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Title	Effects of water temperature on feeding and growth of juvenile marbled flounder <i>Pseudopleuronectes yokohamae</i> under laboratory conditions: evaluation by group- and individual-based methods
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Relation	

1 **Effects of water temperature on feeding and growth of juvenile marbled flounder**  
2 ***Pseudopleuronectes yokohamae* under laboratory conditions: evaluation by group- and**  
3 **individual-based methods**

4

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17 **Abstract** To determine the optimal temperature for juvenile (0 year old) marbled flounder  
18 *Pseudopleuronectes yokohamae*, juveniles of 40–54 mm standard length were reared under  
19 six temperature conditions in the range 8 to 26 °C, using group-based and individual-based  
20 methods. Growth of juveniles increased from 8 to 20 °C and decreased from 20 to 26 °C,  
21 irrespective of the rearing method used. Food intake was greatest at 20 and 24 °C than other  
22 temperatures, while feed conversion efficiency was greater at 20 °C than 24 °C in individual  
23 rearing. Individual rearing provided more information such as individual variations in growth  
24 and food consumption, suggesting the importance of individual-based experiments for  
25 exploring the optimal temperature for fish.

26

27 **Keywords** flatfish; food intake; laboratory experiment; thermal response

28

29 **Introduction**

30

31 Water temperature is an important factor affecting fish feeding and growth and is recognized  
32 as a controlling factor [1]. Thermal effects for a number of species have been investigated  
33 under laboratory conditions [2-4]. For European plaice *Pleuronectes platessa* and European  
34 flounder *Platichthys flesus*, growth rates and food intake are greatest at 18–20 °C [5]. For  
35 Japanese flounder *Paralichthys olivaceus*, growth rates and food intake increase from 20 to  
36 25 °C and decrease from 25 to 30 °C [6]. Such responses to temperature are species specific,  
37 and are important for predicting the impact of climate change on vital rates (e.g. growth,  
38 feeding and metabolism) of the fish species.

39 The optimal temperature for fish has been examined in laboratory experiments in which  
40 multiple juveniles were kept in temperature-conditioned aquaria [5, 7]. Growth was evaluated  
41 by changes in the average size of fish, but not by changes in individual size. Food intake has  
42 also been evaluated based on the total amount consumed by multiple individuals [5-7]. Ideally,  
43 feeding and growth should be examined individually, because variability among individuals is  
44 masked under group-based estimation.

45 The marbled flounder *Pseudopleuronectes yokohamae*, an important pleuronectid flatfish  
46 for coastal fisheries in Japan, was used as the study organism. This species spawns around  
47 winter and juveniles occur in shallow coastal waters from March [8, 9]. Although it has been

48 reported that larval growth of this species is faster under higher temperature conditions within  
49 the range of 10 to 18 °C [10], to the best of our knowledge, few studies have revealed the  
50 growth response of juvenile marbled flounder to temperature. This study aimed (1) to reveal  
51 the effects of water temperature on feeding and growth of marbled flounder, and (2) to  
52 compare the use of group-based and individual-based experimental protocols for assessment  
53 of the optimal temperature for juvenile flatfish.

54

## 55 **Materials and Methods**

56

57 A laboratory experiment was conducted using hatchery-reared juvenile marbled flounder  
58 produced from wild parental fish collected in the Seto Inland Sea, western Japan. In May  
59 2015, 96 cultured juveniles of 40–54 mm standard length (SL) at age 127 days were  
60 accommodated in a 500-l tank in Setouchi Field Science Center Takehara Station (Fisheries  
61 Research Station), Hiroshima University. After acclimation for 2 weeks, juveniles were  
62 transferred to six 100-l aquaria (16 individuals in each) filled with aerated natural seawater  
63 (salinity = 32.6) and were maintained without feeding for 24 h. The water temperature in the  
64 respective aquaria was adjusted to 8, 12, 16, 20, 24, and 26 °C by temperature controllers. The  
65 highest temperature was set at 26 °C because even though the temperature threshold of adult  
66 marbled flounder is considered to be 28 °C [11], some juveniles died at 27 °C in a preliminary

67 experiment. A closed circulation system was used for each aquarium and no sand was used.  
68 Light intensities at the seawater surface in aquaria were 180–2135 lx during the daytime and  
69 approximately 0 lx at night. Water was added to aquaria when the water level decreased due  
70 to evaporation.

71 Two rearing systems, i.e. group-based and individual-based methods (ESM Fig. S1),  
72 were adopted. In the group-based method, 10 fish were kept in each aquarium. In the  
73 individual-based method, fish were isolated individually by using floating plastic cages (27  
74 cm long, 16 cm wide, 11 cm high; top and bottom were flat without mesh, and sides were  
75 covered with 0.85-mm nylon mesh; Fig. S1). Each cage was partitioned into two  
76 compartments using a plastic board. Three cages were floated in each aquarium and six  
77 individuals were kept in the six compartments per aquarium. Prior to accommodation of  
78 juveniles, each juvenile was measured (SL [mm] and wet weight [g]).

79 For both group and individual rearing, excess amounts of commercial pellets (mean  $\pm$   
80 SD =  $10.1 \pm 1.4$  mg,  $N = 10$ , Otohime S2; Marubeni Nisshin Feed, Tokyo, Japan) were fed to  
81 fish three times per day during the daytime (08:00, 12:00, and 18:00 hours). This procedure  
82 was continued for 7 days. The water temperature of each aquarium was measured at the time  
83 of feeding ( $N = 21$ , Table 1). For the individual-based method, both the number of pellets  
84 given to fish and those remaining in each compartment after 1 h were recorded, so that the  
85 food intake (the number of consumed pellets  $\times$  average weight of pellets) could be assessed.

86 For the group-based method, neither the number of pellets given to fish nor the number of  
87 remained pellets remaining were recorded, because the relationship between food  
88 consumption and growth of juveniles cannot be assessed individually and because it was  
89 extremely difficult to count remaining pellets owing to giving numerous amount of pellets.  
90 The remaining pellets and feces were removed from the aquaria once per day (around 19:00)  
91 in order to minimize handling effects.

92 After 24 h from the last feeding on the 7th day, all individuals were measured again.  
93 Three group-reared fish and six individually reared fish died at 26 °C during the time from the  
94 last feeding on the 7th day to the final measurement, possibly due to an unexpected rise in  
95 temperature to 27 °C. These individuals were also included in the analyses after correcting for  
96 shrinkage in SL associated with rigor mortis ( $3.9 \pm 1.3\%$ ,  $N = 30$ ), although the correction  
97 may become the error source of the data. No other fish died during the experiment. For the  
98 individually reared fish, feed conversion efficiency (increment in body weight divided by the  
99 weight of consumed pellets) was determined. Growth in SL and body weight, food  
100 consumption, and feed conversion efficiency were compared between temperatures using the  
101 Steel-Dwass test. To assess the individual variation under each temperature, the coefficient of  
102 variation (CV, %) was also determined for growth in SL and body weight, food intake, and  
103 feed conversion efficiency of individually-reared juveniles.

104

105 **Results**

106

107 In the group-reared fish, the largest increments in average SL and average body weight were  
108 4.7 mm and 0.8 g at 20 °C, respectively (Fig. 1). Increments in average SL and body weight  
109 were greater at 24 °C than at 16 °C.

110 In the individually reared fish, increments in average SL and body weight were also  
111 greatest at 20 °C (mean  $\pm$  SD: 6.7  $\pm$  1.5 mm in SL and 1.1  $\pm$  0.2 g in body weight; Fig. 2,  
112 ESM Table S1). Food intake was significantly higher at  $\geq 20$  °C than at  $\leq 16$  °C. No significant  
113 difference was observed between 20 and 24 °C, but feed conversion efficiency was  
114 significantly greater at 20 °C than 24 °C (Fig. 2d). Feed conversion efficiency was also high  
115 at 16 °C, but food intake and growth were low at this temperature. Individual variation in the  
116 feed conversion efficiency appeared to be large at 12 °C; however, this might be attributable  
117 to the inclusion of data from one individual in which body weight decreased during the  
118 experiment and from one individual with exceptionally high feed conversion efficiency of  
119 3.24 (Table S1). Feed conversion efficiency was lowest at 26 °C. The CV for growth, food  
120 intake, and feed conversion efficiency was consistently lowest at 20 °C in the individual  
121 reared fish (Table S2).

122

123 **Discussion**



124

125 The present study clearly showed that juvenile marbled flounder exhibit the highest growth  
126 performance around 20 °C, as revealed by both group- and individual-based methods. Food  
127 intake was also high at 24 °C, but the increment in body weight was less than 20 °C possibly  
128 due to the higher metabolic costs at higher temperatures. Although only one aquarium was  
129 used for each temperature, the optimal temperature around 20 °C would be robust. Tsuchida  
130 [12] explored the temperature preference for many marine fish species in the laboratory, using  
131 a temperature gradient tanks. Although marbled flounder did not aggregate to a certain  
132 temperature position, the preferred temperature for marbled flounder of 54 mm SL was  
133 estimated to be 20.5 °C, which is similar to the optimal temperature estimated for marbled  
134 flounder in the present study.

135 Water temperature usually exceeds 20 °C from June, while juvenile marbled flounder  
136 appear in an estuary in the Seto Inland Sea from March [9]. In another estuary in the Seto  
137 Inland Sea (34°14' N, 132° 33' E), juveniles around 45 mm SL were observed from middle  
138 April with the temperature around 15 °C (Otsuki et al., unpubl. data 2015). These results  
139 indicate that the temperature juveniles experience is generally lower than the optimal  
140 temperature. Moreover, the result of individually-reared experiment showed the growth  
141 potential of juvenile marbled flounder. The highest growth rate was 1.08 mm SL d<sup>-1</sup>, which  
142 has never been observed in the field [13, 14]. Growth rate is regulated by various factors such

143 as temperature and food [1]. Thus, the growth rate of juveniles in the field should be  
144 investigated further with taking effects of temperature and food into account.

145 Feed conversion efficiency often exceeded 1, possibly because dry pellets were used as  
146 food for juveniles. Similar pattern was also observed for Japanese flounder [7]. To assess  
147 daily food intake of juveniles in the field from the feed conversion efficiency, dry weight in  
148 both food and body weight increment of juveniles should be used for calculating feed  
149 conversion efficiency.

150 Although the optimal temperature for growth of juvenile marbled flounder was common  
151 in group- and individual-based methods, average increment in body weight was higher in  
152 group-reared fish than individually-reared fish at 8–16 °C and 26 °C. Although the reason is  
153 unclear, a possible explanation for the greater growth of group-reared fish is that some fish  
154 foraging food might facilitate other individuals' feeding (social learning [15]) in the  
155 group-based method. On the contrary, the increment in body weight was higher in the  
156 individually reared fish than group-reared fish at 20 °C and 24 °C. The mechanism causing  
157 this difference is also unclear, but the individually-reared fish were not exposed to the  
158 interaction with other individuals by biting caudal fin each other [16, 17] or by competing for  
159 food. Additionally, the consistently lowest CV at 20 °C for growth, food intake, and feed  
160 conversion efficiency may suggest that individual variation becomes small under the optimal  
161 temperature condition. Another possible explanation is that CV becomes small when average

162 value is high even if SD is the same: SD of growth and food intake were similar between  
163 temperatures but average values were greatest under 20 °C (Table S2). The individual-based  
164 method is useful for revealing thermal responses in the feeding and growth of juvenile flatfish,  
165 at least for marbled flounder. This experimental protocol can be applied to future studies  
166 investigating the effects of body size on thermal responses, since the optimal temperature  
167 generally decreases as fish grow [18-20]. Clarifying the ontogenetic changes in the optimal  
168 temperature will cue to understand the habitat shift of fish in relation to the ambient water  
169 temperature.

170

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176

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229

230 Figure captions

231

232 Fig. 1. Growth of juvenile marbled flounder in group-based rearing. (a) Increments in average  
233 standard length (SL), and (b) increments in average body weight.

234

235 Fig. 2. Growth and feeding of juvenile marbled flounder in individual-based rearing. (a)  
236 Increments in standard length (SL), (b) increments in body weight, (c) food intake, and  
237 (d) feed conversion efficiency. Data are shown as the mean + SD.  $N = 6$  for each group.

238 Different letters in italics indicate significant differences between groups (Steel-Dwass  
239 test,  $P < 0.05$ ).

240

Fig. 1.

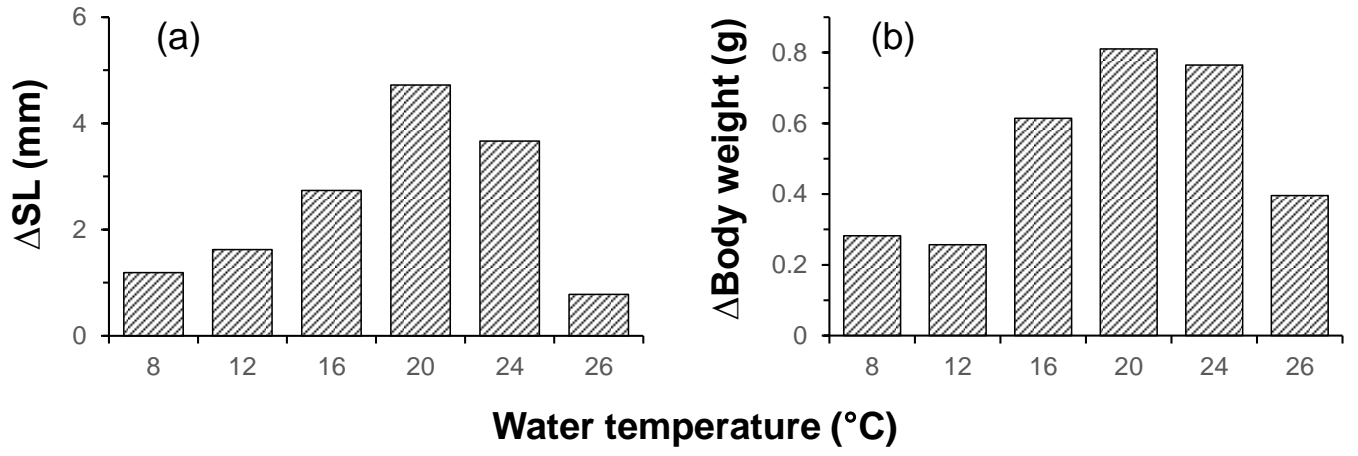
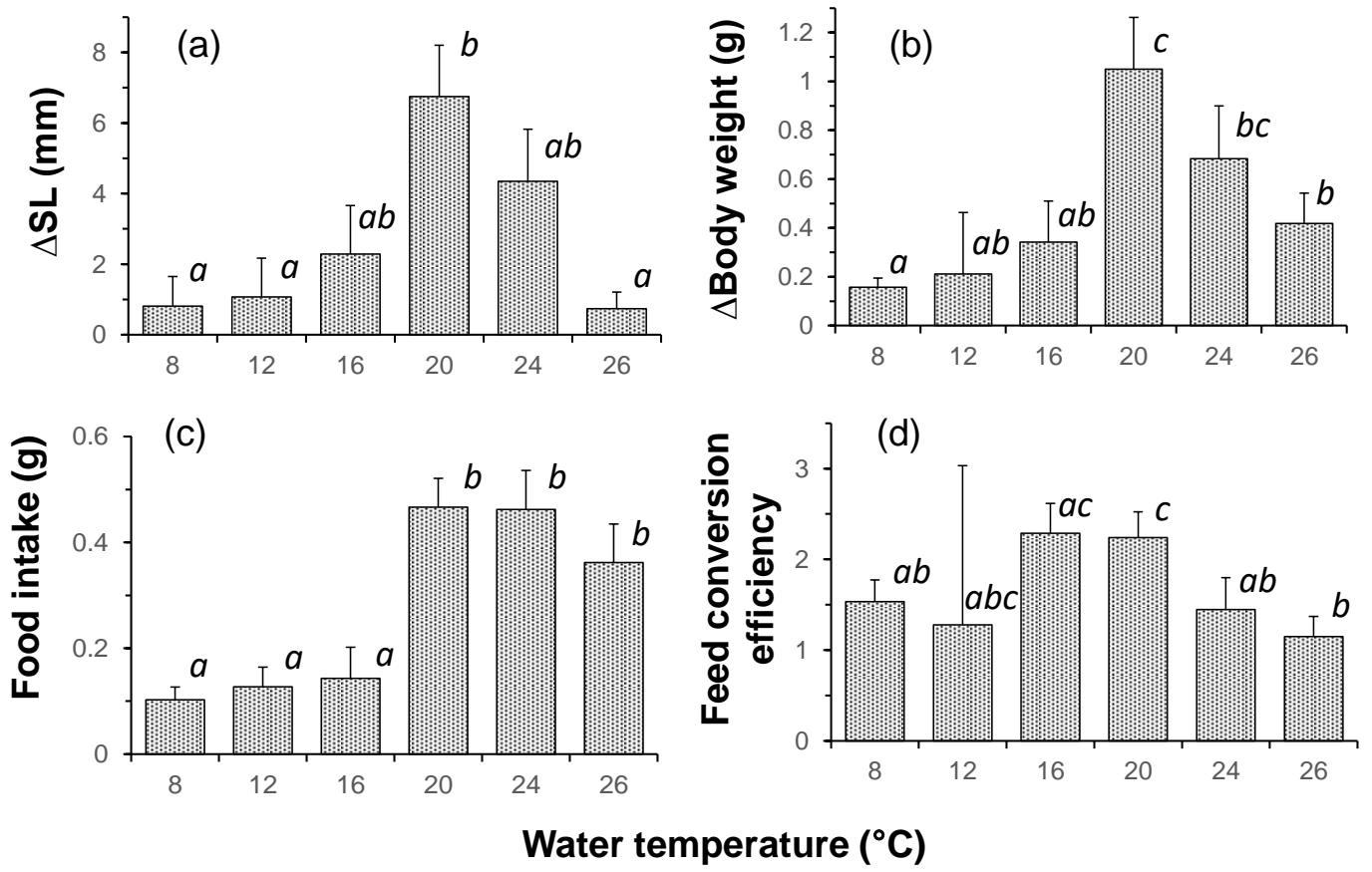




Fig. 2.



**Table 1** Experimental condition for feeding and growth of juvenile marbled flounder

Aquarium	Water temperature (°C)	Group-based				Individual-based			
		Initial SL (mm)	Initial BW (g)	SL at the end (mm)	BW at the end (g)	Initial SL (mm)	Initial BW (g)	SL at the end (mm)	BW at the end (g)
8	8.06 ± 0.17	47.20 ± 3.88	1.62 ± 0.40	48.39 ± 3.73	1.90 ± 0.41	48.42 ± 3.34	1.88 ± 0.46	49.24 ± 3.35	2.04 ± 0.48
12	11.94 ± 0.15	47.45 ± 3.62	1.74 ± 0.37	49.08 ± 3.43	1.99 ± 0.47	47.28 ± 2.48	1.65 ± 0.28	48.36 ± 2.22	1.87 ± 0.28
16	15.80 ± 0.20	47.10 ± 3.57	1.46 ± 0.37	49.84 ± 3.51	2.08 ± 0.47	45.12 ± 2.54	1.43 ± 0.26	47.41 ± 2.33	1.77 ± 0.29
20	20.00 ± 0.73	46.97 ± 3.10	1.56 ± 0.29	51.69 ± 2.83	2.37 ± 0.40	44.88 ± 2.25	1.41 ± 0.22	51.63 ± 2.03	2.46 ± 0.42
24	23.64 ± 0.30	46.42 ± 3.30	1.46 ± 0.30	50.08 ± 3.17	2.22 ± 0.43	45.46 ± 3.06	1.49 ± 0.29	49.81 ± 3.62	2.17 ± 0.44
26	26.09 ± 0.43	48.02 ± 2.76	1.72 ± 0.26	48.80 ± 3.12	2.12 ± 0.30	47.68 ± 2.55	1.74 ± 0.28	48.42 ± 2.57	2.16 ± 0.36

Data are shown as the mean ± SD. SL and BW indicate standard length and body wet weight, respectively. Water temperature was measured three times per day during the feeding period (7 days). Ten and six individuals were maintained in each aquarium used for group-based and individual-based rearing, respectively. Mean standard length (SL) is shown; raw data for individual-based method are presented in Table S1. No significant difference in initial SL was observed between aquaria (Kruskal-Wallis test; group-based:  $P = 0.88$ ; individual-based:  $P = 0.17$ )