

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

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ABSTRACT

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

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

1. Introduction

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

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

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

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

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

2. Materials and Methods

2.2. Laboratory experiment

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

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

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

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

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

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

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

2.2. Collection of wild juveniles

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

Juvenile marbled flounder were collected by scoop nets (35 cm width, 2 mm mesh, 90 cm handle length) and a 1.5 m wide push net with 3 mm mesh at areas with depths <1 m around the spring ebb tide during the day from April to May 2014. In the Kamo River estuary, the density of juveniles was determined from push net collection (see Hata et al., 2016). In the Niko River estuary, the swept area was calculated from the width of the scoop net and the distance walked for collection, and the density was determined. Swept areas per survey were 450 m² in the Kamo River estuary and 133–450 m² in the Niko River estuary. The catch efficiency was assumed to be 1 to determine the juvenile densities. Juveniles were preserved in 99% ethanol and brought to the laboratory. Bottom water temperature and salinity were measured with a digital precision meter Multi 3420 (WTW GmbH, Weilheim). Salinity was not considered as an environmental factor in this study (range; Kamo River estuary: 23–30; Niko River estuary: 24–31).

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

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

2.3. Measurements and data analyses

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

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

where BW_{end} and BW_{start} represent body wet weight at the end and start of the experiment, respectively, and t is the number of feeding days (seven in this study) during the experiment. To determine food consumption, the number of pellets consumed by each individual was calculated as the difference between the numbers of pellets given and those remaining, and was converted to the weight based on the average pellet weight. Daily food intake (DFI, mg d⁻¹) was calculated for each individual from the total consumption of pellets in weight divided by seven.

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

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

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

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

$$\ln \text{SL}_{c-10} = \ln \text{SL}_c + (\ln \text{OR}_{c-10} - \ln \text{OR}_c) (\ln \text{SL}_c - \ln 12.53) (\ln \text{OR}_c - \ln 102.51)^{-1},$$

where *C* is the collection day and *C*–10 is 10 d before collection. Growth rates (mm d^{-1}) were determined from the difference between SL_c and SL_{c-10} .

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

3. Results

3.1. Laboratory experiment

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

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

from 20 to 22 °C. The average OGR at 20 °C was 1.02 mm d⁻¹.

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

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

3.2. Occurrence and growth of juveniles in the field

Water temperature increased from 13.0 to 21.6 °C and from 13.1 to 19.3 °C in the Kamo River estuary and the Niko River estuary, respectively, during April–May (Fig. 3). The temperature was slightly lower in the Kamo River estuary than in the Niko River estuary until 15 May. The density of juveniles was consistently higher in the Niko River estuary (≤ 25.9 inds per 100 m²) than in the Kamo River estuary (≤ 0.9 inds per 100 m²). No juveniles were collected from both sites at the end of May when water temperature reached around 20 °C.

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

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

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 Kamo River estuary and the Niko River estuary, respectively. The SLs of these juveniles were $22.9 \pm 2.7 \text{ mm}$ ($n = 9$) and $35.4 \pm 5.8 \text{ mm}$ ($n = 13$) in the Kamo River estuary and Niko River estuary, respectively.

A quadratic relationship was observed between SL at the end of the experiment and OGR at 14°C in the laboratory experiment:

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

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

4. Discussion

4.1. Ontogenetic change in optimal water temperature

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

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

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

4.2. Occurrence and growth in the field

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

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

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

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

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

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Figure captions

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

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

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

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

Table 1

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

Group	Water temperature (°C) ^a	Initial SL ^a (mm)	Initial BW ^b (g)	Total food intake ^c (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

^a standard length

^b body wet weight

^c total consumption of pellets in dry weight during 7 d of each experiment

Table 2

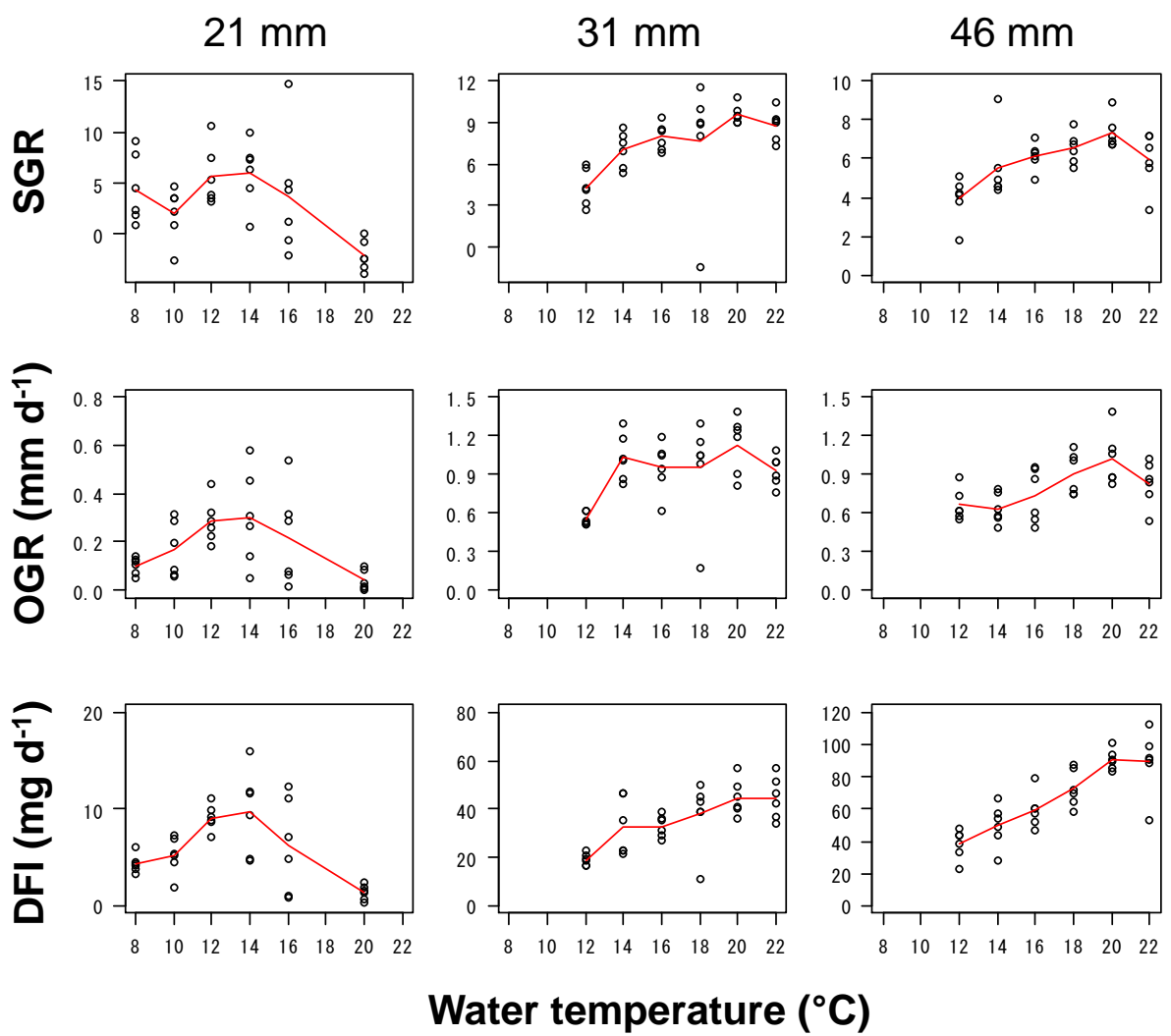
Growth rates (GR) of juvenile marbled flounder in the field in 2014. The GR was estimated from the date–SL relationship and from the otolith microstructure.

Site	Period	WT (°C) ^a	GR from SL ^b (mm d ⁻¹)	GR from otolith ^b (mm d ⁻¹)
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)

^a Water temperature

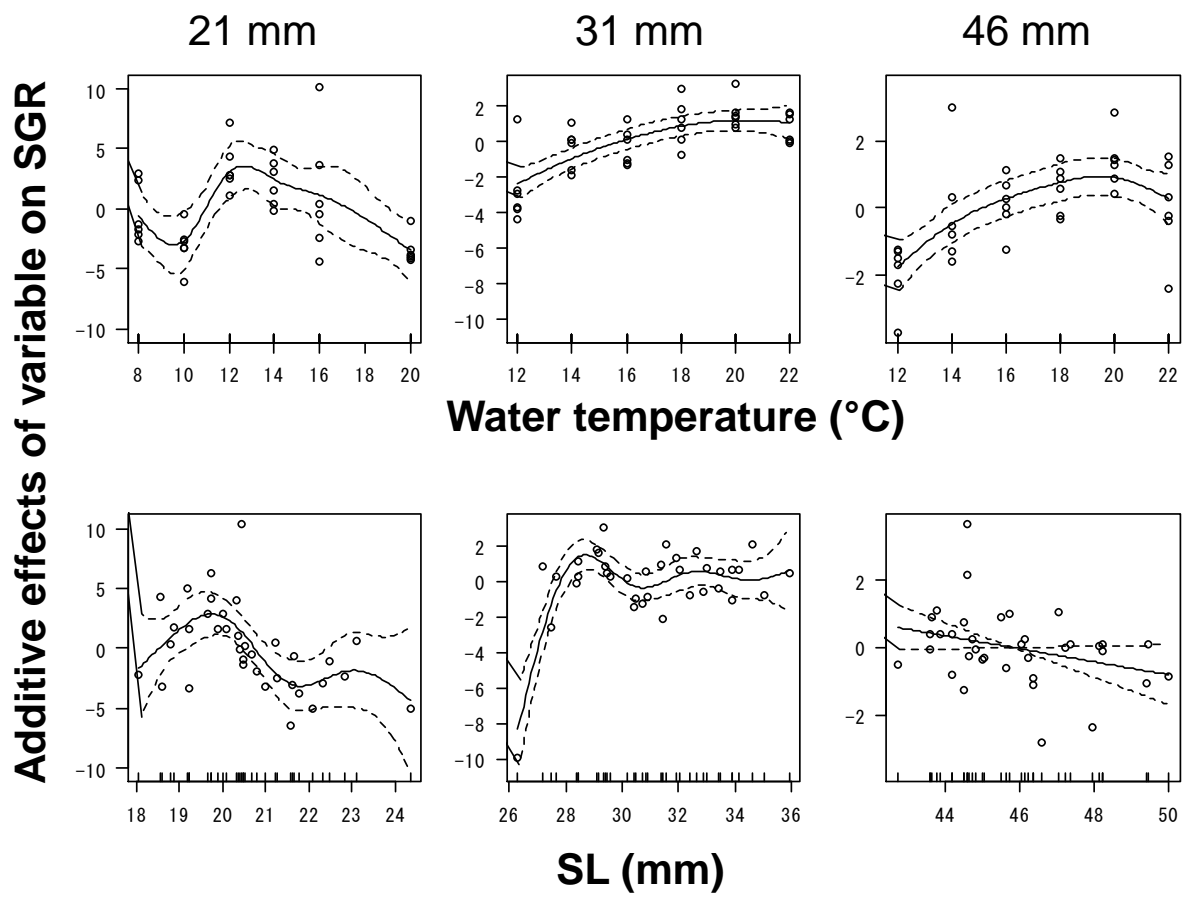
^b Numerals in parenthesis denote sample sizes

495 Fig. 1



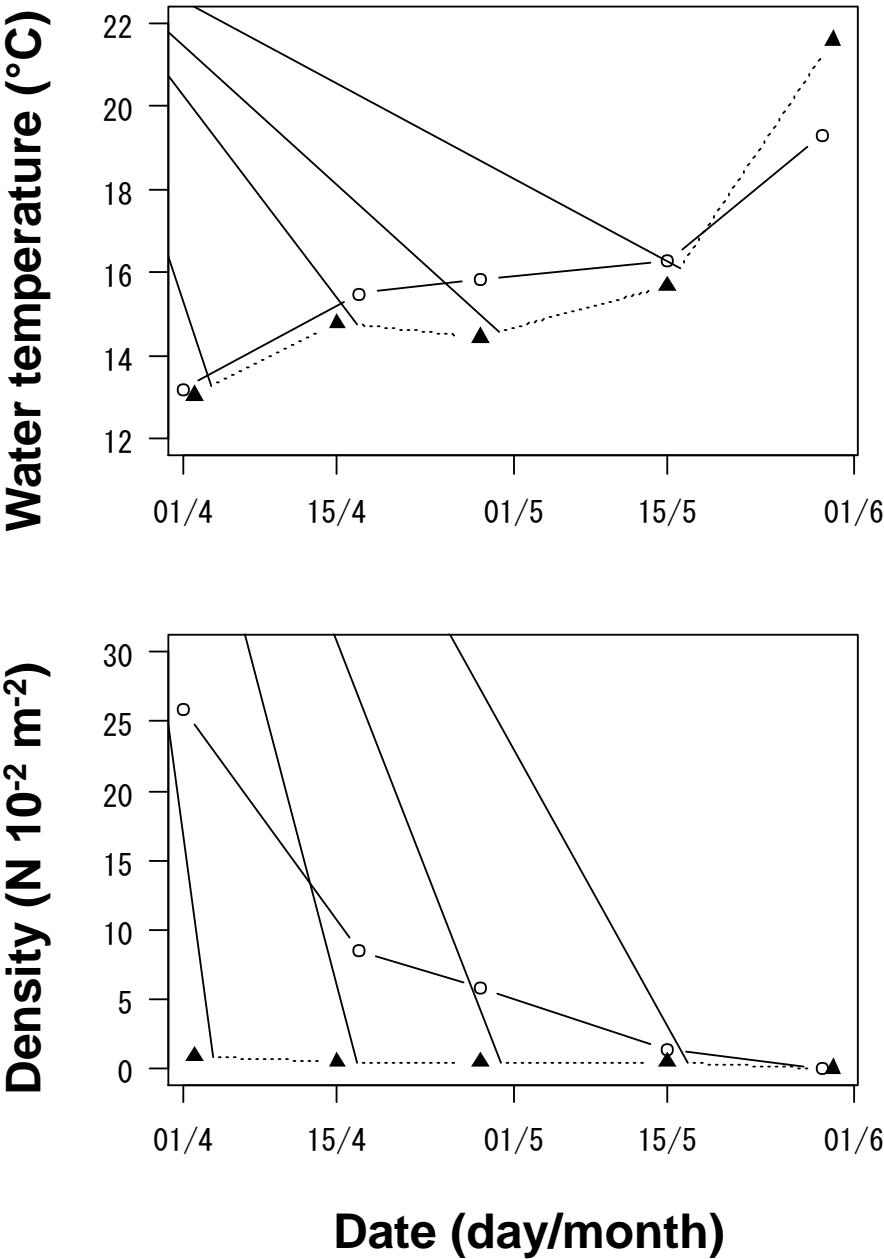
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