Interannual growth differences and growth-selective survival in larvae and juveniles of marbled sole *Pseudopleuronectes yokohamae*

Mikimasa Joh[1],*, Mitsuhiro Nakaya[2], Naoto Yoshida[3], Tetsuya Takatsu[4]

[1]Abashiri Fisheries Research Institute, Hokkaido Research Organization, 1-1-1 Masu-ura, Abashiri, Hokkaido, 099-3119, Japan
[2]Faculty of Fisheries, Hokkaido University, 3-1-1 Minatocho, Hakodate, Hokkaido, 041-8611, Japan
[3]Nippon Suisan Kaisha Ltd, Ohtemachi, Chiyoda, Tokyo 100-8686, Japan
[4]Faculty of Fisheries Sciences, Hokkaido University, 3-1-1 Minatocho, Hakodate, Hokkaido, 041-8611, Japan

ABSTRACT: Flatfishes drastically change their habitat, body form, and feeding during metamorphosis; thus, the early juvenile and larval stages are viewed as being critical for early survival. However, to the best of our knowledge, no studies have tested the growth-mortality hypothesis for both larval and juvenile stages of flatfishes. Therefore, we investigated the relationship between growth rate and environmental factors and tested the hypothesis for both larval and juvenile stages of marbled sole *Pseudopleuronectes yokohamae* in Hakodate Bay, Japan from 2001 to 2003. For both larval and juvenile stages, otolith growth correlated with water temperature. Eye-migrating larvae were defined as survivors of planktonic life ($SV_{pelagic}$). Large juveniles captured in late June and July were defined as survivors of shrimp predation ($SV_{juvenile}$). To test the growth-mortality hypothesis, otolith growth was compared between the SV and the original population. During the pelagic larval stage, growth-selective survival was not detected in any of the 3 yr. During the early juvenile stage, fast-growing individuals survived selectively in 2002 but not in 2003. In 2002, population growth of juveniles was slow because water temperatures were low. Thus, juveniles in 2002 required time to exceed the size spectrum that is vulnerable to shrimp predation; consequently, the individuals that grew more rapidly were able to survive selectively. Our results show the importance of the early juvenile stage for the survival of flatfishes.

KEY WORDS: Flatfish · Growth-selective survival · Juveniles · Larvae · Prey density · *Pseudopleuronectes yokohamae* · Temperature

INTRODUCTION

Fluctuations in the recruitment level of marine fishes are mainly determined by variations in survival during the early life stages (Houde 1987). Anderson (1988) summarized the theory of recruitment in fishes and hypotheses pertaining to causes of recruitment fluctuation and suggested that the growth-mortality hypothesis was a rational concept for recruitment research and a solid basis for future study. This hypothesis states that fast-growing individuals can pass the spectrum of somatic size that is vulnerable to predation and, thus, increase their chance of survival. Therefore, understanding the growth pattern during early life and the relationship between growth and environmental factors is fundamental to understanding recruitment and survival. Moreover, we can assess the growth-mortality hypothesis by comparing growth between survivors and their original population (OP).

The relationships between growth and survival of larvae have been investigated for numerous species (Campana 1996, Meekan & Fortier 1996, Bergenius et al. 2002, Takahashi & Watanabe 2004, Takasuka et al. 2004a,b), and the mortality of the larval stage is many times greater than that of later stages. Larvae of flatfish species are planktonic, thus their habitat is the 3-dimensional water column. After metamorphosis, flatfish larvae become juveniles and their habitat shifts to the 2-dimensional sea floor. When this shift occurs, their morphology and feeding habits change dramatically. In addition, there are many benthic organisms on the sea floor, and predation of flatfish juveniles by small decapods such as crangonid shrimp has been reported for many flatfish species (van der Veer et al. 1991, Witting & Able 1995, Oh et al. 2001, Taylor & Collie 2003). Thus, mortality during the early juvenile stage may be as high as or higher than that in the planktonic larval stage for flatfishes (van der Veer et al. 2000). Hovenkamp (1992) investigated the relationship between growth and mortality during the planktonic larval stage of plaice *Pleuronectes platessa*, and Fox et al. (2007) studied birth-date selectivity in the survival of this species. However, to the best of our knowledge, no studies have tested the growth-mortality hypothesis for both larval and juvenile stages of flatfishes.

Marbled sole *Pseudopleuronectes yokohamae* is a temperate flatfish species distributed in coastal areas ranging from Japan to the Yellow and Bohai Seas, with latitudinal expansion from the southern part of Hokkaido Island to Kyushu Island. Marbled sole is an important species in bottom-trawl and gill net fisheries. Hakodate Bay, which lies at the southern end of Hokkaido Island, is the northernmost habitat of marbled sole (Fig. 1). The spawning season of marbled sole in this area is early spring (February to April). Environmental factors here are variable in spring, and this may affect survival of marbled sole larvae and juveniles.

The ecology of the early life stages of marbled sole has been studied previously in Hakodate Bay. For example, Nakaya et al. (2004a,b, 2007) studied predation on marbled sole larvae and juveniles by the sand shrimp *Crangon uritai*, and Joh et al. (2005) described daily periodicity of otolith ring formation. In late March 2003, Oyashio Coastal Water, which is characterized by low temperature (<3°C) and low salinity (≤33.0), flowed into Hakodate Bay, and water temperature in late March became extremely low. Joh et al. (2009) suggested that almost all larvae hatched earlier in the season may have died due to the extremely low water temperature. To date, however, the growth patterns of wild *Pseudopleuronectes yokohamae* larvae and juveniles have not been studied, nor has the importance of growth for survival been investigated.

In the present study, we investigate the growth patterns of marbled sole larvae and juveniles, the relationship between environmental factors and their growth, and the relationship between pelagic larval growth and planktonic larval duration (PLD). Additionally, we investigate growth-selective survival during both the pelagic larval and early juvenile stages.
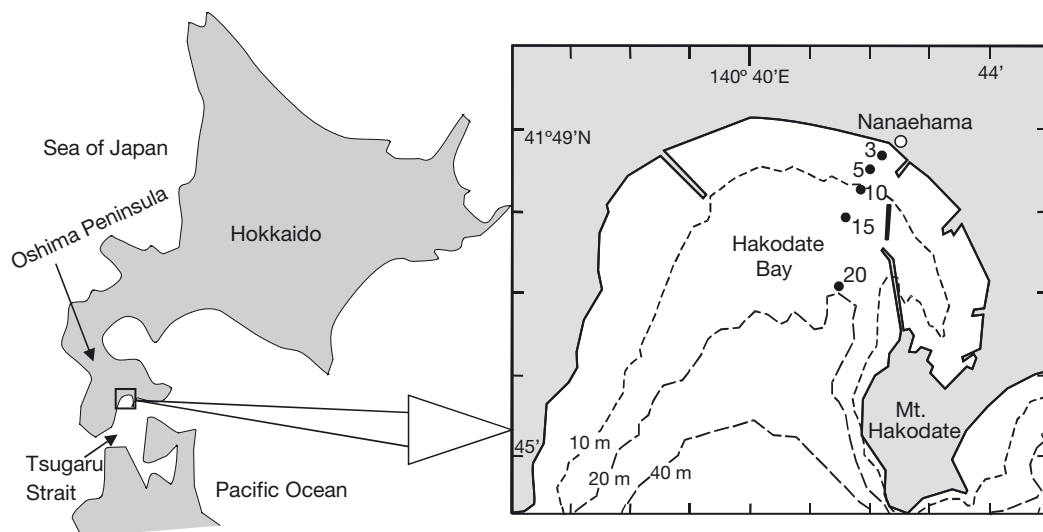

Fig. 1. Location of sampling stations and isobaths in Hakodate Bay, Japan. Solid circles indicate sampling stations used in the present study (numerals show the depth at each station)

MATERIALS AND METHODS

Study area, sampling, and treatment of specimens

Hakodate Bay is a small bay (ca. 66.9 km^2) located at the southern end of Hokkaido Island (Fig. 1). Adult marbled sole females spawn demersal adhesive eggs on the bottom along the 30 m isobath in the eastern part of the bay mouth near Mt. Hakodate (Fig. 1). Pelagic larvae are abundant in the eastern part of Hakodate Bay, but are scarce in the western part and outside of the bay (Nakagami et al. 2001). Settled juveniles are abundant off Nanaehama in the inner section of Hakodate Bay. Therefore, larvae and juveniles were collected along a transect of 5 stations beginning at Nanaehama out towards the spawning ground (Joh et al. 2009). These sampling stations were at depths of 3, 5, 10, 15, and 20 m, respectively.

Sampling for marbled sole larvae and small juveniles was conducted from March to May in 2001, 2002, and 2003 (Table 1). Pelagic larvae were collected in triplicate oblique tows of a plankton net (net opening diameter: 80 cm; mesh aperture: 0.33 mm; a flow meter was installed at the mouth) at each station. Larvae and juveniles distributed near and on the sea floor were collected by simultaneous tows with 2 Hirota's sledge nets (mouth opening: 60 × 40 cm; mesh aperture: 0.50 mm; Hirota et al. 1989). Sampling for larger juveniles was carried out in June and July. Simultaneous tows with 2 sledge nets were conducted 2 or 3 times at each station. Larvae and juveniles were preserved in a 90% ethanol solution. The densities of larvae collected by plankton net were converted to number per m^2 based on the rotations of the flow meter, the mouth opening of the net (0.50 m^2), and the length of warp. The density of juveniles collected by sledge net was converted to number per 100 m^2. The catch efficiency of the sledge net was assumed to be 1 (Joh et al. 2009).

Digital calipers were used to measure notochord length for larvae and standard length (SL) for juveniles. We used the lapilli for otolith microstructure analysis. Throughout the larval and juvenile stages, the lapilli of marbled sole can be observed without being sectioned (Joh et al. 2005). Both sides of the lapillar otoliths are similar in shape during the larval stage, but the lapilli of large juveniles are bilaterally asymmetrical (Joh et al. 2005). Therefore, we used ocular side lapilli for otolith measurements for the juveniles. The number of otolith increments was counted and the increment widths were measured using an otolith measurement system (ARP Ver. 5.00, Ratoc System Engineering Co.). See Joh et al. (2005,

Table 1. Numbers of sampling casts and the density of larvae collected with a plankton net and density of larvae and juveniles collected with a sledge net. Numbers in parentheses represent the density of juveniles; (–) no sampling cast

Year	No. of casts		Density	
	Plankton net	Sledge net	Plankton net (ind. m^{-2})	Sledge net (ind. 100 m^{-2})
2001				
9 Mar	15	10	0.1	0 (0)
26 Mar	15	10	8.9	0.5 (0)
6 Apr	15	14	0.7	0 (0)
16 Apr	15	14	0	0 (0)
25 Apr	15	14	1.1	0.8 (0.0)
8 May	10	16	0	0.9 (1.1)
23 May	4	19	0	0 (0.1)
10 Jul	–	18	–	0 (0.8)
2002				
13 Mar	15	5	18.5	3.7 (0)
25 Mar	15	10	16.9	10.1 (0)
5 Apr	15	14	61.3	73.7 (0)
15 Apr	13	8	2.2	10.3 (2.6)
24 Apr	15	18	0.1	4.1 (2.2)
6 May	12	16	0	0.3 (2.4)
20 May	–	18	–	0 (0.9)
17 Jun	–	12	–	0 (1.4)
28 Jun	–	12	–	0 (1.8)
26 Jul	–	12	–	0 (2.3)
2003				
6 Mar	15	10	13.6	0.3 (0)
14 Mar	15	12	59.8	9.9 (0)
25 Mar	15	10	6.4	3.8 (0)
1 Apr	15	12	5.7	0 (0)
15 Apr	15	12	4.5	1.8 (0)
25 Apr	15	12	0.2	3.2 (0)
6 May	15	16	0.1	1.3 (0.4)
23 May	–	14	–	0.1 (0.6)
9 Jun	–	8	–	0 (2.8)
30 Jun	–	10	–	0 (2.0)
30 Jul	–	13	–	0 (1.6)

2009) for details of otolith preparation and analysis for marbled sole.

Water temperature and prey density

On each sampling day, water temperature was measured using a Seabird CTD system (SBE-19, Sea Bird Electronics) in every 3 or 5 m depth layer from the sea surface to the sea floor (i.e. 0 and 3 m at Stn 3; 0 and 5 m at Stn 5; 0, 5, and 10 m at Stn 10; 0, 5, 10, and 15 m at Stn 15; 0, 5, 10, 15, and 20 m at Stn 20). In Hakodate Bay, pelagic larvae of marbled sole are scarce in the 0 m depth layer (Nakagami et al. 2001). Thus, for each sampling date, the mean water temperature for pelagic larvae was calculated for all lay-

ers except for the 0 m depth layer. Because juvenile marbled sole inhabit the sea floor, the mean water temperature for juveniles was calculated from temperatures measured on the sea floor at the 5 stations on each sampling date. Unfortunately, we could not measure water temperature daily; therefore, water temperature had to be estimated between 2 sampling dates. We assumed that the change in temperature between 2 sampling days was linear, and we interpolated the water temperature on each day between 2 sampling dates based on each line. In this way, we obtained a sequence of daily water temperature for each year and generated a 'temperature calendar.'

Using these data, we estimated the temperature that individual larvae and juveniles experienced. For each larva, the date on which otolith ring formation began was estimated based on otolith microstructure analysis, and the temperature trajectory from that date to the sampling date was extracted from the temperature calendar. For juveniles, the date on which each individual reached the juvenile stage was estimated from otolith microstructure analysis (see 'Definition of larval and juvenile ages'), and the temperature trajectory from that date to the sampling date was extracted from the temperature calendar. Individual data were then pooled by year, and the mean experienced water temperature was calculated for each age (see Fig. 3).

For pelagic larvae, the densities of copepod nauplii (the principle prey item for pelagic larvae) were estimated for each sampling day (Joh et al. 2009). Using the same method as that used to generate the temperature calendar, we assumed that the change in prey densities between 2 sampling days was linear, and we obtained the daily sequence of prey density for each year. Based on this daily sequence of prey density, the prey density trajectory for each pelagic larva was estimated individually.

Definition of larval and juvenile ages

For marbled sole, the onset of daily otolith increment formation occurs at the time of transition from endogenous to exogenous nutritional sources (Joh et al. 2005). In the present study, the age of larva was defined as the number of days after the onset of otolith ring formation (larval age: LA), not as the days after hatching.

Settlement of marbled sole larvae occurs before the completion of eye migration (Fukuhara 1988). On the lapillus, the outer check is formed at the onset of eye migration; thus, the rings formed inside and outside of the outer check are thought to be formed during the pelagic and demersal phases, respectively (Joh et al. 2005). For eye-migrating larvae, we measured the distance from the outer check to the otolith edge of the ocular side lapillus, and we pooled these measurements for the 2001 to 2003 year classes. Mean distance for the largest 10% of individuals was 17.1 µm. Therefore, we assumed that the first otolith ring in the juvenile stage formed 17.1 µm outside of the outer check. We defined the number of otolith rings formed outside that first ring as juvenile age (JA), not as the age after hatching.

Interannual growth differences and planktonic larval duration (PLD)

The otolith radii and body lengths were positively correlated for pelagic larvae, eye-migrating larvae, and juveniles. However, we did not express that relationship as a single formula throughout the larval and juvenile stages (Fig. 2). Therefore, we did not estimate somatic growth trajectories using back-calculation techniques. Instead, we treated the width of the otolith increment for each age (WA: width at age) as an alternative measure of somatic growth, as in Searcy & Sponaugle (2000) and Robert et al. (2007, 2010). In late March 2003, low temperature and low salinity Oyashio Coastal Water flowed into the study area; thus water temperature in this area declined considerably. Joh et al. (2009) reported that pelagic larvae that hatched before 17 March 2003 (i.e. the early hatched cohort) did not appear in the nutri-
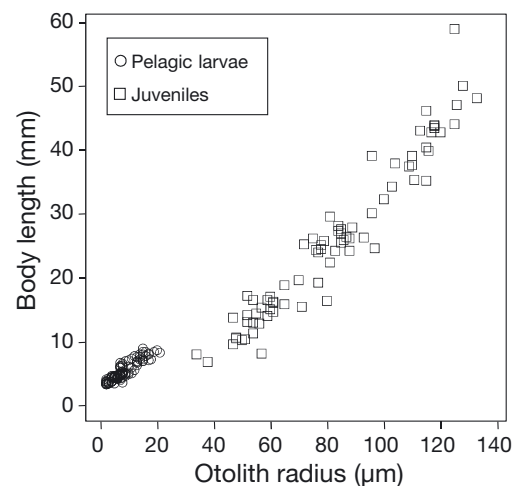

Fig. 2. *Pseudopleuronectes yokohamae*. Relationship between otolith radii and body lengths

tional transition date distribution (NTDD) after April. They suggested that almost all of the early hatched cohort may have died because of the critical decline in temperature. Therefore, we did not include the early hatched cohort collected in March 2003 in the analysis of the present study.

The growth patterns of pelagic larvae in the 3 yr classes were compared using repeated measures MANOVA, which is suitable for analysis of longitudinal data such as successive increment width data from fish otoliths (Chambers & Miller 1995). Repeated measures MANOVA allows for differences in the number of fish individuals among year classes, but it requires that the length of repeated measurements in each individual be equal. In the present study, we used ≥10 d LA larvae and compared WAs during 0 to 10 d LA. Similarly, we used ≥30 d JA juve-

niles and compared otolith growth during 0 to 30 d JA (Table 2).

To investigate the effect of water temperature and prey density on the growth of pelagic larvae, we used a generalized linear model (GLM). Individual mean WA, mean experienced temperature, and mean prey density for 0 to 10 d LA in 2001–2003 were pooled. Mean WA was used as the response variable, and mean experienced temperature, prey density, and the interaction term as explanatory variables. The response variable was assumed to follow a normal distribution. To minimize the number of parameters, model selection was conducted by maximizing the goodness of fit given by Akaike's information criterion (AIC; Akaike 1974). Analyses were carried out using the statistical software R, version 2.15.1 (http://cran.r-project.org/). To investigate the relationship

Table 2. Numbers of larvae and juveniles collected, used for growth comparison among years and for the comparison between survivor (SV) and original population (OP)

Date	No. of larvae			No. of juveniles		
	Collected	Interannual growth comparison	Comparison between OP and (SV)	Collected	Interannual growth comparison	Comparison between OP and (SV)
2001						
9 Mar	6	0	0 (0)	0	0	0 (0)
26 Mar	403	78	0 (0)	0	0	0 (0)
6 Apr	21	8	1 (0)	0	0	0 (0)
16 Apr	2	1	1 (0)	0	0	0 (0)
25 Apr	54	27	12 (5)	1	0	0 (0)
8 May	26	6	0 (5)	30	10	0 (0)
23 May	0	0	0 (0)	4	3	0 (0)
10 Jul	0	0	0 (0)	23	21	0 (0)
2002						
13 Mar	407	98	0 (0)	0	0	0 (0)
25 Mar	511	87	9 (0)	0	0	0 (0)
5 Apr	2922	88	18 (1)	0	0	0 (0)
15 Apr	207	32	8 (18)	44	10	0 (0)
24 Apr	127	53	0 (32)	66	44	0 (0)
6 May	8	6	0 (1)	65	39	3 (0)
20 May	0	0	0 (0)	26	15	6 (0)
17 Jun	0	0	0 (0)	28	23	0 (0)
28 Jun	0	0	0 (0)	37	23	0 (11)
26 Jul	0	0	0 (0)	45	40	0 (18)
2003						
6 Mar	350	112	0 (0)	0	0	0 (0)
14 Mar	1604	115	0 (0)	0	0	0 (0)
25 Mar	231	64	0 (0)	0	0	0 (0)
1 Apr	179	58	3 (0)	0	0	0 (0)
15 Apr	125	44	12 (0)	0	0	0 (0)
25 Apr	68	44	12 (8)	0	0	0 (0)
6 May	48	17	0 (14)	17	4	0 (0)
23 May	2	1	0 (1)	15	9	1 (0)
9 Jun	0	0	0 (0)	37	29	7 (0)
30 Jun	0	0	0 (0)	33	31	0 (31)
30 Jul	0	0	0 (0)	34	33	0 (31)

between the experienced water temperature and juvenile growth, individual mean WA and mean experienced temperature for 0 to 30 d JA in 2001–2003 were pooled, and the correlation between temperature and growth was analyzed.

The number of rings formed on the otoliths of eye-migrating larvae and juveniles from the start of ring formation to the end of pelagic life was defined as the PLD, and the relationship between growth during the pelagic larval phase and PLD was investigated.

Growth-selective survival

Marbled sole larvae settle on the sea floor during the eye-migrating stage (Fukuhara 1988), and these larvae are thus assumed to have survived pelagic larval life. We defined the eye-migrating larvae captured in late April–May as survivors ($SV_{pelagic}$). We compared their growth trajectories during the early pelagic larval stage (0 to 10 d LA) with those of their original population ($OP_{pelagic}$), i.e. the planktonic larval population that satisfied the following conditions: (1) larvae were collected from March to early April, (2) the nutritional transition date (NTD) of larvae was within the range of the NTDD for $SV_{pelagic}$, and (3) larvae were >10 d LA.

The sand shrimp *Crangon uritai* preys on metamorphosing larvae and juveniles (Nakaya et al. 2004a,b, 2007); to date, other demersal predators of marbled sole larvae and juveniles have not been found in Hakodate Bay (Nakaya 2004). Marbled sole juveniles >15.4 mm SL were not vulnerable to predation by *C. uritai* (Joh et al. 2009). Thus, we defined large juveniles (20.2 to 59.7 mm SL) collected in late June and July as survivors of predation ($SV_{juvenile}$), and we compared their otolith growth from 0 to 20 d JA with that of their original population ($OP_{juvenile}$). $OP_{juvenile}$ refers to small juveniles that satisfied the following conditions: (1) juveniles were collected from April to early June, (2) the date on which each juvenile started its juvenile life was within the range of dates on which $SV_{juvenile}$ started their juvenile life, and (3) individuals >20 d JA and <15.4 mm SL. We did not compare growth trajectories between $SV_{juveniles}$ and $OP_{juveniles}$ in 2001 because we did not collect sufficient numbers of small juveniles that satisfied the conditions for the original population.

If there is a wide NTDD range for $SV_{pelagic}$ and also a wide range of dates on which survivors began their juvenile lives, the environmental factors experienced by the early and the late cohorts of SV might differ. In such a case, dividing the SVs into seasonal subgroups may be a more appropriate way of conducting the analysis. However, the small size of our samples, together with other constraints (see 'Discussion'), meant that this was not appropriate in our case.

Comparison of early pelagic larvae and large juvenile densities

To evaluate interannual differences in survivorship from the nutritional transition (NT) stage to the end of shrimp predation, we compared the density of NT-stage larvae and large juveniles collected in July. In each year, the density of large juveniles collected in July was plotted against the density of larvae at the NT stage, and the slope was calculated. If the density of large juveniles is low but the density of larvae is high (i.e. low survivorship), the slope is low. The slope was defined as the survival index, and the index values were compared among years.

RESULTS

Water temperatures and prey densities experienced by larvae and juveniles

The water temperatures experienced by pelagic larvae 0 to 10 d LA differed among the 3 yr (repeated measures MANOVA: F: 225.7, p < 0.001). Mean temperature at 0 d LA was 7.1°C in 2001, 8.0°C in 2002, and 5.6°C in 2003, and the temperatures increased with time. The mean temperatures for the 0 to 10 d LA interval were high in 2002 (8.4°C; Fig. 3), medium in 2001 (7.5°C), and low in 2003 (6.1°C).

The prey densities experienced by pelagic larvae (0 to 10 d LA) also differed among the 3 yr (Fig. 3; repeated measures MANOVA: F: 574.6, p < 0.001). The mean prey densities experienced by pelagic larvae were high in 2001 (61.6 ind. l^{-1}), medium in 2003 (50.8 ind. l^{-1}), and low in 2002 (19.7 ind. l^{-1}).

The water temperatures experienced by juveniles (0 to 30 d JA) differed among the 3 yr (repeated measures MANOVA: F: 16.37, p < 0.001). The mean temperatures that juveniles (0 to 12 d JA) experienced were 9.7°C in 2002, and 2003 and 9.3°C in 2001 (Fig. 3). After 13 d JA, the increase in temperature in 2002 was slower than in the other years, and the mean temperatures experienced by juveniles of 13 to 30 d JA were low in 2002 (10.4°C), medium in 2001 (11.1°C), and high in 2003 (11.6°C).
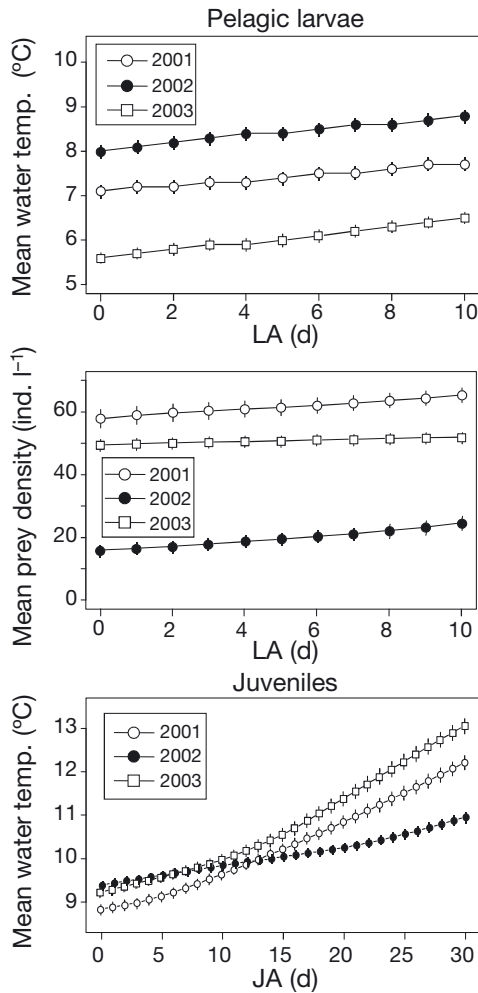
Fig. 3. *Pseudopleuronectes yokohamae*. Mean water temperature that pelagic larvae (top panel) and juveniles (bottom panel) experienced and mean prey densities (ind. l^{-1}) that pelagic larvae experienced (middle panel). The ages of larvae and juveniles were defined as the number of the days after the onset of otolith ring formation (larval age: LA) and the number of otolith rings formed outside the first increment in juvenile stage as juvenile age (JA). Vertical bars indicate SE

Comparison of otolith growth among year classes

In all year classes, the WAs were roughly constant for individuals aged 0–10 d LA. The mean WAs (±SD) for this age group (2001: 0.56 ± 0.12 µm; 2002: 0.60 ± 0.12 µm; 2003: 0.56 ± 0.12 µm) differed among year classes (repeated measures MANOVA: *F*: 6.88, p = 0.001). The mean WA in 2002 was significantly higher than those in 2001 and 2003 (Fig. 4), but there was no significant difference between the WAs in 2001 and 2003 for this age group (Scheffe post hoc comparison: range of p-values: 0.36 to 0.99).

Table 3. Generalized linear model analysis for the effect of water temperature and prey density on the growth of pelagic larvae. Temp and Prey: mean water temperature and prey density that pelagic larvae experienced during 0–10 d LA. temp: prey is an interaction term

Variables	Coefficient	Standard error	p-value
Temp	6.610×10^{-2}	2.435×10^{-2}	0.007
Prey	7.311×10^{-3}	4.287×10^{-3}	0.09
Temp: prey	9.126×10^{-4}	5.362×10^{-4}	0.09
Models	AIC		
Full	−361.5		
Temp + Prey	−360.6		
Temp	−362.6		
Prey	−344.1		

The effect of water temperature and prey density on the growth of pelagic larvae was analyzed using the GLM (Table 3), and the AIC of the model in which only water temperature was included as the explanatory variable was smallest among the models tested.

The mean WAs (±SD) for individuals of 0 to 30 d JA (2001: 1.56 ± 0.35 µm; 2002: 1.58 ± 0.41 µm; 2003: 1.77 ± 0.40 µm) differed among year classes (Fig. 4; repeated measures MANOVA: *F*: 19.47, p < 0.001). For most ages, the WAs in 2003 were higher than those in other years. From 2 to 13 d JA, the WAs in 2002 were medium and those in 2001 were low. However, from 14 to 18 d JA, the WAs in 2001 were similar to those in 2002, and the former became higher than the latter after 19 d JA. In the 2002 and 2003 year classes, the WAs of older juveniles decreased gradually. The WAs in 2001, however, decreased from 0 to 10 d JA, increased from 11 to 25 d JA, and then decreased slightly after 26 d JA. The mean WA for individuals of 0 to 30 d JA was significantly positively correlated with water temperature (r = 0.39, p < 0.001; Fig. 5).

The mean (±SE) PLDs of eye-migrating larvae and juveniles were 23 (±0.56) in 2001, 20 (±0.21), and 22 (±0.30) in 2003. The PLD and the mean WA during pelagic life were significantly negatively correlated (r = −0.46, p < 0.001).

Growth-selective survival

In 2001 to 2003, the WAs for 0 to 10 d LA did not differ between $SV_{pelagic}$ and $OP_{pelagic}$ (repeated measures MANOVA: 2001, 2002, and 2003: p = 0.91, 0.34, and 0.08, respectively; Fig. 6).

For the 2002 year class, the WAs of SV$_{juvenile}$ were significantly higher than those of OP$_{juvenile}$ (repeated measures MANOVA: *F*: 6.58, p = 0.01; Fig. 6). For the 2003 year class, the WAs of SV$_{juvenile}$ were similar to those of OP$_{juvenile}$ (repeated measures MANOVA: *F*: 0.52, p = 0.47). In 2002 and 2003, the WAs of both groups decreased with age.

Comparison of early pelagic larvae and large juvenile densities

The densities of larvae in the NT stage in March and April 2001, 2002, and 2003 were 2.1, 12.5, and 3.6 ind. m^{-2}, respectively. The densities of large juveniles collected in July 2001, 2002, and 2003 were 0.77, 2.25, and 1.57 ind. 100 m^{-2} respectively (Fig. 7). Thus, the survival indices in 2001, 2002, and 2003 were 0.36, 0.18, and 0.46, respectively. In 2002, larvae and large juveniles were abundant, but the survival index was the lowest among the 3 yr studied.

DISCUSSION

The effects of environmental factors on growth

The growth of pelagic larvae was highest in 2002 when water temperature was highest. For other flat-

fish species, positive relationships between water temperature and larval growth rate have been reported from rearing experiments (Japanese flounder *Paralichthys olivaceus*: Seikai et al. 1986; summer flounder *Paralichthys dentatus*: Burke et al. 1999; sole *Solea solea*: Lagardere 1989) and in the field (plaice larvae: Hovenkamp 1989, 1990). Generally, prey abundance is an important factor affecting larval growth. Copepod nauplii are major prey items for marbled sole larvae (Nakagami 2001). However, in the present study, the density of copepod nauplii was not an explanatory variable for the growth of pelagic larvae. Thus, our results suggest that water temperature may be a key factor affecting the growth of marbled sole pelagic larvae and that larvae may grow fast in the high water temperature range observed in 2001 to 2003. In contrast, the prey densities observed in 2001 to 2003 may not affect the growth of marbled sole larvae.

Pelagic larvae experienced distinctly different water temperatures in 2001 and 2003 (mean: 7.5 and 6.0°C, respectively). However, the mean WAs of pelagic larvae did not differ significantly between these 2 yr (mean: 0.56 µm in both years). Nakagami (2001) investigated the effect of water temperature and prey density on feeding by marbled sole pelagic larvae in Hakodate Bay from 1997 to 2000. He found that marbled sole larvae could not feed on prey organisms satisfactorily in water <7.5°C even if prey density was high. Moreover, he suggested that almost all larvae in 1999 might have starved and died due to failure to feed caused by the low water temperature. Feeding condition data for pelagic larvae in 2001 to 2003 were not available; however, water temperatures were 7.5°C in 2001 and <7.5°C in 2003. Therefore, the growth rate of pelagic larvae in 2001 and 2003 may have been low due to failure to feed caused by low water temperatures.

The PLD of eye-migrating larvae and juveniles was negatively correlated with growth during pelagic
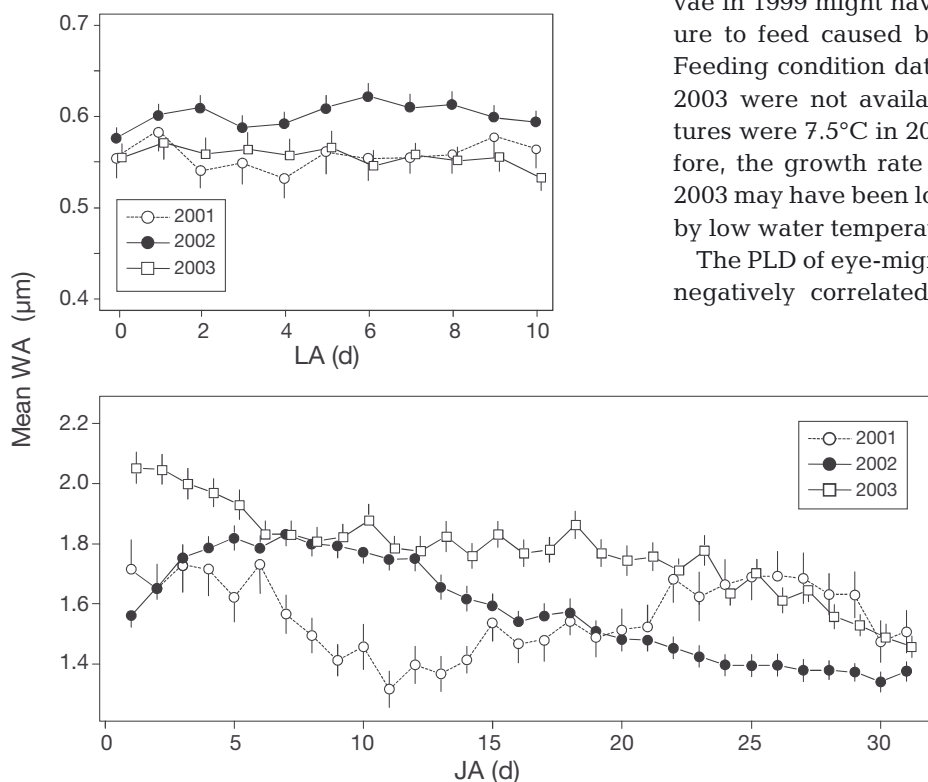

Fig. 4. *Pseudopleuronectes yokohamae*. Width of otolith rings at each age (WA: width at age) in pelagic larval stages (upper panel) and juvenile stage (lower panel). LA: larval age; JA: juvenile age (for definitions see Fig. 3). Vertical bars indicate SE (for the sake of clarity not all are shown)
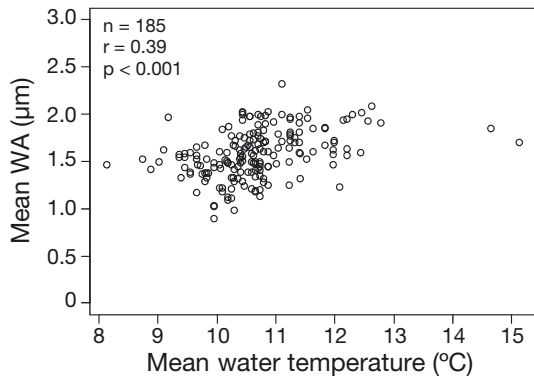
Fig. 5. *Pseudopleuronectes yokohamae*. Relationship between mean water temperatures experienced and mean width of otolith rings at age (WA) for individuals of 0–30 d JA

life. The present study showed that high water temperature may have enhanced the growth of pelagic larvae and that fast growth quickened larval development and shortened the PLD. In a rearing experiment, Mutsutani (1988) found a positive relationship between high water temperature and larval growth, and a negative relationship between larval growth and the length of PLD for marbled sole (range: 10 to 18°C). In the field, larval growth of North Sea plaice was high, and the number of days until the completion of metamorphosis was small in a warm year (Hovenkamp & Witte 1991).

The investigation of the effects of larval growth and the length of PLD on the body size of newly settled juveniles allowed us to examine whether such larval features affect size-selective mortality during the early juvenile phase. However, the relationship between otolith radii and body length was non-linear during the pelagic and eye-migrating stages (Joh et al. 2005). Similar non-linearity has been reported for
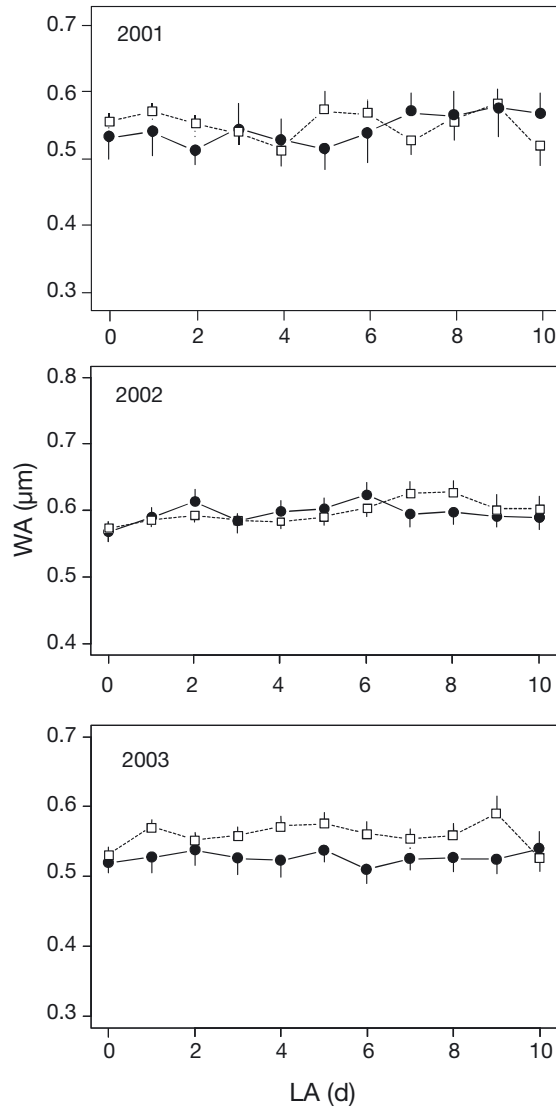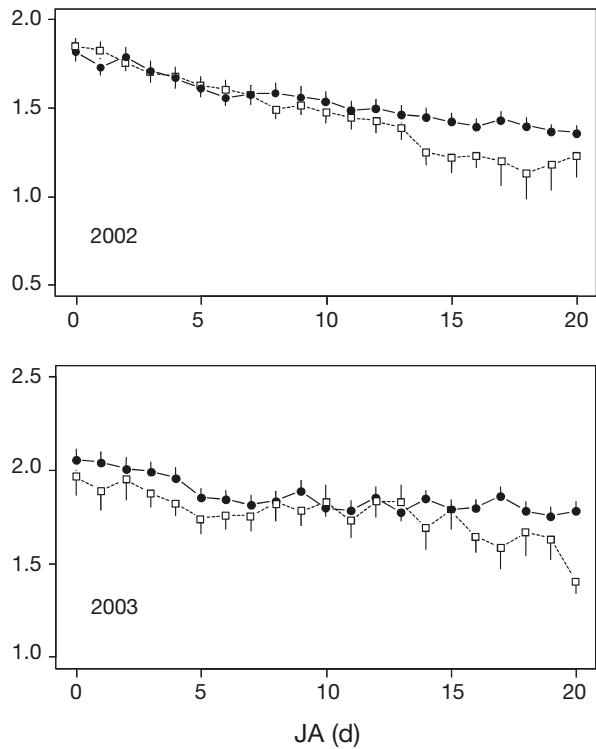

Fig. 6. *Pseudopleuronectes yokohamae*. Width of otolith rings at age (WA) of survivors (SV) and their original population (OP) in the pelagic larval stage (left panels) and juvenile stage (right panels). LA: larval age; JA: juvenile age (for definitions see Fig. 3). Vertical bars indicate SE (for the sake of clarity not all are shown)
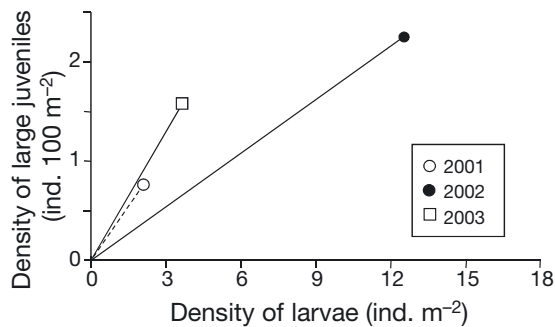
Fig. 7. *Pseudopleuronectes yokohamae*. Relationship between the densities of larvae at the nutritional transition stages (March and April) and large juveniles collected in July

many flatfish species. This non-linear relationship precludes the back calculation of body length by the biological intercept method (Campana 1990). Thus, we could not examine the effects of larval growth and the length of PLD on the body size of newly settled juveniles.

Juvenile growth and water temperature were positively correlated. Moreover, the patterns of seasonal and interannual change in otolith growth in the 2001 and 2002 year classes were similar to seasonal and interannual changes in water temperature. These results suggest that water temperature strongly affected the growth of juveniles, as it did for larvae. Positive relationships between water temperature and the growth of juveniles have been reported for other flatfish species (sole and plaice: Amara 2003). Conversely, May & Jenkins (1992) reported that the relationships between water temperature and the growth of juvenile flounder *Rhombosolea tapirina* were positive for the early cohort that hatched in winter but negative for the late cohort that hatched in spring. The relationship between water temperature and the growth of larvae and juveniles is usually dome shaped (Boehlert 1981), and growth rate increases with the rise of temperature below the most suitable temperature but decreases beyond the optimum. In the present study, the growth rate of juvenile marbled sole was high during the warmer year. Thus, in Hakodate Bay, the highest water temperature observed in the present study might be below the optimum.

Generally, prey abundance is one of the important factors affecting the growth of fish juveniles (Karakiri et al. 1989, 1991, Malloy et al. 1996). However, van der Veer (1986) reported that prey abundance did not affect the growth of juvenile plaice. The principal prey item for small marbled sole juveniles in May to August in Hakodate Bay is demersal harpacticoid

copepods (Nakagami et al. 2000). We did not investigate the effect of prey abundance on marbled sole juveniles in the present study because of the difficulty in sampling these copepods. The effects of prey abundance on the growth of marbled sole juveniles need to be evaluated in rearing experiments in the future.

Growth-selective survival

In the growth comparison between SV and OP, the larvae and juveniles included in OP were limited by their NTD or by the date when they became juveniles. If there is a wide NTDD range for $SV_{pelagic}$ and also a wide range of dates on which survivors began their juvenile lives, the environmental factors experienced by the early and the late cohorts of SV might differ. In this case, dividing the SVs into seasonal sub-groups may be a more appropriate way of conducting the analysis. However, the span of days between the 25th and 75th percentiles of the NTD of $SV_{pelagic}$ was relatively narrow (9 d in 2001, 8 d in 2002 and 2003; Fig. 8). The span of days between the 25th and 75th percentiles of the day when individuals of $SV_{juveniles}$ became juveniles was also narrow (9 d in 2002, 7 d in 2003), and the changes in water temperature were relatively stationary. Thus, we did not conduct sub-grouping in the present study. Water temperature during the larval phase in 2003 was lower in the early phase than in the late phase (upper panel in Fig. 8), so the survival pattern might differ between the early and the late cohorts of $SV_{pelagic}$. However, sub-grouping could not be conducted for $SV_{pelagic}$ in 2003 because of the small numbers of specimens.

During the early pelagic stage, growth-selective larval survival was not detected for any of the year classes, although water temperature was low in 2003. The growth-mortality hypothesis is based on the concept that fast-growing larvae and juveniles have the advantage of high survival by growing large enough to avoid predation (Anderson 1988). However, predators of marbled sole pelagic larvae have not been identified to date, and predation risk has not been estimated in Hakodate Bay and its vicinity.

Starvation might be another source of larval and juvenile mortality. Almost all larvae that underwent NT before 17 March 2003 were thought to have died due to failure to feed caused by extremely low water temperature (Joh et al. 2009). However, larvae that underwent NT after 17 March 2003 could have survived to the juvenile stage, and growth-selective mortality was not detected for larvae in 2003.
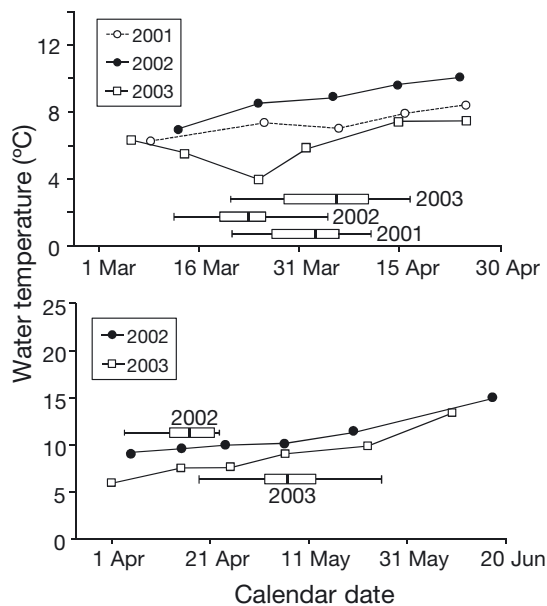
Fig. 8. Seasonal changes in water temperature in the water column exclusive of the surface layer (upper panel), and on the sea floor (lower panel). Horizontal box plots in the upper panel show the nutritional transition date range for surviving eye-migrating larvae (SV$_{pelagic}$) and those in the lower panel show the range of dates on which large juveniles collected in June and July (SV$_{juveniles}$) started demersal life. Whiskers indicate the minimum and maximum values. Boxes indicate the 25th and 75th percentiles and the vertical bars in the boxes indicate the mean

Among flatfish species, the PLD of marbled sole is relatively short, and this may be another reason for the absence of growth-selective survival of pelagic larvae. Of the 120 flatfish species that inhabit the northern Pacific, only 5 (including marbled sole) spawn adhesive and demersal eggs. This characteristic probably prevents the risk of advection of eggs (i.e. passive transport) to unsuitable locations (Rijnsdorp & Witthames 2005). Moreover, eye-migrating larvae of marbled sole start their demersal life before the completion of metamorphosis (Fukuhara 1988). Therefore, the PLD of marbled sole larvae may be shorter than that of other flatfishes that spawn floating eggs and start their demersal life after the completion of eye migration.

In the early juvenile stage, growth-selective survival was detected in 2002 but not in 2003. Sand shrimps are known to be predators of demersal larvae and juveniles for some flatfish species (van der Veer et al. 1991, Witting & Able 1995, Oh et al. 2001, Taylor & Collie 2003). In Hakodate Bay, predation on demersal larvae and juveniles of marbled sole by *Crangon uritai* has been reported (Nakaya et al.

2004a,b, 2007), but no other predator has been observed (Nakaya 2004). Therefore, we propose that growth-selective survival of the juvenile stage may occur in relation to predation by *C. uritai*. Population growth of juveniles was slow in 2002 because of low water temperature. Thus, juveniles required time to exceed the size spectrum that is vulnerable to shrimp predation; the individuals that grew more rapidly were able to survive, thereby exhibiting growth-selective survival. In contrast, survival in the juvenile stage was not growth related in 2003. In 2003, water temperature experienced by pelagic larvae was low, but it quickly increased during the early juvenile stage, thus allowing juveniles to grow quickly in the warmer environment. In 2003, juveniles likely passed more rapidly through the risky size spectrum preferred by shrimp, and consequently growth-selective survival was not detected in 2003.

Comparison of early pelagic larvae and large juvenile densities

The survival index in 2002 was 0.18 and was the lowest among the 3 yr classes (2001 and 2003: 0.36 and 0.46, respectively). This means that survivorship between the completion of the NT and the end of the phase vulnerable to predation by sand shrimps was likely low in 2002. The growth rate of pelagic larvae and water temperature were high in 2002 but low in 2001 and 2003. Additionally, survival of the pelagic larval stage was not growth selective in any of the years studied. These results suggest that the difference in survivorship between 2002 and 2003 was not related to the pelagic larval stage.

Population growth rate of juveniles and water temperature were lowest in 2002, and survival of the early juvenile stage was growth selective in 2002 (but not in 2003). These findings suggest that for marbled sole in Hakodate Bay, interannual fluctuations in cumulative survivorship throughout the pelagic larval and early juvenile stages were more affected by growth and survival of the juvenile stage than those of the pelagic larval stage. Although we did not evaluate the occurrence of density-dependent mortality of early juveniles in 2002, predation on larval and juvenile marbled sole by *Crangon uritai* is reportedly density independent, size specific, and spatial-distribution-overlap dependent (Nakaya et al. 2004a, 2007).

Joh et al. (2009) suggested that almost all of the pelagic larvae hatched before 17 March 2003 may have died due to the critical decline of temperature

caused by intrusion of cold Oyashio Coastal Water. Therefore, we propose that the pelagic larval stage is also an important period for survival of the population under extremely severe conditions. The Tsugaru Warm Current flows into the bay throughout the year. Hakodate Bay is the northernmost habitat of marbled sole. The spawning season of marbled sole in Hakodate Bay starts in late winter and ends in early spring (February to April), when water temperatures are lowest throughout a year. We suggest that the large volume of thermal transport by the Tsugaru Warm Current to the bay probably sustains the reproduction of marbled sole in Hakodate Bay.

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