups, 4 weeks for the 88 g fish group, and 5 weeks for the 176 g fish group. The water temperature of each aquarium was then gradually changed to 10, 15, 20, 25, or 30°C over a 5 day period. These temperatures were controlled within $\pm 1^\circ\text{C}$ during the experimental period. In the experiments, fish were fed a commercial pelleted diet for flounder (Higashimaru Foods Inc.) until satiation twice a day for 20 days. The diet contained about 60% crude protein, 9% crude lipid, and 14% crude ash on a dry basis. In order to estimate the amount of diet consumed by the fish in the 4 and 16 g groups, the number of pellets not consumed by the fish were counted after each feeding. This was multiplied by the average pellet weight, and the result was subtracted from the weight of pellet supplied. The fish in the 88 and 176 g groups were tagged with colored plastic beads to identify individual fish, and the weight of consumed diet was estimated from the number of pellets consumed by each fish and the average pellet weight.

The daily growth rate, the daily feeding rate, and the feed conversion efficiency (gross) were calculated as follows:

Table 1. The source hatcheries of Japanese flounder used in this study

Body weight group	Hatchery
4 g	Mano-Machi Fisheries Cooperative Association (Niigata Pref.)
16 g	Uchiumi Fisheries Biotech. R & D Center Nippon Formula Feed Manufacturing Co., Ltd. (Ehime Pref.)
88 g	Mano-Machi Fisheries Cooperative Association (Niigata Pref.)
176 g	Shizuoka Prefectural Thermal Effluent Utilization Research Center (Shizuoka Pref.)

Table 2. Initial and terminal body weights, and survival rates of Japanese flounder

Body weight group	Test temp. (°C)	Initial number of fish	Initial body weight (g)	Terminal body weight (g)	Survival rate (%)
4 g	10	30	4.0 ± 0.7	5.7 ± 1.3	93
	15	30	4.1 ± 1.1	8.8 ± 2.5	70
	20	30	4.8 ± 1.0	16.2 ± 4.8	63
	25	30	4.6 ± 1.0	17.0 ± 5.8	90
	30	30	4.7 ± 1.1	12.4 ± 4.7	100
16 g	10	25	14.3 ± 0.8	18.4 ± 1.8	100
	15	25	14.9 ± 1.3	27.9 ± 3.4	100
	20	25	17.3 ± 1.2	39.3 ± 4.2	100
	25	25	17.0 ± 1.3	37.1 ± 6.7	100
	30	25	16.1 ± 1.2	29.3 ± 3.4	100
88 g	10	20	90.7 ± 25.5	94.1 ± 26.7	100
	15	20	89.3 ± 26.6	115.1 ± 34.8	100
	20	19	88.5 ± 29.0	119.7 ± 36.6	100
	25	19	88.1 ± 20.3	115.2 ± 22.2	100
	30	20	83.4 ± 23.0	87.9 ± 21.6	100
176 g	10	10	173 ± 33	184 ± 35	100
	15	10	175 ± 25	224 ± 32	100
	20	10	182 ± 40	239 ± 52	100
	25	10	179 ± 30	251 ± 42	100
	30	10	173 ± 33	184 ± 30	100

Initial and terminal body weights were expressed as means ± standard deviations.

$$\text{Daily growth rate (\%)} = \frac{W_t - W_0}{t \times (W_t + W_0)/2} \times 100$$

$$\text{Daily feeding rate (\%)} = \frac{\sum f_k}{t \times (W_t + W_0)/2} \times 100$$

$$\text{Feed conversion efficiency (\%)} = \frac{\text{Daily growth rate}}{\text{Daily feeding rate}} \times 100$$

t : feeding days (day)

W_0 : initial fresh weight of fish (g)

W_t : terminal fresh weight of fish (g)

f_k : dry weight of diet consumed by fish at each feeding (g)

Body weights were measured at the beginning and at the end of experiments, after the fish were starved for about 36 h in the 4 g group and for about 60 h in the other groups.

After the feeding experiment, the fish from the 176 g group were reared under experimental temperatures without feeding for 8 days to examine the effect of temperature on the decrease in body weight. The daily decrease rate in body weight was calculated in the same way as the daily growth rate.

Oxygen Consumption Rate

The oxygen consumption rate of flounder was examined at 10, 20, and 30°C with fish 145 to 325 g in body weight. The origin of the fish was the same as the 176 g group, and fish were reared for more than 40 days at each temperature. Oxygen consumption was measured by the constant flow method⁷⁾ with 3 fish at 10 and 20°C, and with 4 fish at 30°C. Oxygen consumption rate was continuously monitored for 96 h, and the value at the lowest level was adopted as the standard metabolism of each fish.

Results

Initial and terminal body weights, and survival rate are shown in Table 2. At every temperature tested, the mean body weights of all weight groups increased during the experimental period. Some fish from the 4 g group died from scuticociliatosis,⁸⁾ while the survival rate of the other weight groups was 100%.

The daily growth rate is shown in Fig. 1. The growth rate of the 4 g group increased with temperature from 10 to 25°C with the highest rate of 5.8% at 25°C, and decreased

Fig. 1. Daily growth rate of Japanese flounder at different temperatures.

Data represent means and standard deviations of individual rates for the 88 and 176 g groups. Fish were fed a commercial diet to satiation twice a day for 20 days.

slightly at 30°C. For the 16 g group, the highest daily growth rate of 3.9% was obtained at 20°C, and the growth rate showed a slight decline at 25 and 30°C. In the 88 g group, the daily growth rate at 20°C (1.5%) was significantly higher than those at 10 and 15°C (Mann-Whitney test, $p < 0.05$). However, there was no significant difference in the rate between 20 and 25°C. In contrast to the results for smaller fish (4 and 16 g groups), a much lower growth rate (0.3%) was observed at 30°C than at 20 and 25°C for the 88 g group ($p < 0.05$). The daily growth rate for the 176 g group at 25°C (1.7%) was significantly higher than those at the other temperatures ($p < 0.05$). As observed for the 88 g group, the growth rate of the 176 g group at 30°C (0.3%) dropped sharply above 25°C.

The daily feeding rates are shown in Fig. 2. For all weight groups, the rate increased with temperature up to 25°C and decreased at 30°C.

Feed conversion efficiency is shown in Fig. 3. The efficiency of the 4 and 16 g groups increased with temperature up to 20°C then decreased linearly. However, for the 88 g group, the highest efficiency was obtained at 15°C and decreased at higher temperatures. The efficiency of the 88 g group at 10 and 30°C was significantly lower than at other temperatures ($p < 0.05$). The efficiency of the 176 g group tended to decrease gradually with temperature

Fig. 2. Daily feeding rate of Japanese flounder at different temperatures. Details are shown in the footnote of Fig. 1.

from 10 to 25°C, and was much lower at 30°C.

The daily decrease rate for body weight of the 176 g group during starvation is shown in Fig. 4. The rate was almost constant from 10 to 25°C, but was much higher at 30°C than at the lower temperatures ($p < 0.05$).

The oxygen consumption rates of flounder from 145 to 325 g at 10, 20, and 30°C are shown in Fig. 5. At each temperature, no obvious relationship was observed between the rate and the weight of fish examined. The rate at 30°C was markedly higher than at other temperatures ($p < 0.05$), while no significance was observed in the rate between 10 and 20°C.

Discussion

The daily growth rate of Japanese flounder reared under laboratory conditions at about 20°C has been reported to be 2.5 and 1.6% for 16 and 88 g fish,⁹⁾ and 2.2 and 1.4% for 15 and 84 g fish, respectively.¹⁰⁾ The rates obtained at 20°C in this study with fish of corresponding size (3.9% for the 16 g group, and 1.5% for the 88 g group) were similar to those reported previously.

For all weight groups, the daily growth rate increased with temperature up to 20 or 25°C, and there was little difference in the rates between these temperatures. However, the rate decreased at 30°C. The daily feeding rate also increased with temperature up to 25°C, while the feed

Fig. 3. Feed conversion efficiency of Japanese flounder at different temperatures. Details are shown in the footnote of Fig. 1.

conversion efficiency decreased above 20°C. Therefore, it can be concluded that the optimum temperature for the growth of Japanese flounder of the sizes tested is between 20 and 25°C. This was similar to the results of another study⁴⁾ which observed the highest daily growth rate at 21°C with flounder of about 220 g.

In this study, the optimum temperature for growth of the flounder did not appear to be affected by the size of fish tested. However, the daily growth rate and feed conversion efficiency at the highest temperature of 30°C decreased more in the larger flounder (88 and 176 g) than in the smaller ones (4 and 16 g). For commercial flounder farms, reduction in growth rate during the summer season is often observed especially in larger fish, causing problems for flounder culture. The results of this study may have demonstrated this phenomenon experimentally.

The difference in the effects of high temperature on growth rate between smaller fish and larger fish is considered to be caused by the difference in energy budget. The energy budget in fish is generally described by the following equation¹¹⁾:

$$I_e = A + C + F, \quad (1)$$

where I_e is energy ingested, A is anabolism, C is catabolism, and F is feces. As the digestibility of the energy ingested (d) can be described as $d = (I_e - F)/I_e = (A + C)/I_e$, A is

Fig. 4. Daily decrease rate in body weight of Japanese flounder during starvation at different temperatures.

Data represent means and standard deviations. Ten fish were used and were starved for 8 days at each temperature. Initial body weights of fish examined were 204 ± 39 g (mean $\pm$ S.D.) at 10°C, 245 ± 39 g at 15°C, 297 ± 67 g at 20°C, 342 ± 57 g at 25°C, and 201 ± 28 g at 30°C.

Fig. 5. Oxygen consumption rate of Japanese flounder at different temperatures.

Data represent means and standard deviations. Body weights of fish examined were 247 ± 16 g (mean $\pm$ S.D., $n=3$) at 10°C, 289 ± 37 g ($n=3$) at 20°C, and 171 ± 29 g ($n=4$) at 30°C.

expressed as follows:

$$A = d \times I_e - C. \quad (2)$$

A and I_e can be described as $A = k_1 \times G$ and $I_e = k_2 \times I$, where k_1 is energy content per unit weight of fish, G is weight increment in the fish, k_2 is energy content per unit weight of feed, and I is weight of feed ingested. Therefore, the

Fig. 6. Relationship between daily feeding rate and daily growth rate of Japanese flounder at different temperatures for the 88 g group.

Fig. 7. Relationship between daily feeding rate and daily growth rate of Japanese flounder at different temperatures for the 176 g group.

following equations can be derived:

$$k_1 G = d \times k_2 I - C, \\ G = d \times (k_2/k_1) \times I - (1/k_1) \times C. \quad (3)$$

From Eq. (3),

$$g = d \times (k_2/k_1) \times f - (1/k_1) \times c \quad (4)$$

where g is daily growth rate, f is daily feeding rate, and c is catabolic rate per day. From Eq. (4), the lower g of larger fish at 30°C may be caused by lower d or f , or higher c .

The daily growth rates of the 88 and 176 g groups are plotted against their feeding rates in Figs. 6 and 7. The relationships between feeding rate and growth rate can be expressed by the following equations:

88 g group

$$\begin{aligned} 10^\circ\text{C}: g &= 1.34f - 0.20 \quad (r^2 = 0.55) \\ 15^\circ\text{C}: g &= 1.57f - 0.08 \quad (r^2 = 0.82) \\ 20^\circ\text{C}: g &= 1.33f - 0.02 \quad (r^2 = 0.90) \\ 25^\circ\text{C}: g &= 1.52f - 0.60 \quad (r^2 = 0.87) \\ 30^\circ\text{C}: g &= 1.41f - 1.21 \quad (r^2 = 0.68) \end{aligned}$$

176 g group

$$\begin{aligned} 10^\circ\text{C}: g &= 1.36f + 0.01 \quad (r^2 = 0.48) \\ 15^\circ\text{C}: g &= 1.54f - 0.15 \quad (r^2 = 0.96) \\ 20^\circ\text{C}: g &= 1.61f - 0.32 \quad (r^2 = 0.82) \end{aligned}$$

$$25^{\circ}\text{C}: g = 1.31f - 0.07 \quad (r^2 = 0.84)$$

$$30^{\circ}\text{C}: g = 1.47f - 0.80 \quad (r^2 = 0.91)$$

In both weight groups, the slopes of the regression line were not significantly different for the temperatures tested (*F*-test). Therefore, it is likely that the digestibilities of the flounder of the 88 and 176 g groups were not greatly affected by temperature between 10 to 30°C. Constant digestibilities independent of temperature have also been reported with black rockfish *Sebastes inermis*,¹²⁾ jack mackerel *Trachurus japonicus*,¹³⁾ filefish *Navodon modestus*,¹⁴⁾ and walleye pollock *Theragra chalcogramma*.¹⁵⁾

For the daily feeding rate of larger fish, there was little difference between 15 or 20°C and 30°C. Comparing the growth rate at the same feeding level by the regression lines of Figs. 6 and 7, the growth rate at 30°C is still much lower than the rates at other temperatures. Therefore, the difference in feeding rate is probably not the major cause of the low growth rate in larger fish at 30°C.

On the other hand, for the regression lines between the daily growth rate and the daily feeding rate of larger fish, the intercepts on the *Y*-axis at 30°C were significantly lower than at other temperatures ($p < 0.05$). Therefore, the catabolic rate of larger fish appears to be definitely higher at 30°C than at lower temperatures. Much higher catabolic rates for larger fish at 30°C were also found in the rates of reduction in body weight during starvation and oxygen consumption. The much lower growth rate of larger fish at 30°C was therefore probably caused by their higher catabolic rate.

This study has identified the probable cause of the drop in growth rate for larger flounder at higher temperatures. However, further investigation is necessary to clarify the cause of different effects of high temperature on growth between smaller and larger flounder.

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