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Title	Ontogenetic changes in the optimal temperature for growth of juvenile marbled flounder <i>Pseudopleuronectes yokohamae</i>
Author(s)	Tomiyaama, Takeshi; Kusakabe, Kazushi; Otsuki, Noriko; Yoshida, Yusei; Takahashi, Satoshi; Hata, Masaki; Shoji, Jun; Hori, Masakazu
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Relation	



1 **Ontogenetic changes in the optimal temperature for growth of juvenile marbled**  
2 **flounder *Pseudopleuronectes yokohamae***

3

4 Takeshi Tomiyama<sup>1\*</sup>, Kazushi Kusakabe<sup>1</sup>, Noriko Otsuki<sup>1</sup>, Yusei Yoshida<sup>1</sup>, Satoshi  
5 Takahashi<sup>1</sup>, Masaki Hata<sup>1</sup>, Jun Shoji<sup>1</sup>, Masakazu Hori<sup>2</sup>

6

7 <sup>1</sup> Graduate School of Biosphere Science, Hiroshima University, Higashi-Hiroshima 739-8528,  
8 Japan

9 <sup>2</sup> National Research Institute of Fisheries and Environment of Inland Sea, Japan Fisheries  
10 Research and Education Agency, Hatsukaichi, Hiroshima 739-1452, Japan

11

12

13 \*Corresponding author: T. Tomiyama

14 Tel: +81-82-424-7941

15 Email: [tomiyama@hiroshima-u.ac.jp](mailto:tomiyama@hiroshima-u.ac.jp) (T. Tomiyama)

16 **ABSTRACT**

17

18 We conducted a laboratory experiment and field collections of juvenile marbled flounder  
19 (*Pseudopleuronectes yokohamae*) to assess growth rates of juveniles. In the laboratory,  
20 cultured juveniles of approximately 31 and 46 mm standard length grew fastest at 20 °C,  
21 while juveniles of approximately 21 mm grew faster at 12–14 °C than at 20 °C. This result  
22 confirmed that optimal temperature for growth of juveniles changes ontogenetically. Juveniles  
23 were also collected from shallow estuaries during April to May, during which the water  
24 temperature increased from 13 to 22 °C. The increase in optimal temperature is advantageous  
25 for juveniles, because both juvenile size and ambient temperature increase as the season  
26 progresses. Growth rates of juveniles were nearly maximum under given temperatures in the  
27 field when assessed from otolith microstructure. Thus, juveniles grow at high rates in the  
28 field, although ambient temperatures are not necessarily optimal for growth.

29

30 **Keywords:** flatfish; nursery ground; thermal optima; specific growth rate

31

## 32 1. Introduction

33

34 Shallow estuaries function as nursery grounds for juvenile flatfish (Able et al., 2005).  
35 Nursery grounds usually provide good conditions in temperature, salinity, prey availability,  
36 and predation risk for juveniles (Bergman et al., 1988). Juvenile growth has been assessed to  
37 evaluate the quality of nursery habitats (Gibson, 1994; Sogard et al., 2001; Kurita et al.,  
38 2017). If there is no density-dependent effects on growth, food would not be a limiting factor  
39 and the growth of juveniles would be determined solely by prevailing water temperature. This  
40 “maximum growth/optimal food condition” (MG/OFC) hypothesis (Van der Veer and Witte,  
41 1993; Van der Veer et al., 1994) has been tested in many studies (reviewed by Nash and  
42 Geffen, 2015). However, it has rarely been considered whether or not the temperature they  
43 actually experience is optimal for growth. To achieve the greatest growth in the field,  
44 juveniles may prefer habitats with optimal temperature conditions.

45 Optimal temperature for growth changes ontogenetically in many species. Declines in  
46 optimal temperature associated with increases in fish size have been demonstrated for  
47 demersal fishes such as Atlantic cod *Gadus morhua* (Björnsson et al., 2001) and turbot  
48 *Scophthalmus maximus* (Imsland et al., 1996; Árnason et al., 2009). On the other hand, the  
49 optimal temperature for growth was consistent between 20 and 25 °C in juvenile Japanese  
50 flounder *Paralichthys olivaceus* with sizes from 4 to 176 g (Iwata et al., 1994) or with sizes  
51 from 0.02 to 0.25 g (Seikai et al., 1997). It was also demonstrated that the effects of  
52 temperature and body size on maximum growth was different between European plaice  
53 *Pleuronectes platessa* and European flounder *Platichthys flesus* (Fonds et al., 1992); optimal  
54 temperature decreases are associated with size increases in plaice, but even large-sized  
55 flounder (>20 cm) are less sensitive to high temperatures. Thus, the influence of body size on  
56 optimal temperature would vary among species.

57 Marbled flounder *Pseudopleuronectes yokohamae* is a commercially important  
58 pleuronectid flatfish in Japan. Adult fish spawn from December–February in the Seto Inland  
59 Sea, western Japan (Tanda et al., 2008), and juveniles appear in shallow nursery grounds from  
60 March–June (Hata et al., 2016). Although the optimal temperature for growth of juveniles >40  
61 mm standard length (SL) has been reported to be 20 °C (Kusakabe et al., 2017), no  
62 information on the post-settlement ontogenetic change in their optimal temperature is  
63 available.

64 This study aimed to: (1) test whether optimal temperatures shift ontogenetically, (2)  
65 clarify the temperature that juveniles actually experience in the field, and (3) assess growth

66 performance of juveniles in the field in relation to their potential growth under given  
67 temperatures. We conducted a laboratory experiment in which juveniles were reared under  
68 various temperatures with excess food. We also collected wild juveniles from the field and  
69 their growth was assessed from their change in body size and otolith daily increments, and  
70 tested the MG/OFC hypothesis for marbled flounder.

71

## 72 **2. Materials and Methods**

73

### 74 2.2. Laboratory experiment

75

76 We conducted a laboratory experiment to reveal the maximum growth of juvenile  
77 marbled flounder with excess food at various temperatures. Hatchery-reared juveniles were  
78 used for the experiment. Parental fish were caught by gillnet and set-net fisheries from the  
79 Seto Inland Sea, Japan. Larvae hatched from artificially fertilised eggs at 13.1 °C in January  
80 2016 and were fed on rotifers until larval settlement around 27 days after hatching (DAH).  
81 *Artemia* nauplii were fed to fish from 23 to 93 DAH. Commercial pellets were also fed to fish  
82 from 60 DAH. Settled juveniles were kept at around 13.5 °C before the experiment in April.  
83 Thereafter, the water temperature gradually rose to 18.7 °C before the experiment in June.

84 We carried out the experiment three times in April, May, and June 2016, using different  
85 size classes of  $20.6 \pm 1.4$  mm SL (mean  $\pm$  SD;  $0.15 \pm 0.04$  g in body wet weight [BW], 88  
86 DAH),  $31.0 \pm 2.4$  mm ( $0.64 \pm 0.14$  g, 120 DAH), and  $45.8 \pm 1.9$  mm ( $1.96 \pm 0.28$  g, 150  
87 DAH), hereafter assigned to 21, 31, and 46 mm, respectively. These size classes were selected  
88 because juveniles within these size ranges are usually observed in estuaries.

89 We reared juveniles individually, using the protocol of Kusakabe et al. (2017). We used  
90 six 100-l aquaria in which water temperature was adjusted using thermostats with electronic  
91 aquarium heaters and chillers, and each aquarium was filled with filtered seawater with a  
92 salinity of 32 and aerated. Initially, we used temperatures of 8, 12, 16, 20, 24, and 26 °C for  
93 juveniles of 21 mm, but no juvenile survived at 24 and 26 °C within 24 h of the start of the  
94 experiment. Therefore, 10 and 14 °C were used instead. For juveniles of 31 and 46 mm,  
95 temperatures were adjusted to 12, 14, 16, 18, 20, and 22 °C.

96 Three cages of 27 cm  $\times$  16 cm  $\times$  11 cm (length  $\times$  width  $\times$  height) with sides covered in  
97 0.85 mm nylon mesh were set afloat in each 100-l aquarium. Each cage was partitioned into  
98 two compartments by a plastic board. Juveniles were measured (SL to the nearest 0.01 mm  
99 and BW to the nearest 0.01 g) and were accommodated individually at each compartment. Six

100 juveniles were used for each aquarium and each experiment, and juveniles were starved for  
101 the first 24 h for acclimation. They were then fed commercial pellets (For juveniles of 21 mm:  
102 Otohime S1, 0.62 mg per particle; for juveniles of 31 and 46 mm: Otohime S2, 1.01 mg per  
103 particle; Marubeni Nisshin Feed, Tokyo, Japan) three times per day (08:00, 13:00, and 18:00)  
104 for seven days. Pellets were manually fed to fish until satiation, and those that remained 1 h  
105 after feeding were collected each time. The numbers of pellets both given and remaining were  
106 recorded. After 24 h from feeding at 18:00 on the seventh day, juveniles were collected and  
107 were immediately anaesthetised with 0.1% FA-100 (a pharmaceutical preparation of 10%  
108 eugenol; Tanabe Pharmaceutical Co., Japan) before measurements.

109 The laboratory experiments were carried out without tank replicates. Although the results  
110 of the 46 mm class (see results) were quite similar to those of the previous study using  
111 juveniles of 40–54 mm SL (Kusakabe et al., 2017), tank replicates should be considered in  
112 future studies to enhance the quality of the experiment.

113 All experimental protocols and animal care followed the guidelines of the Hiroshima  
114 University Animal Research Committee (number CD001825).

115

## 116 2.2. Collection of wild juveniles

117

118 The Kamo River estuary (34° 19' N, 132° 53' E) and the Niko River estuary (34° 14' N,  
119 132° 33' E) off Hiroshima, western Japan, were chosen as the study sites. Widths of both  
120 rivers at the mouth were less than 50 m. Tidal range was approximately 4 m.

121 Juvenile marbled flounder were collected by scoop nets (35 cm width, 2 mm mesh, 90  
122 cm handle length) and a 1.5 m wide push net with 3 mm mesh at areas with depths <1 m  
123 around the spring ebb tide during the day from April to May 2014. In the Kamo River estuary,  
124 the density of juveniles was determined from push net collection (see Hata et al., 2016). In the  
125 Niko River estuary, the swept area was calculated from the width of the scoop net and the  
126 distance walked for collection, and the density was determined. Swept areas per survey were  
127 450 m<sup>2</sup> in the Kamo River estuary and 133–450 m<sup>2</sup> in the Niko River estuary. The catch  
128 efficiency was assumed to be 1 to determine the juvenile densities. Juveniles were preserved  
129 in 99% ethanol and brought to the laboratory. Bottom water temperature and salinity were  
130 measured with a digital precision meter Multi 3420 (WTW GmbH, Weilheim). Salinity was  
131 not considered as an environmental factor in this study (range; Kamo River estuary: 23–30;  
132 Niko River estuary: 24–31).

133 In 2015, no juveniles were collected from the Niko River estuary. Similarly, only seven

134 total individuals were collected there (one individual in April and six in May) in 2016.  
135 Therefore, we only used samples from 2014 for analyses.

136

### 137 2.3. Measurements and data analyses

138

139 In the laboratory experiment, all juveniles were measured (SL, BW) after  
140 anaesthetization with 0.1% FA-100. Juvenile growth was assessed based on differences in SL  
141 and BW data at the beginning and the end of the experiment. Observed growth rate (OGR,  
142 mm d<sup>-1</sup>) was calculated from the increment of SL divided by seven (feeding period). Specific  
143 growth rate (SGR, % d<sup>-1</sup>) was determined as follows, following Fonds et al. (1995):

$$144 \quad \text{SGR} = (\ln(\text{BW}_{\text{end}}) - \ln(\text{BW}_{\text{start}})) / t \times 100,$$

145 where BW<sub>end</sub> and BW<sub>start</sub> represent body wet weight at the end and start of the experiment,  
146 respectively, and t is the number of feeding days (seven in this study) during the experiment.  
147 To determine food consumption, the number of pellets consumed by each individual was  
148 calculated as the difference between the numbers of pellets given and those remaining, and  
149 was converted to the weight based on the average pellet weight. Daily food intake (DFI, mg  
150 d<sup>-1</sup>) was calculated for each individual from the total consumption of pellets in weight divided  
151 by seven.

152 Juvenile growth generally shows a unimodal peak under various temperatures (Fonds et  
153 al., 1992), and quadratic regressions are often used to express the relationship between  
154 maximum growth and water temperature (Imsland et al., 1996; Seikai et al., 1997; Árnason et  
155 al., 2009; Schram et al., 2013). However, growth seems to decrease rapidly from the unimodal  
156 peak to higher temperature (Fonds et al., 1992). Therefore, to generate the temperature-  
157 growth relationship, a generalised additive model (GAM) with Gaussian family and identity-  
158 link function was used for each size class in the laboratory experiment. SGR was used as the  
159 response variable. Because body size influences food intake and growth (Fonds et al., 1992;  
160 Yamashita et al., 2001), water temperature and initial SL were used as initial explanatory  
161 variables, and explanatory variables were selected based on the Akaike information criterion.  
162 Statistical analyses were carried out with R software (R Core Team, 2014). The model  
163 selection was conducted using the package ‘MuMIn’.

164 For field-collected specimens, SL of juveniles was measured to the nearest 0.1 mm in the  
165 laboratory. Correction of SL was made for shrinkage due to dehydration by ethanol  
166 preservation. Preliminarily the rate of shrinkage by 99% ethanol was determined for juveniles  
167 of 31–68 mm SL (n = 18, 3.34 ± 2.07%, mean ± SD), although this value was lower than that

168 for marbled flounder juveniles preserved in 70% ethanol (5.6%) in a previous report (Lee et  
169 al., 2012). SL was compared between sites using the Mann-Whitney *U* test for each season  
170 (early April, middle April, late April, and middle May).

171 To estimate the growth rates of juveniles in the field, two methods were applied. First,  
172 we used the regression coefficient from the relationship between date and SL in each estuary.  
173 Alternatively, we estimated growth from the otolith microstructure analysis. The daily ring  
174 formation was validated for lapilli of marbled flounder (Joh et al., 2005). Lapillus of the  
175 ocular side of juveniles collected from each estuary in mid-April was extracted from each  
176 individual under a microscope and mounted on a glass slide with nail enamel without  
177 polishing. The maximum otolith radius (OR,  $\mu\text{m}$ ) and increment widths of the outer 10 daily  
178 rings of the lapillus were measured using an otolith measurement system (Ratoc System  
179 Engineering Inc.), following Tomiyama et al. (2007). The SL of juveniles 10 d before  
180 collection was back calculated using the biological intercept method (Campana and Jones,  
181 1992). The SL and OR of the smallest individual fish (12.53 mm SL, 102.51  $\mu\text{m}$  in OR) were  
182 used as the intercept. The equation was:

183 
$$\ln \text{SL}_{c-10} = \ln \text{SL}_c + (\ln \text{OR}_{c-10} - \ln \text{OR}_c) (\ln \text{SL}_c - \ln 12.53) (\ln \text{OR}_c - \ln 102.51)^{-1},$$
  
184 where *C* is the collection day and *C*-10 is 10 d before collection. Growth rates ( $\text{mm d}^{-1}$ ) were  
185 determined from the difference between  $\text{SL}_c$  and  $\text{SL}_{c-10}$ .

186 Average temperature during early April to middle April was 13.9 °C in the Kamo River  
187 estuary and 14.4 °C in the Niko River estuary. Therefore, the OGR in all size classes at 14 °C  
188 in the laboratory experiment was used for evaluation of juvenile growth in the field.

189

### 190 **3. Results**

191

#### 192 **3.1. Laboratory experiment**

193

194 No juveniles died at 8–20 °C (size class: 21 mm) and at 12–22 °C (31 and 46 mm)  
195 during the experiment. The average BW increased during the experiment for all size classes  
196 except for juveniles of 21 mm reared at 20 °C (Table 1).

197 OGR in 21-mm juveniles increased when temperature was increased 8 to 14 °C, but  
198 decreased when temperature was increased from 14 to 20 °C (Fig. 1). The average OGR was  
199 0.30  $\text{mm d}^{-1}$  at 14 °C. The OGR in 31-mm juveniles was higher at 14–20 °C than at 12 and  
200 22 °C. The average OGR at 14, 16, 18, and 20 °C were 1.03, 0.95, 0.95, and 1.13  $\text{mm d}^{-1}$ ,  
201 respectively. The OGR in 46-mm juveniles increased from 14 to 20 °C and rapidly decreased



202 from 20 to 22 °C. The average OGR at 20 °C was 1.02 mm d<sup>-1</sup>.

203 SGR and DFI showed similar patterns to OGR. However, DFI of 31- and 46-mm  
204 juveniles were similar between 20 and 22 °C, although SGR and OGR of juveniles of both  
205 sizes were lower at 22 °C than at 20 °C.

206 In the GAM analysis, both water temperature and SL were adopted as explanatory  
207 variables for all size classes (for 21 mm class: Deviance explained = 64.5%, Generalised  
208 Cross Validation [GCV] score = 12.1, Scale est. = 8.8, n = 36; for 31 mm class: Deviance  
209 explained = 77.9%, GCV score = 2.4, Scale est. = 1.9, n = 36; for 46 mm class: Deviance  
210 explained = 50.9%, GCV score = 1.5, Scale est. = 1.3, n = 36). Water temperature was  
211 adopted consistently as a significant factor ( $P < 0.001$ ) for all size classes. The initial SL was  
212 also adopted consistently in the selected model, although it was not significant for the 46 mm  
213 class (for 21 mm class:  $P < 0.05$ ; for 31 mm class:  $P < 0.001$ ; for 46 mm class:  $P = 0.08$ ).  
214 SGR of juveniles was greatest around 12–13 °C in 21 mm class, while it was greatest around  
215 19–20 °C in 31 and 46 mm classes (Fig. 2). SGR tended to decline with increasing SL,  
216 although this tendency was not clear for the 31-mm class.

217

### 218 3.2. Occurrence and growth of juveniles in the field

219

220 Water temperature increased from 13.0 to 21.6 °C and from 13.1 to 19.3 °C in the Kamo  
221 River estuary and the Niko River estuary, respectively, during April–May (Fig. 3). The  
222 temperature was slightly lower in the Kamo River estuary than in the Niko River estuary until  
223 15 May. The density of juveniles was consistently higher in the Niko River estuary ( $\leq 25.9$   
224 inds per 100 m<sup>2</sup>) than in the Kamo River estuary ( $\leq 0.9$  inds per 100 m<sup>2</sup>). No juveniles were  
225 collected from both sites at the end of May when water temperature reached around 20 °C.

226 Juveniles were consistently smaller in the Kamo River estuary than in the Niko River  
227 estuary (Fig. 4). SL was significantly greater in the Niko River except for during May (early  
228 April:  $29.2 \pm 5.6$  mm [mean  $\pm$  SD] in the Niko River,  $21.3 \pm 4.5$  mm in the Kamo River,  $U =$   
229  $3986$ ,  $P < 0.001$ ; middle April:  $35.6 \pm 5.3$  mm in the Niko River,  $22.9 \pm 4.1$  mm in the Kamo  
230 River,  $U = 2791$ ,  $P < 0.001$ ; late April:  $47.0 \pm 10.9$  mm in the Niko River,  $29.5 \pm 1.8$  mm in  
231 the Kamo River,  $U = 260$ ,  $P < 0.001$ ; May:  $57.5 \pm 17.5$  mm in the Niko River,  $37.1 \pm 5.7$  mm  
232 in the Kamo River,  $U = 6$ ,  $P = 0.2$ ).

233 Daily growth rates in SL, estimated from the relationship between date and SL, were  
234 0.24 and 0.63 mm d<sup>-1</sup> in the Kamo River estuary and the Niko River estuary, respectively  
235 (Table 2). Daily growth rates of juveniles collected in mid-April, as estimated from the otolith

236 microstructure analysis, were  $0.54 \pm 0.18 \text{ mm d}^{-1}$  (mean  $\pm$  SD) and  $0.98 \pm 0.18 \text{ mm d}^{-1}$  in the  
237 Kamo River estuary and the Niko River estuary, respectively. The SLs of these juveniles were  
238  $22.9 \pm 2.7 \text{ mm}$  ( $n = 9$ ) and  $35.4 \pm 5.8 \text{ mm}$  ( $n = 13$ ) in the Kamo River estuary and Niko River  
239 estuary, respectively.

240 A quadratic relationship was observed between SL at the end of the experiment and OGR  
241 at  $14 \text{ }^{\circ}\text{C}$  in the laboratory experiment:

$$242 \quad \text{OGR} = 0.1501 \text{ SL} - 0.0019 \text{ SL}^2 - 2.0339 \quad (n = 18, r^2 = 0.62)$$

243 Based on this equation, the maximum growth rates for  $22.9 \text{ mm}$  and  $35.4 \text{ mm}$  juveniles at  
244  $14 \text{ }^{\circ}\text{C}$  were predicted to be  $0.41 \text{ mm d}^{-1}$  and  $0.90 \text{ mm d}^{-1}$ , respectively. Thus, the growth rates  
245 of juveniles were lower than maximum under the given temperature when estimated from the  
246 temporal change in SL, but were higher than the maximum when estimated from the otolith  
247 daily increments.

248

## 249 **4. Discussion**

250

### 251 4.1. Ontogenetic change in optimal water temperature

252

253 This study revealed that optimal temperatures for growth vary with increasing body size  
254 in juvenile marbled flounder. To our knowledge, this study is the first to demonstrate that the  
255 optimal temperature was lower in small juveniles ( $12\text{--}14 \text{ }^{\circ}\text{C}$  for  $21 \text{ mm SL}$ ) than in larger  
256 juveniles ( $20 \text{ }^{\circ}\text{C}$  for  $31$  and  $46 \text{ mm SL}$ ). The inverse tendency, namely optimal temperature  
257 declines associated with size increases, has been observed for other species (Imsland et al.,  
258 1996; Björnsson et al., 2001; Hurst et al., 2010). Additionally,  $21\text{-mm}$  juveniles did not  
259 survive at  $24 \text{ }^{\circ}\text{C}$ , while juveniles of  $40\text{--}54 \text{ mm SL}$  survived even at  $26 \text{ }^{\circ}\text{C}$  (Kusakabe et al.,  
260 2017), indicating that high temperature tolerance also changes ontogenetically. This  
261 corresponds with seasonal changes in water temperature in the field, because the temperature  
262 is around  $11\text{--}13 \text{ }^{\circ}\text{C}$  in March when juveniles are small (mostly  $<25 \text{ mm SL}$ ), and thereafter  
263 increases to  $20 \text{ }^{\circ}\text{C}$  by the end of May when juveniles have grown to over  $40 \text{ mm SL}$ .  
264 Furthermore, this ontogenetic change in the thermal optima is applicable to juvenile culture:  
265 juvenile growth can be accelerated by taking ontogenetic shifts in optimal temperature into  
266 consideration (Imsland et al., 2007).

267 The temperature optima shifted with size increases in juveniles, but it should be noted  
268 that optimal temperature may also be affected by other factors. For example, thermal  
269 experience affects temperature tolerances and preferences (Jobling, 1981). The difference in

270 recently experienced temperatures between the 21-mm (around 13 °C) and 46-mm juveniles  
271 (around 18 °C) might affect the difference in optimal temperature. Additionally, juveniles of  
272 similar sizes with different temperature experiences may show different temperature  
273 responses. The 21-mm juveniles being kept around 13 °C did not survive at 24 °C and their  
274 food intake was quite low even at 20 °C in the laboratory in April (Fig. 1). In contrast,  
275 juveniles around 20 mm SL, acclimated to natural temperatures of 15–20 °C during May–  
276 June, survived at 24 and 26 °C, and their food intake was greatest at 24 °C (Kusakabe,  
277 unpublished data). This result suggests that thermal responses are affected not only by the size  
278 of juveniles, but also by experienced temperatures; the duration after settlement was also  
279 different between the two groups (around 60 days in the former 21-mm juveniles and 110  
280 days in the latter 20-mm juveniles). In the field, juveniles of different cohorts would  
281 experience different temperature conditions and would show different thermal responses.  
282 Actually, cohort-specific patterns in feeding, growth, and survival have been observed for  
283 other flatfish species in the field (Geffen et al., 2011; Tomiyama, 2012). Inter-cohort variation  
284 in the thermal history and growth should be considered in future studies.

285

#### 286 4.2. Occurrence and growth in the field

287

288 Juveniles were found around intertidal areas until the end of May. It is noteworthy that  
289 juveniles disappeared from the field in June when water temperature exceeded 20 °C. A  
290 similar pattern has also been observed in European plaice that emigrate to deeper waters to  
291 avoid temperatures above 20 °C, and may be explained by the decreased optimal temperature  
292 with increasing size of fish (Fonds et al., 1992). As the optimal temperature for growth of 46-  
293 mm juveniles is 20 °C, juveniles may emigrate from shallow areas before the water  
294 temperature reaches 20 °C and select habitats cooler than the thermal optima. It is suggested  
295 that fish select such cooler habitats under limited food conditions (Teal et al., 2012). Another  
296 example of such inconsistencies has been reported for Dover sole *Solea solea*: temperature  
297 preferences exceeded the temperature optimum for growth of 22.7 °C (Schram et al., 2013).  
298 However, temperature is a constraint for habitat use of fish (Furey and Rooker, 2013; Furey et  
299 al., 2013; Freitas et al., 2016), and the mechanisms of habitat selection and emigration from  
300 the nursery grounds are still important issues.

301 This study indicates that juveniles grow at a nearly maximum rate under given  
302 temperatures, although the growth rates differed between the two sites. This difference may  
303 be due to differences in habitat quality, as inferred for European plaice (Fox et al., 2014). It

304 should be noted that our collections were made at depths <1 m around ebb tide. Growth of  
305 juvenile European plaice increases with depth at a small spatial scale (Ciotti et al., 2013b).  
306 Additionally, juveniles that reach sufficient size for refuge from predation would move to  
307 deeper areas (Gibson et al., 2002; Ryer et al., 2010). Thus, body size or growth may be  
308 influenced by depths, and investigations of juveniles inhabiting deeper waters should be  
309 included in future studies.

310 This study also showed that growth rates estimated by otolith microstructure analysis  
311 were greater than those estimated from the length-frequency distribution. This may be due to  
312 the earlier emigration of larger fish from shallow areas. Thus, underestimation of growth  
313 evaluation by length data should be taken into consideration. The MG/OFC hypothesis has  
314 been discussed in many studies (Nash and Geffen, 2015), and the hypothesis has been  
315 accepted in several cases (Kamermans et al., 1995; Van der Veer et al., 2001; Sogard et al.,  
316 2001; Amara et al., 2001; Reichert, 2003; Tomiyama et al., 2007) but not necessarily in others  
317 (Pihl, 1989; Van der Veer and Witte, 1993; Berghahn et al., 1995; Hurst and Abookire, 2006;  
318 Freitas et al., 2012; Ciotti et al., 2013a). The density dependency of juvenile growth is  
319 dependent on year-class strength. Growth assessment of juveniles in the field with regard to  
320 maximum potential growth estimated through laboratory experiments or energy budget  
321 models is essential.

322 In conclusion, this study demonstrated the ontogenetic change in the optimal temperature  
323 for the growth of juvenile marbled flounder, from 12–14 °C for 21 mm to 20 °C for 46 mm  
324 juveniles. In the field, juveniles seemed to experience temperature cooler than optimal, but  
325 growth was estimated to be nearly maximum under the given temperatures.

326

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335

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461

462 Figure captions

463

464 Fig. 1. Specific growth rate (SGR), observed growth rate (OGR), and daily food intake  
465 (DFI) of marbled flounder of three size classes in the laboratory experiment. Solid lines  
466 show changes in the average values at each temperature.

467

468 Fig. 2. Relationships between explanatory variables and specific growth rates of marbled  
469 flounder in the laboratory experiment, as obtained by generalised additive models (GAMs;  
470 21 mm:  $r^2 = 0.53$ ; 31 mm:  $r^2 = 0.72$ ; 46 mm:  $r^2 = 0.45$ ). Additive effects were generated  
471 through generalised additive models. Dotted lines indicate 95% confidence intervals.

472

473 Fig. 3. Temporal changes in water temperature and the density of juvenile marbled flounder  
474 in 2014. Data in the Kamo River estuary and the Niko River estuary were shown by solid  
475 triangles with dotted lines and by open circles with solid lines, respectively.

476

477 Fig. 4. Length-frequency distribution of marbled flounder collected from the Kamo River  
478 estuary and the Niko River estuary in 2014. Numerals show sample sizes.

479



480 Table 1  
 481 Experimental condition and results in the laboratory experiment. The number of juveniles  
 482 used for each group was consistently 6, and no juvenile died during the experiment. Data are  
 483 shown by mean  $\pm$  SD.

Group	Water temperature (°C) <sup>a</sup>	Initial SL <sup>a</sup> (mm)	Initial BW <sup>b</sup> (g)	Total food intake <sup>c</sup> (mg)	SL at end (mm)	BW at end (g)
21 mm						
8 °C	8.0 $\pm$ 0.17	20.1 $\pm$ 0.6	0.13 $\pm$ 0.03	30.4 $\pm$ 6.8	20.8 $\pm$ 0.7	0.18 $\pm$ 0.02
10 °C	9.8 $\pm$ 0.20	19.4 $\pm$ 0.7	0.12 $\pm$ 0.02	36.3 $\pm$ 13.7	20.6 $\pm$ 1.2	0.14 $\pm$ 0.03
12 °C	11.9 $\pm$ 0.20	21.0 $\pm$ 1.4	0.17 $\pm$ 0.04	63.7 $\pm$ 9.5	23.0 $\pm$ 1.3	0.25 $\pm$ 0.03
14 °C	13.8 $\pm$ 0.23	20.7 $\pm$ 1.6	0.15 $\pm$ 0.05	68.0 $\pm$ 31.0	22.8 $\pm$ 2.3	0.23 $\pm$ 0.08
16 °C	15.9 $\pm$ 0.49	21.2 $\pm$ 1.7	0.15 $\pm$ 0.04	43.5 $\pm$ 34.2	22.7 $\pm$ 1.8	0.20 $\pm$ 0.07
20 °C	19.7 $\pm$ 0.24	21.1 $\pm$ 1.7	0.19 $\pm$ 0.05	9.4 $\pm$ 5.6	21.3 $\pm$ 1.8	0.17 $\pm$ 0.04
31 mm						
12 °C	11.7 $\pm$ 0.41	30.1 $\pm$ 2.6	0.56 $\pm$ 0.13	133.2 $\pm$ 16.9	33.9 $\pm$ 2.6	0.75 $\pm$ 0.14
14 °C	14.2 $\pm$ 0.17	31.1 $\pm$ 2.3	0.65 $\pm$ 0.12	227.9 $\pm$ 83.7	38.3 $\pm$ 3.2	1.07 $\pm$ 0.28
16 °C	15.9 $\pm$ 0.16	31.3 $\pm$ 1.9	0.67 $\pm$ 0.13	229.9 $\pm$ 31.7	38.0 $\pm$ 2.3	1.17 $\pm$ 0.22
18 °C	17.8 $\pm$ 0.28	30.8 $\pm$ 3.4	0.63 $\pm$ 0.14	265.0 $\pm$ 96.1	37.5 $\pm$ 5.3	1.13 $\pm$ 0.37
20 °C	19.7 $\pm$ 0.16	31.1 $\pm$ 2.2	0.67 $\pm$ 0.13	312.6 $\pm$ 51.4	39.1 $\pm$ 2.4	1.31 $\pm$ 0.28
22 °C	21.8 $\pm$ 0.16	31.7 $\pm$ 2.8	0.68 $\pm$ 0.19	312.8 $\pm$ 60.6	38.2 $\pm$ 3.1	1.26 $\pm$ 0.33
46 mm						
12 °C	12.1 $\pm$ 0.14	46.0 $\pm$ 2.0	2.09 $\pm$ 0.30	268.2 $\pm$ 61.9	50.7 $\pm$ 1.5	2.73 $\pm$ 0.29
14 °C	14.1 $\pm$ 0.19	47.1 $\pm$ 3.0	2.13 $\pm$ 0.43	349.5 $\pm$ 93.1	51.5 $\pm$ 3.4	3.11 $\pm$ 0.53
16 °C	16.2 $\pm$ 0.16	45.3 $\pm$ 1.1	1.80 $\pm$ 0.25	414.1 $\pm$ 77.4	50.4 $\pm$ 2.2	2.77 $\pm$ 0.41
18 °C	18.2 $\pm$ 0.12	45.4 $\pm$ 1.2	1.87 $\pm$ 0.16	511.1 $\pm$ 79.7	51.8 $\pm$ 1.6	2.95 $\pm$ 0.23
20 °C	20.2 $\pm$ 0.13	45.4 $\pm$ 2.2	1.90 $\pm$ 0.27	633.8 $\pm$ 43.5	52.6 $\pm$ 1.5	3.17 $\pm$ 0.34
22 °C	22.0 $\pm$ 0.21	45.6 $\pm$ 1.1	1.98 $\pm$ 0.16	624.7 $\pm$ 137.0	51.4 $\pm$ 1.5	3.02 $\pm$ 0.46

484 <sup>a</sup> standard length

485 <sup>b</sup> body wet weight

486 <sup>c</sup> total consumption of pellets in dry weight during 7 d of each experiment

487

488

489 Table 2

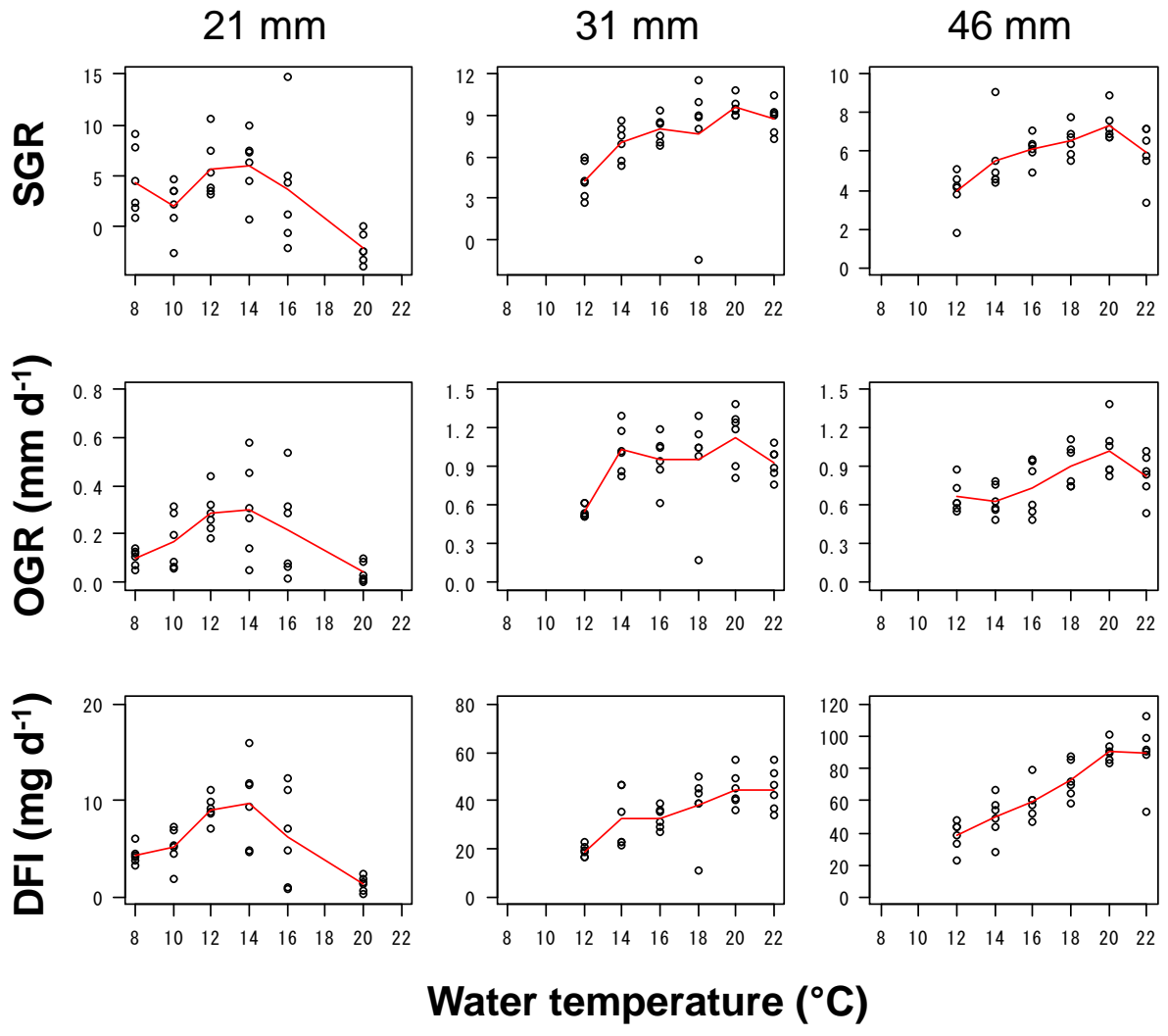
490 Growth rates (GR) of juvenile marbled flounder in the field in 2014. The GR was estimated  
491 from the date–SL relationship and from the otolith microstructure.

Site	Period	WT (°C) <sup>a</sup>	GR from SL <sup>b</sup> (mm d <sup>-1</sup> )	GR from otolith <sup>b</sup> (mm d <sup>-1</sup> )
Kamo River	April 2 – May 15	13.1–15.7	0.24 (227)	0.54 (9)
Niko River	April 1 – May 15	13.2–16.3	0.63 (96)	0.98 (13)

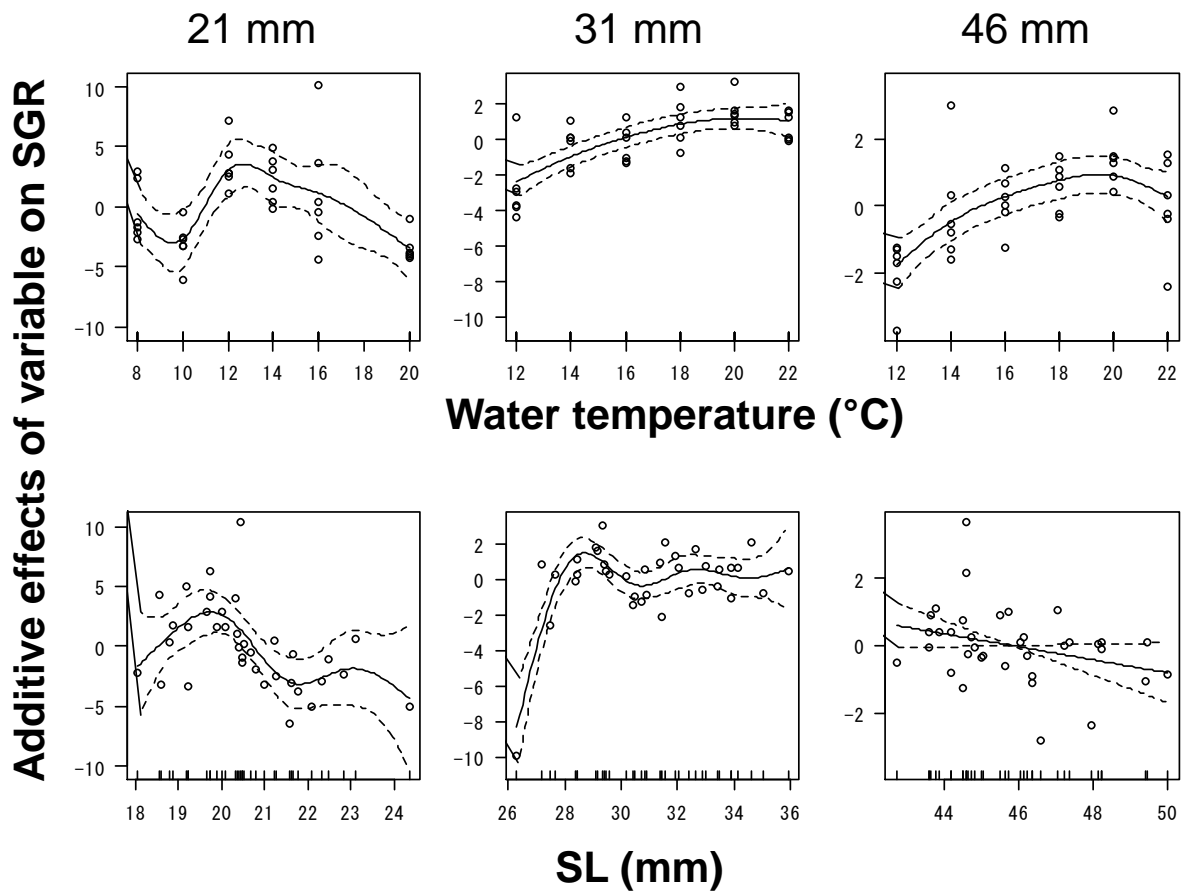
492 <sup>a</sup> Water temperature

493 <sup>b</sup> Numerals in parenthesis denote sample sizes

494

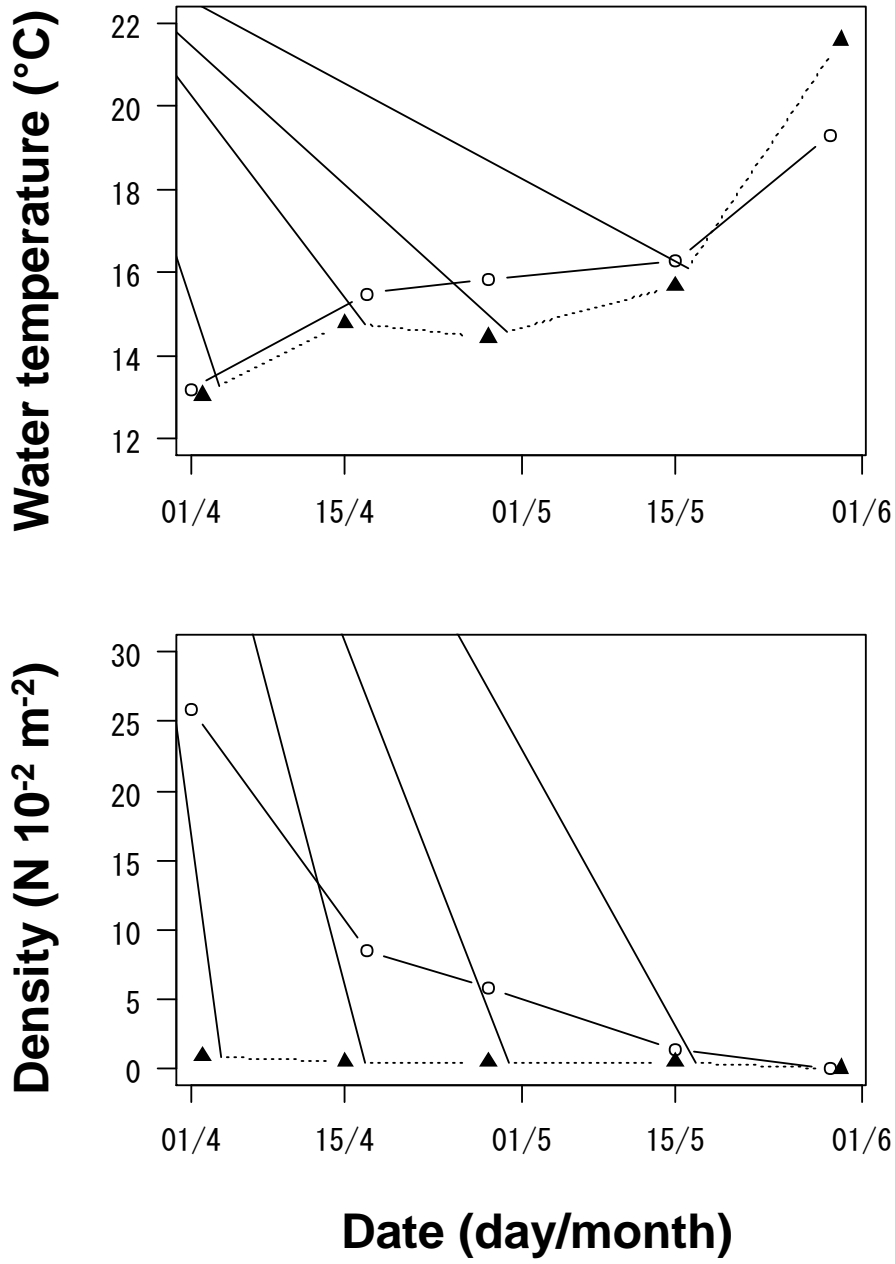


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