RESEARCH ARTICLE

P. Guido · M. Omori · S. Katayama · K. Kimura

Classification of juvenile rockfish, *Sebastes inermis*, to *Zostera* and *Sargassum* beds, using the macrostructure and chemistry of otoliths

Abstract Young-of-the-year (YOY) *Sebastes inermis* use *Zostera* and *Sargassum* beds as nursery grounds, although it is not known which habitat YOY prefer. In this study, YOY *S. inermis* were accurately assigned to *Zostera* or *Sargassum* beds by two approaches: the width and length of the otolith nucleus and the composition of trace elements in otoliths. The otolith nucleus was initially opaque and then showed a marked shift to hyaline deposition once YOY settled in the nursery grounds. The first hyaline zone (FHZ) was deposited earlier in *Zostera* beds (from mid-May to early June) than in *Sargassum* beds (around mid-summer). Likewise, irrespective of settlement year, the FHZ was formed at both significantly younger ages and shorter back-calculated sizes (total length, TL) in the *Zostera* bed (overall mean: 131 ± 3 days; 2.5 ± 1.7 mm TL) than in the *Sargassum* bed (overall mean: 158 ± 12 days; 61.3 ± 1.00 mm TL). YOY collected in the *Zostera* bed were born earlier (mainly in January) than YOY from the *Sargassum* bed (mainly in February). In addition, a significant linear relationship was found between the age at formation of the FHZ and nucleus dimensions, suggesting that nucleus dimensions were a reliable macroscopic indicator of the time of formation of the FHZ and, consequently, also an indicator of the nursery where YOY grew. Linear discriminant function analysis (LDFA) based on the width and length of the otolith nucleus could distinguish juveniles from *Zostera* (88– 96%) and *Sargassum* (96–97%) beds with a high degree of accuracy. In the other approach, six detectable trace elements (Li, Mn, Ni, Cu, Zn, and Ba) in otoliths of YOY collected in the nursery grounds were measured by high-resolution, inductively coupled mass spectrometry. LDFA based on the trace elemental composition separated YOY from three nurseries with 100% of accuracy. Findings suggest that both the trace elemental composition and nucleus dimensions of otoliths can be used as natural tags of the nursery grounds of *S. inermis*, offering a considerable potential for answering questions on habitat use and the contribution of nursery grounds to the adult stock.

Communicated by T. Ikeda, Hakodate

P. Guido (✉) · M. Omori · S. Katayama · K. Kimura
Department of Applied Bio-Science, Faculty of Agriculture,
Tohoku University, 1-1 Tsutsumi-dori, Amamiya-machi,
Aoba-ku, Sendai 981-8555, Japan
E-mail: guido@affrc.go.jp
Tel.: +81-88-8325146
Fax: +81-88-8313103

Present address: P. Guido
Kuroshio Research Division,
National Research Institute of Fisheries Science,
Sanbashi-dori 6-1-21, Kochi 780-8010, Japan

Introduction

Macrophytes, such as large algae and seagrasses, are important nursery areas for many fish species in various shallow coastal zones (Adams 1976; Bell and Westoby 1988; Jenkins et al. 1996; Levin and Hay 1996; Schaffelke and Klumpp 1996). Such species include rockfishes, which after their planktonic stage settle simultaneously into seagrass and algal beds, migrating with growth to deeper habitats, where they contribute to coastal fisheries (Love et al. 1991; Moser and Boehlert 1991). Knowledge of the contribution of each nursery ground is mandatory for fisheries management and for developing measures to protect nursery areas affected by human activities. However, quantifying the proportion of adult fish that grew in a given nursery ground is difficult without marking and recapture procedures (Haines and Modde 1996; Frederick 1997), which are expensive and time consuming. An alternative would be to find indicators acting as natural tags of the nursery grounds where the fish lived.

The hyaline zone of otoliths from temperate fishes generally reflects seasonal changes in water temperature and changes in the growth rate of the fish (William and

Bedford 1974; Jerald 1983; Casselman 1987; Schramm 1989; Beckman and Wilson 1995). Consequently, in addition to temperature, many factors triggering a reduction in growth might, in part, be responsible for the appearance of the hyaline zone, e.g. reproduction, lack of food, or environmental perturbation. Recently, Pearson (1996) found that the timing of hyaline zone formation in a rockfish was unrelated to reproduction and, instead, appeared to be linked to increasing water temperature. In rockfishes, the nucleus is generally opaque, encompassing the period from hatching through the larval and early juvenile stages during the first year of life, when the first hyaline zone (FHZ) is deposited. This is the case in *Sebastes. inermis*, in which the FHZ forms after young-of-the-year (YOY) settle into *Zostera* and *Sargassum* beds (Plaza et al. 2001). In Sendai Bay, northern Japan, both habitats are shallow (to about 2 m in depth), although *Zostera* beds are confined to more enclosed areas with soft bottoms and *Sargassum* beds generally occupy the subtidal area on hard sea bottoms along the rocky shore. Hence, the enclosed nature of *Zostera* beds, presumably with higher water temperature than that in *Sargassum* beds, may trigger the formation of the FHZ earlier. In addition, it is reasonable to expect a direct relationship between dimensions of the otolith nucleus (i.e. width and length) and the timing of formation of the FHZ; consequently, the nucleus dimensions may potentially provide a natural tag for YOY growing in *Zostera* and *Sargassum* beds.

On the other hand, the ability of otoliths to record chronological events applies also to the elemental composition of the ambient water to which the fish are exposed (Kalish 1989; Dove et al. 1996; Gillanders and Kingsford 1996; Milton et al. 1997; Campana 1999). Otoliths are formed from the crystallization of calcium carbonate in layers over time. The major component is calcium carbonate ($\sim$90%); the remainder is composed of an organic matrix of minor elements ($>$100 ppm) and trace elements ($<$100 ppm, most $<$10 ppm) (Campana 1999). The calcium carbonate and the trace elements seem to be derived from the water; hence, trace element composition in otoliths may be a natural tag, because it reflects the characteristics of the water where the fish lives (Campana 1999; Rooker et al. 2001, 2003).

The aims of this research were to evaluate two new approaches for assigning YOY *S. inermis* to *Zostera* and/or *Sargassum* beds: (1) by measuring the width and length of the otolith nucleus and (2) by determining the composition of trace elements in otoliths. First, we estimated the timing of formation of the FHZ and its relationship with nucleus dimensions. Then, we tested whether the width and length of the otolith nucleus can act as natural tags of the nearshore nursery grounds where the YOY grew. In parallel, we used high-resolution, inductively coupled plasma mass spectrometry (HR-ICPMS) to evaluate whether the elemental composition in otoliths of YOY *S. inermis* can be used as a natural tag as well.

Materials and methods

Timing of formation of the first hyaline zone

The date of formation of the FHZ was analyzed in settled YOY collected from the Matsushima *Zostera* bed from March to August in 1999–2001 and from the Kitsunezaki *Sargassum* bed in 2000 (July) and 2001 (August) (Fig. 1; Table 1). Samples from the *Zostera* bed were collected in the daytime with a small trawl net that had a total wing length of 14 m, a height of 1.5 m, a 5-mm mesh at the wing, and a 3-mm mesh at the cod end. Two 5-m ropes were attached to each end of the wings and tied to two long poles fixed to the front and rear on the right side of a small-engine boat. A 150-m rope was mounted in a rotator device equipped with a cylindrical part and a crank. A bamboo stick, to which the end of the 150-m rope was tied, was sunk in the mud bottom. The whole rope was released as the boat was driven covering the 150-m rope, and then the boat was turned perpendicular to the rope. At this time, the net was deployed from the left side and was then pulled by turning the crank manually across the 150-m rope (see Plaza et al. 2002 for a schematic diagram of the sampling procedures). Samples from the *Sargassum* bed were collected in set nets installed overnight in inshore waters
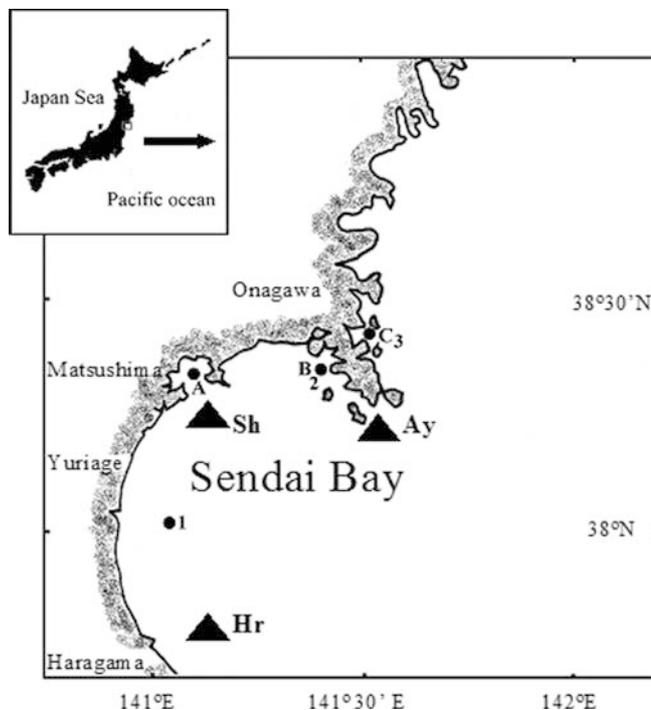

Fig. 1 *Sebastes inermis*. Map showing the nursery grounds in northeast Miyagi, Japan, in which young-of-the-year (YOY) were collected in the summer of 2000 and 2001. *Numerals close to the letters* denote the stations at which surface-water temperature was recorded (*A* Matsushima *Zostera* bed; *B* Kitsunezaki *Sargassum* bed; *C* Onagawa *Sargassum* bed; *triangles* locations where the adult fish where collected; *Ay* Ayukawa; *Sh* Shichigahama; *Hr* Haragama; *1* Yuriage)

Table 1 *Sebastes inermis*. Summary statistics (in order of research purposes) of fish collected in *Zostera* and *Sargassum* beds (juveniles) and along the rocky shore (adults) in Sendai Bay, northern Japan [*N* numbers of fish in which the nucleus dimensions were measured; *1* timing of formation of the first hyaline zone (FHZ), age and back-calculated size at FHZ, birth dates; *2* relationships between the FHZ and the nucleus dimensions of otoliths; *3* linear discriminant function analysis (LDFA) using otolith dimensions; *4* classification of adults using the otolith dimensions; *5* LDFA using the trace element composition of otoliths]

Habitat	Location	*N*	Year	Collectiion date	Purpose	Mean total length (±SD, mm)	Range
Sargassum	Kitsunezaki	116	2000	12 Jul	1[a], 3[a]	64.77 ± 3.23	53–72
		101	2001	21 Aug	1, 3	74.59 ± 3.60	67–83
	Onagawa	40	2001	1–5 Aug	3, 5[a]	70.14 ± 4.23	64–81
Zostera	Matsushima	150[b]	1999	23 Jun & 24 Aug	1[a], 2[a]	64.69 ± 6.25	44–80
		105	2000	Mar–Aug	1, 2[a], 3	64.69 ± 7.64	45–81
		96	2001	11 Jun–2 Jul	1, 2, 3	53.15 ± 6.75	44–75
Rocky shore	Ayukawa	772	2000–2001	7 Oct–9 Mar	4	200.12 ± 14.36	14–26
	Shichigahama	282	2000–2001	7 Oct–9 Mar	4	200.55 ± 17.54	13–27
	Haragama	300	2000–2001	7 Oct–9 Mar	4	210.75 ± 17.92	16–29

[a]Subsamples from "*N*" were analyzed (indicated in their respective results)
[b]In this case "*N*" denotes YOY collected

close to areas where *Sargassum* algae are the main vegetation. Juveniles were preserved in 95% ethanol so that the otoliths could be removed later. The total length (TL) was measured to the nearest 0.01 mm, without correction for shrinkage. All otoliths examined showed an opaque nucleus, with a hyaline zone at the edge. Sagittae were thin enough for estimating the date of formation of the first hyaline zone (DFHZ) by subtracting the daily increments in the hyaline zone from the collection date (Fig. 2). Otoliths were put in a small dish containing distilled water and observed on a dark background through a dissecting microscope connected to an image analysis system (Quantimet 600, Leica). A preliminary examination of the ventral edge of 30 otoliths found no significant difference (ANOVA, $P > 0.05$) in the mean DFHZ between right and left sagittae, although to keep consistent, the daily increments in the hyaline zone were counted on the ventral edge of the left sagittae. The DFHZ was previously measured in whole otoliths, and then otoliths were polished until the nucleus became clearly visible, with 800–2,000-grit lapping films and 4,000-grit grinding paste. Growth increments were counted and measured under a light microscope connected to the image analysis system at magnifications of ×400–1,000. Increments were counted twice across the area of distinctive increments. More detailed information on otolith preparation and age validation for this species were given by Plaza et al. (2001).

To get a better picture of the seasonal variations in surface-water temperature in Sendai Bay, we examined monthly data from April to August of 1998–2001 recorded at three stations by the Miyagi Prefectural Research Center. We compared these data with records of temperature obtained in the Matsushima *Zostera* bed in the 4 years studied (Fig. 1).

Age and size at the formation of the first hyaline zone

Both the age at formation of the first hyaline zone (AFHZ) and the size at formation of the first hyaline zone (SFHZ) were studied in the same nursery grounds and years as the DFHZ. The AFHZ was estimated by subtracting the daily hyaline increments from the total age. In addition, we focused on the relationship of the AFHZ to birth date and to width and length of the nucleus. For this purpose we used a subsample of 100 YOY caught in the Matsushima *Zostera* bed in 2001, because samplings were continuous enough to get juve-
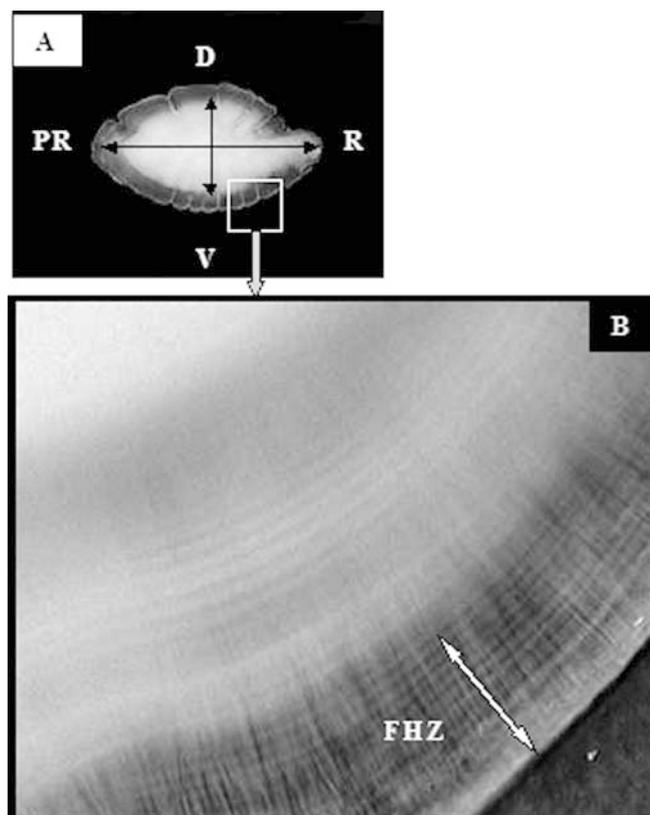

Fig. 2A, B *Sebastes inermis*. Macrostructure (**A**) and microstructure (**B**) of a left sagitta from a YOY (60 mm total length) collected in summer in the Kitsunezaki *Sargassum* bed. *Horizontal and vertical arrows* denote the nucleus length and width. The *white oblique arrow* denotes the days subtracted from the catching date to estimate the date at formation of the first hyaline zone (*FHZ*) (*PR* postrostrum; *R* rostrum; *D* dorsal; *V* ventral)

niles at different ages. The parturition season of *Sebastes inermis* starts in late December; hence, 1 December was set as day 1 to transform birth dates into numerical data.

Since a linear relationship between otolith size and fish size has been described for this species after settlement into seagrass beds (Plaza et al. 2001), we were able to back-calculate the SFHZ. The biological intercept method (Campana and Jones 1992; Campana 1999) was used in the back-calculations as follows:

$$SFHZ = L_c + (W_{hz} - W_c)(L_c - 6.63)(W_c - W_i)^{-1} \quad (1)$$

The value of 6.63 is the mean total length measured from larvae at parturition under rearing conditions (Plaza et al. 2001), W_i is the otolith width at parturition (i.e. the otolith diameter due to the roundness of saggitae at parturition), W_{hz} is the nucleus width at the formation of the first hyaline zone, and L_c and W_c are the fish size and otolith width at the time of capture, respectively.

Not all the otoliths for which otolith dimensions were measured could be used for estimations of the AFHZ and SFHZ. To estimate the AFHZ the otolith must be polished; hence, the sampling size depends upon the readability of otolith increments after polishing. Some otoliths were discarded due to poor resolution of their otolith increments. Therefore, subsamples used for estimating AFHZ were smaller than those used for estimating SFHZ because the width of otolith at parturition, at the time of FHZ, and at the time of catching (the three variables needed to estimate the SFHZ) could be measured in most of the discarded otoliths. In both cases, however, subsamples were sufficiently large to apply the statistical tests needed (see "Statistical analysis") to reach readable conclusions.

Nucleus dimensions and trace elements of otoliths

YOY *S. inermis* collected in the three nearshore nursery grounds (Matsushima *Zostera* bed, Kitsunezaki *Sargassum* bed, and Onagawa *Sargassum* bed) in the summer of 2001 were used for comparison (Fig. 1; Table 1). The left and right sagittae were removed with plastic forceps, cleaned of any adhering tissue in Milli-Q water, and stored in polystyrene culture clusters. The left sagita of each pair was used for examining the otolith macrostructure and the right one for microchemistry. The width and length of the nucleus from 169 YOY were measured to the nearest 0.01 mm with the image analyzer. The nucleus width was measured from the dorsal to the ventral edge, and the nucleus length was measured along the postrostrum and rostrum axes (Fig. 2). The left sagittae from a subsample of 18 YOY (Matsushima: $N=8$, mean TL $=63.5\pm2.7$ mm; Kitsunezaki: $N=5$, TL $=69.3\pm4.9$ mm; Onagawa: $N=5$, TL $=77.1$ ±1.3 mm) were used for analysis of trace elements. They were cleaned for a few seconds in 1% HNO_3 solution and then rinsed repeatedly in Milli-Q water.

Otoliths were then weighed on an analytical balance to the nearest 0.01 mg. Dried sagittae were then dissolved in a solution of 100 µl HNO_3 and 300 µl Milli-Q water in 10-ml polycarbonate tubes. Then, 100 µg l^{-1} of Indium solution at a concentration of 1 µg l^{-1} was added as an internal standard. Milli-Q water was added to give a final concentration of 1% HNO_3. Blanks were prepared following the same procedures, but without otoliths. Elemental concentrations were measured by high-resolution ICPMS (Element, Finnigan MAT, Germany) in medium-resolution mode (R $=3,000$). A preliminary study suggested that six elements (Li, Mn, Ni, Cu, Zn, and Ba) were detectable by HR-ICPMS. Sample solutions were introduced into the ICPMS with a high-efficiency pneumatic nebulizer. The ICPMS was calibrated by making working standards from multi-element solution standards (XSTC-1, -7, -8, and -331; SPEX Certi-Prep, USA). Standards were made in 5% HNO_3 containing Indium at 1 µg l^{-1} at concentrations of 0.1, 1, 5, and 10 µg l^{-1}. The standards and blanks were run at the beginning of each session. Samples were measured at random, and a new blank was also measured at the end of the measurements. The detection limits for the trace metals of interest, determined as three times the SD of filter blanks and converted to a weight basis (values in ppm), were as follows: (Li[7] 0.062, Mn[55] 0.126, Ni[62] 0.060, Cu[63] 0.042, Zn[66] 0.051, Ba[137] 0.045). More than 80% of the concentrations measured in this study were at least one order of magnitude higher than the detection limits.

To understand the annual variability of nucleus dimensions, we extended the analysis to dry-preserved otoliths collected in the Kitsunezaki *Sargassum* bed and Matsushima *Zostera* bed in 2000 (Table 2). Unfortunately, an analysis during the same year was not possible for trace elements of otoliths. Birth dates of YOY for the LDFA using nucleus dimensions, as well as those of YOY collected in Matsushima Bay in 1999, were back-calculated, subtracting the total age in days from the calendar day of sampling. Birth date distributions were not corrected for mortality effects, because survival rates during the larval stage are unknown for *S. inermis*. Numerical data of birth dates were compared using two samples Kolmogorov–Smirnov test (K–S, Sokal and Rohlf 1995).

Statistical analysis

One-way ANOVA (Sokal and Rohlf 1995; Zar 1998) was used to test for significant differences in AFHZ and SFHZ. The Shapiro–Wilks and Bartlett tests were used to test for normality and homogeneity of variance in the ANOVA procedures, and log transformations were made when it was necessary. Tukey's honestly significant differences (HSD) test was used for multiple comparisons. One-way ANOVA was also used to test for significant differences in each element and in the width and length of the otolith nucleus among nursery grounds.

Table 2 *Sebastes inermis*. Mean age and mean back-calculated size at first hyaline zone formation of young-of-the year caught in three nursery grounds in Sendai Bay, northern Japan (*S, Sargassum* bed; *Z, Zostera* bed; *T*, Tukey's honestly significant differences test at $P < 0.05$, *different letters* show significant differences among collections)

Location	Bed	Year	Mean ± SD	Range	N	T
Mean age (days)						
Kitsunezaki	S	2000	146 ± 6	135–156	66	a
		2001	170 ± 13	140–194	101	b
Onagawa	S	2001	157 ± 8	143–171	40	c
Matsushima	Z	1999	134 ± 11	115–158	60	d
		2000	129 ± 13	90–162	105	d
		2001	132 ± 13	96–162	96	d
Mean back-calculated size (mm)						
Kitsunezaki	S	2000	62.1 ± 3.5	51–71	66	f
		2001	62.4 ± 4.4	53–74	101	f
Onagawa	S	2001	60.1 ± 3.5	55–67	40	f
Matsushima	Z	1999	48.8 ± 4.7	38–57	60	g
		2000	49.5 ± 4.4	45–58	92	g
		2001	46.4 ± 5.8	35–62	96	g

Linear discriminant function analysis (LDFA) was used to evaluate whether trace elements or nucleus dimensions can accurately indicate the nursery origin of juvenile *S. inermis*. The error rate of the LDFA was evaluated using the "leave-one-out method" (cross-validation), because it is markedly superior for small data sets than the "hold-one-out method" (Stone 1977).

Finally, 1,354 adults collected from three locations in Sendai Bay were categorized by LDFA into *Zostera* or *Sargassum* origin, based on the nucleus dimensions. Adults were caught using angling and gill nets through cooperative research with fishermen in three locations along the coast of Sendai Bay from October 2001 to March 2002 (Ayukawa, Shigahama and Haragama; Fig. 1).

Parallel to the classification by LDFA and in order to evaluate the discriminatory characteristics of the nucleus dimensions, the frequency distributions of the widths and lengths of the otolith nuclei from all fish collected were compared for the three locations along the rocky shore. To assess the variability when mixing year classes, the frequency distributions of nucleus dimensions of 3-year-old fish were also analyzed. Fish were aged using surface readings after placing the sagittae in a capsule containing distilled water and then observing them using an image analysis system supported by a stereomicroscope. The opaque zone was taken as being the annulus, because it is formed once a year for this species (Hatanaka and Iizuka 1962; Yokogawa et al. 1992).

Taxonomic status of *S. inermis*

The rockfish *S. inermis* has several color morphotypes, which have, to date, been considered to indicate intraspecific modification caused by environmental factors. Recently studies have also found morphological and genetic differences among the three color variations of *S. inermis* (morphotype A: body reddish, morphotype B: body blackish, and morphotype C: body dark brownish), suggesting reproductive isolation (Kai and Nakabo 2002; Kai et al. 2002). Consequently, the current taxonomic status quo of *S. inermis* is unknown. The three morphotypes are recognizable in settled juveniles (the ontogenetic stage considered in our study). Hence, it is important to mention that this research was focused on blackish *S. inermis*, the only morphotype found in Sendai Bay during the study period.

Results

Seasonal fluctuations in water temperature

In general, surface-water temperature followed the same seasonal tendency in the 4 years analyzed, ranging from 7°C in early April to about 29°C in August. Surface-water temperatures were higher in the Matsushima *Zostera* bed than at the other sites. In particular, temperatures at Matsushima (from May to July) were over 5°C higher than in Kitsunezaki and Onagawa *Sargassum* beds (Fig. 3).

Timing of formation of the first hyaline zone

ANOVA of the DFHZ showed significant differences between the Matsushima *Zostera* and the Kitsunezaki *Sargassum* beds (ANOVA, $P < 0.05$). The FHZ began to be formed from mid-May to early June in the Matsushima *Zostera* bed for the 3 years analyzed, but in summer in the Kitsunezaki *Sargassum* bed (Fig. 4). Further, hyaline zones were formed earlier in this *Sargassum* bed in 2000 than in 2001. However, these differences may be a result of an underestimation of the mean dates, because in 2000 YOY were caught in July, and most otoliths were fully opaque.

Age and size at the first hyaline zone formation

Because the sampling design was not consistent among nurseries and years, one-way ANOVA was used to test for significant differences in both AFHZ and SFHZ. AFHZ ranged from 90 to 194 days, varying significantly between *Zostera* (overall mean: 131 ± 3 days) and *Sargassum* (overall mean: 158 ± 12 days) beds (ANOVA, $F_{5, 493} = 137.7$, $P < 0.001$; Table 2). Mean AFHZ did not
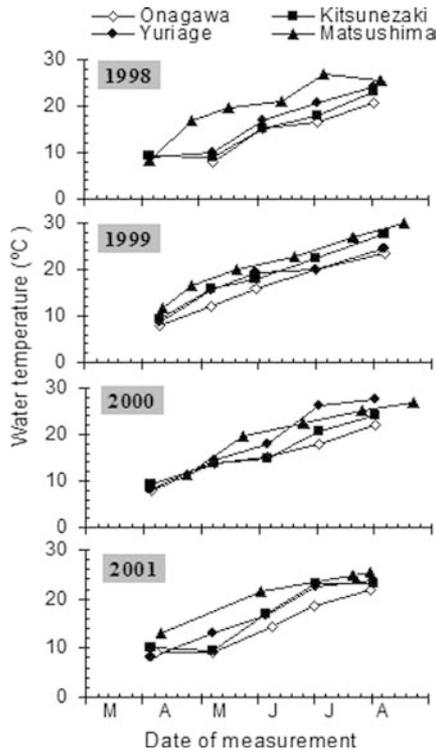
Table 3 *Sebastes inermis.* Linear models ($Y = a + bX$) and parameter estimates in 100 YOY collected in summer 2001 in Matsushima Bay, northern Japan (*AFHZ* age at formation of first hyaline zone; *SE* standard error)

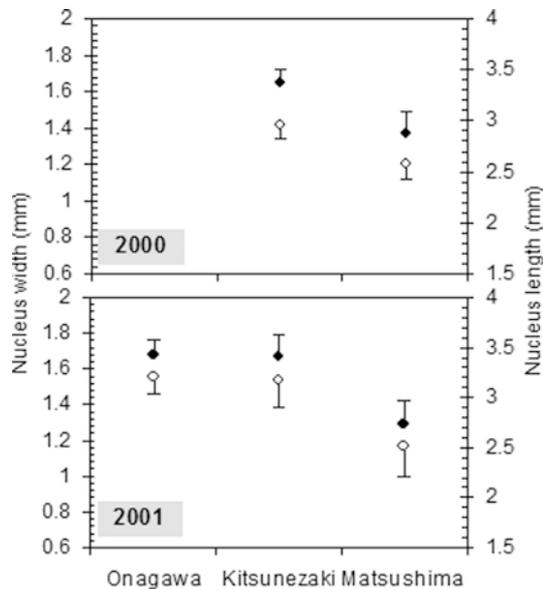| Regression	a	(SE)	b	(SE)	r^2	F
AFHZ on birth date	168.99	(2.855)	−0.73	(0.053)	0.66	190.33
AFHZ on nucleus width	32.35	(4.523)	76.56	(3.506)	0.83	476.89
AFHZ on nucleus length	37.61	(6.292)	40.40	(2.719)	0.71	220.70
Nucleus width on birth date	1.72	(0.036)	−0.01	(0.001)	0.62	152.54
Nucleus length on birth date	3.09	(0.059)	−0.01	(0.001)	0.66	187.24

Fig. 5 *Sebastes inermis.* Mean values of nucleus width (*filled circles*) and length (*open circles*) of otoliths from YOY collected in *Zostera* (Matsushima) and *Sargassum* beds (Onagawa and Kitsunezaki) during the summers of 2000 and 2001

2000
$$Sargassum = -167.05 + 103.19 \times \text{width} + 55.108 \times \text{length}$$
$$Zostera = -121.315 + 77.20 \times \text{width} + 52.69 \times \text{length}$$
2001
$$Sargassum = -103.96 + 100.0 \times \text{width} + 10.74 \times \text{length}$$
$$Zostera = -59.85 + 75.52 \times \text{width} + 8.30 \times \text{length}$$

$$(2)$$

The 1,354 adults collected at three sites in Sendai Bay (Ayukawa, Shichigahama, and Haragama; Fig. 1) were classified at the same time using the classification functions of 2000 and 2001. For this purpose the width and length values of the otolith nucleus of each fish were entered into both the *Sargassum* and *Zostera* equations in a given year. Then, the fish was classified as belonging to the nursery ground with the higher score. The percentage of the 1,354 adults for each location assigned to the Matsushima *Zostera* bed using the 2000 and 2001 classification functions was > 50% in all locations (Fig. 8).

Frequency distributions of the widths and lengths of the otolith nuclei of adults showed a bimodal tendency. Only the nucleus length of fish collected in Shichigahama was normally distributed at $\alpha = 0.01$ (Fig. 9). The bimodal tendency was clearer when only 3-year-old fish were used, suggesting that the major overlap occurred by mixing year classes in the analysis. In addition, birth dates of YOY used in the LDFA and those of YOY collected in Matsushima Bay in 1999 showed an overall range from late December to early March. YOY collected in the *Zostera* beds were extruded earlier (mainly in January, except for 2001) than YOY collected in *Sargassum* beds (mainly in February), although most histograms were significantly different in shape and range between nursery grounds and years (Fig. 10; two-sample K–S, $P < 0.001$, Table 5).

Otolith microchemistry

There were significant differences in otolith weight among habitats (Matsushima: 3.16 ± 0.16; Kitsunezaki: 6.70 ± 1.58; Onagawa: 3.69 ± 0.59 mg; ANOVA, $F_{2, 15} = 38.5$, $P < 0.001$). Hence, otolith weight was used as a covariate in the ANOVA analysis. The effect of otolith weight was removed by using the common within-group slope of otolith weight (independent variable) regressed against elemental concentration (dependent variable).

Table 4 *Sebastes inermis.* Jackknifed percentage of correct classifications predicted by linear discriminant function analysis for YOY into three nearshore nursery grounds in Sendai Bay, northern Japan, based on nucleus dimensions in 2000 and 2001, as well as trace element composition of otoliths in 2001 (*asterisk* no sampling)

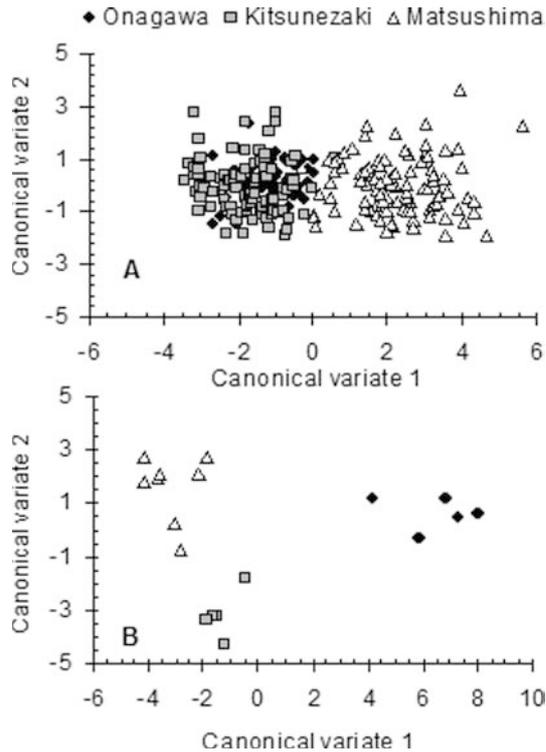| Site	Onagawa	Kitsunezaki	Matsushima
Nucleus dimensions 2000			
Kitsunezaki *Sargassum* bed ($n = 116$)	*	96	4
Matsushima *Zostera* bed ($n = 105$)	*	12	88
Onagawa *Sargassum* bed ($n = 40$)	3	97	0
Nucleus dimensions 2001			
Kitsunezaki *Sargassum* bed ($n = 101$)	1	97	2
Matsushima *Zostera* bed ($n = 96$)	0	5	95
Trace element composition 2001			
Onagawa *Sargassum* bed (n = 5)	100	0	0
Kitsunezaki *Sargassum* bed (n = 5)	0	100	0
Matsushima-*Zostera* bed (n = 8)	0	0	100

Fig. 6A, B *Sebastes inermis*. Plots of the first two canonical discriminant functions for nucleus dimensions (**A**) and trace elemental composition (**B**) from otoliths of YOY collected in *Zostera* (Matsushima) and *Sargassum* beds (Onagawa and Kitsunezaki) during the summer of 2001

The data for each element were examined for homogeneity of variance and, where appropriate, were transformed by $\log_e(x)$. Univariate and multivariate ANOVA
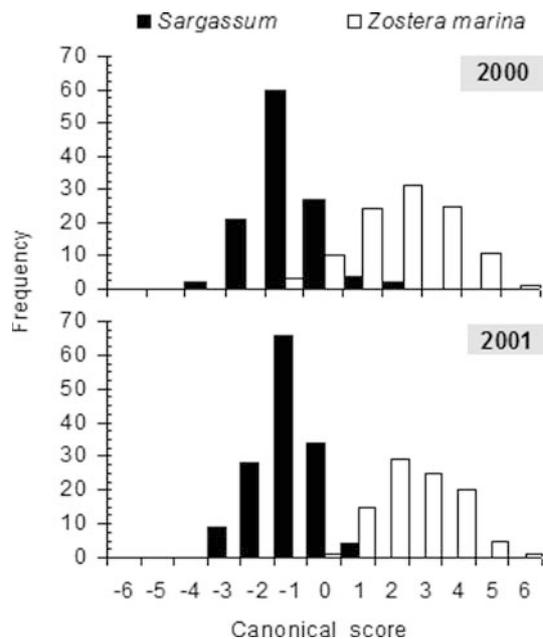

Fig. 7 *Sebastes inermis*. Frequency distribution of canonical scores from the discriminant function analysis showing separation of YOY collected from the Matsushima *Zostera marina* beds and *Sargassum* beds in the summers of 2000 and 2001
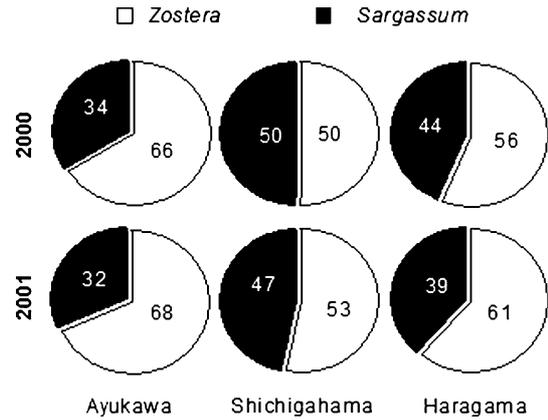

Fig. 8 *Sebastes inermis*. Adults collected at four sites along Sendai Bay ($n = 1,354$) assigned to *Zostera* and *Sargassum* beds by means of classification functions derived from the widths and lengths of otolith nuclei of YOY collected in 2000 and 2001 in each nursery ground. *Numbers inside the pie graphs* denote percentages

for trace elements in YOY *S. inermis* showed significant differences among the three sites studied ($P < 0.05$). A multiple-comparison test showed the most significant differences ($P < 0.001$) between Onagawa and Matsushima. Elemental concentrations from Kitsunezaki showed an intermediate level, depending on the element, and only Li and Ni were significantly different among the three habitats (Fig. 11). The six elements were entered into a three-group LDFA, which significantly and completely separated the three sites (Wilks' lambda $= 0.0081$, approximately $F_{12,20} = 16.73$, $P < 0.001$; Table 4). When multivariate separation was reduced to two-dimensional CDA, the *Sargassum* beds (Kitsunezaki and Onagawa) were separated on the first canonical variate and the *Zostera* bed (Matsushima Bay) was separated on the second (Fig. 6B).

Discussion

Trace elemental composition of otoliths

To date, most applications of otolith microstructure have been focused on recognition of anadromy (Kalish 1990; Secor 1992; Thresher et al. 1994; Secor et al. 1995; Arai and Tsukamoto 1998), age validation (Kalish et al. 1997; Campana and Jones 1998), and stock identification in adult fishes (Campana et al. 1994; Edmond et al. 1989; Edmond and Fletcher 1997); although some attempts to use otolith microchemistry as a natural tag in juveniles growing in estuarine environments and rocky reef nursery grounds have been made using the concentrations of trace metals (Gillanders and Kingsford 1996) and the Sr:Ca molar ratio of otoliths (Thorrold et al. 1998a; Yamashita et al. 2000). Estuaries and rocky reefs seem to be distinctive enough to allow these approaches, because the Sr:Ca molar ratio is expected to vary significantly between these habitats, owing to the differences in salinity. However, in more homogeneous

Fig. 9 *Sebastes inermis.* Histograms of widths and lengths of the otolith nuclei of all adults (*total*) and 3-year-old fish (*3+fish*) collected at three sites along the rocky shore in Sendai Bay, northern Japan (*W* Shapiro–Wilks' test for normality)
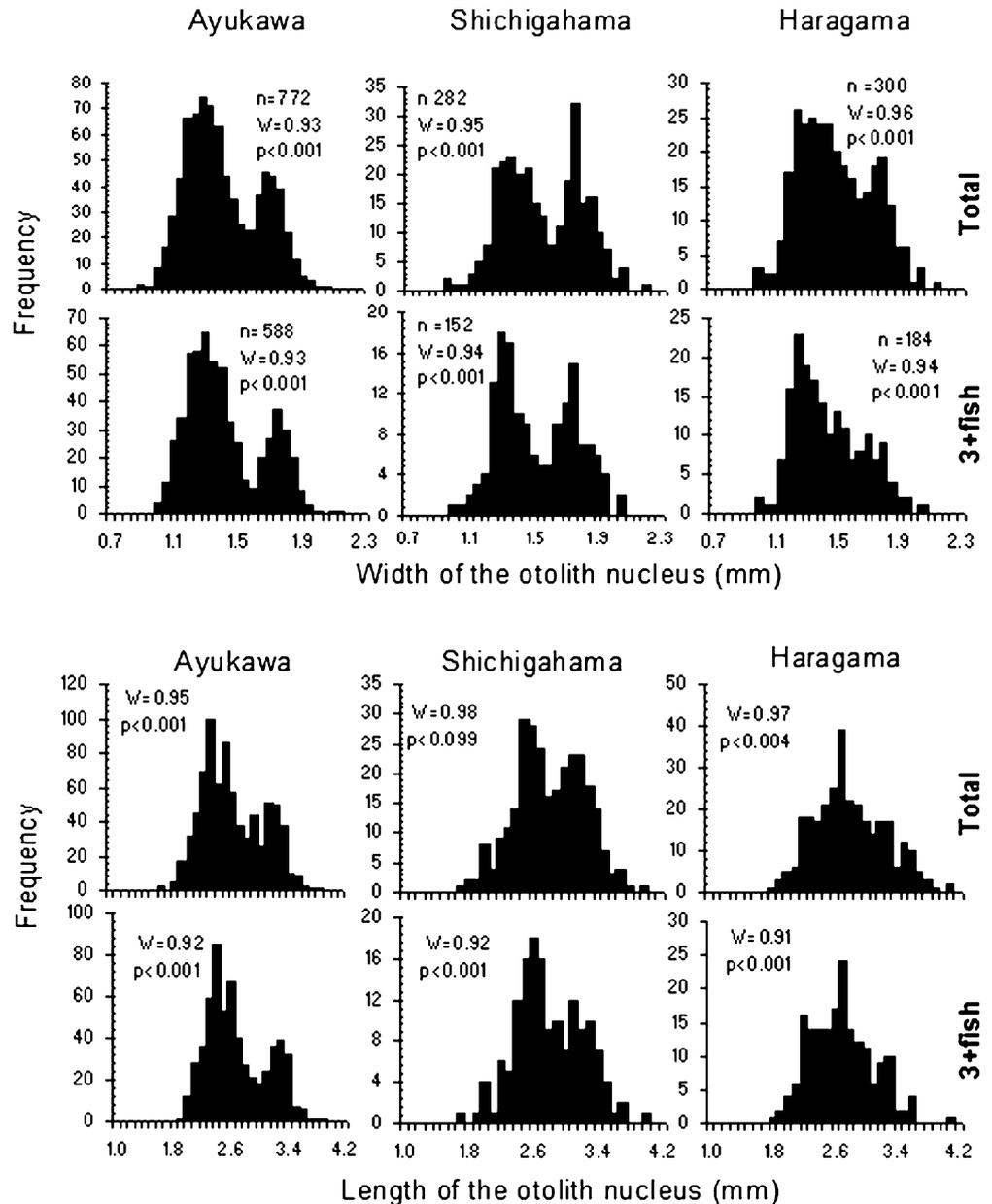
marine nursery grounds, having a similar range of salinity, the Sr:Ca ratio might not vary sufficiently for discrimination. In fact, in a preliminary attempt we did not find significant differences in the Sr:Ca molar ratio among our three nursery grounds (Fig. 12). Therefore, the significant variability among nursery grounds found in trace elements in otoliths of YOY suggests that trace elements may be used as a natural tag when shallow nursery areas are more homogeneous, even between *Sargassum* beds only a few kilometers apart. Furthermore, we dissolved whole otoliths, which cover the common planktonic period of 2–3 months offshore. Hence, it seems that the post-settlement period (about 3 months) was sufficient to produce significant differences in the trace elemental composition among habitats. The high accuracy found was perhaps aided by the fact that the six trace elements detectable in this species

(Li, Mn, Ni, Cu, Zn, and Ba) are feasible for use as natural markers (Campana 1999), as well as the high sensitivity of the HR-ICPMS. Although it is well known that ICPMS analyses based on dissolved otoliths can provide precise and accurate measurements of trace elements at concentrations < 10 µg g^{-1} (Dove et al. 1996; Campana 1999), the precision might still be improved by using HR-ICPMS. Likewise, the high discriminatory power of otolith elemental compositions underlined in this study agrees with recent works using solution-based ICPMS procedures to classify juvenile Atlantic bluefin tuna (*Thunnus thynnus*) into their nursery grounds at a wider spatial scale from eastern and western Atlantic regions (Rooker et al. 2001, 2003). On the other hand, it is difficult to hypothesize about the observed differences in trace elements among the three habitats, because mechanisms generating geographic
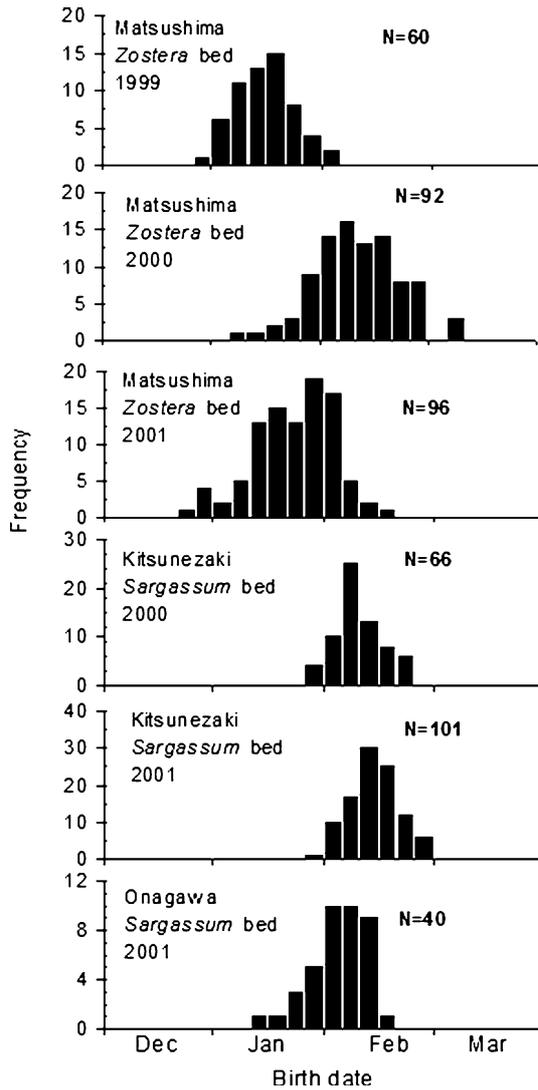
Fig. 10 *Sebastes inermis*. Frequency distribution of birth dates of YOY collected in three nursery grounds along Sendai Bay, northern Japan

differences in trace element composition of otoliths are not well understood (Thorrold et al. 1998a, 1998b) and because data on the elements in the environment are lacking. However, the elements that occurred at greater concentrations in otoliths of YOY from the Matsushima

Table 5 *Sebastes inermis*. Maximum differences of the two-sample Kolmogorov–Smirnov test for birth date distributions of YOY collected in three nursery grounds in Sendai Bay, northern Japan (*Ma* Matsushima *Zostera* bed; *Ke* Kitsunezaki *Sargassum* bed; *On* Onagawa *Sargassum* bed; *superscripts*: [a]$P = 0.048$, [b]$P = 0.008$, [c]$P = 0.131$; *no superscript* $P > 0.001$)

Location	Ma-1999	Ma-2000	Ma-2001	Ke-2000	Ke-2001
Ma-2000	0.795				
Ma-2001	0.419	0.557			
Ke-2000	0.967	0.182[a]	0.724		
Ke-2001	0.957	0.234[b]	0.787	0.157[c]	
On-2001	0.792	0.352	0.437	0.361	0.456

Zostera bed (Cu, Zn, and Ba) have also been found at high concentrations in estuarine seagrass beds (Gillanders and Kingsford 1996). On the other hand, it is important to emphasize that the concentrations of trace elements reported here are stationary. Therefore, further research is needed to evaluate to what extent the discriminatory ability of trace elements can be extended across years, due to the fact that significant interannual variability in trace elemental fingerprints have been reported in otoliths of other species (Milton et al. 1997; Rooker et al. 2003).

Timing of formation of the first hyaline zone

The FHZ of YOY *Sebastes inermis* was formed at a relatively fixed time in the Matsushima *Zostera* and Kitsunezaki *Sargassum* beds each year, although significantly later in the *Sargassum* bed than in the *Zostera* bed. Likewise, the FHZ was formed at larger sizes and at older ages in the *Sargassum* bed than in the *Zostera* bed. These facts suggest that the FHZ formation seems to be environmentally triggered rather than ontogenetically mediated. Furthermore, we found that the differences in the timing of formation of the FHZ seem to be linked to the higher water temperatures found in the Matsushima *Zostera* bed. The influence of water temperature in the formation of otolith zones is already known. Changes in water temperature of about 4°C induced a mark on otoliths of several fishes (Neilson and Geen 1985; Schramm 1989; Volk et al. 1990; Munk et al. 1993; Letcher and Terrick 1998). Furthermore, we found that a decrease of 4°C produced a shift from hyaline to opaque deposition in otoliths of reared *S. inermis* (Plaza et al. 2001). Hence, the opposite shift is highly probable for wild fish. In addition, the time of formation of the first hyaline zone for this species seems also to be related to a reduction in fish growth rate in the *Zostera* beds (Plaza et al. 2001). A reduction in growth in earlier stages of fishes is a consequence of two main variables: food availability and temperature (Love et al. 1985; Smith 1985; Miller et al. 1988; Nishimura and Yamada 1988; Oxenford et al. 1994). Likewise, it is well known that the
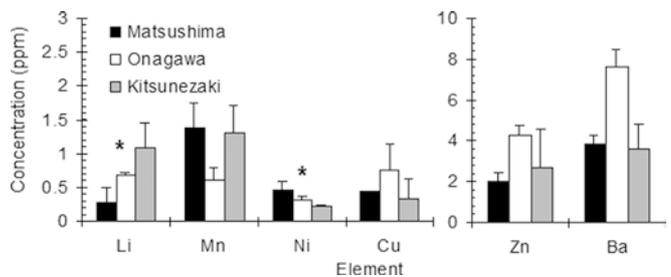

Fig. 11 *Sebastes inermis*. Mean values for concentrations of trace elements in otoliths from YOY collected in *Zostera* (Matsushima) and *Sargassum* beds (Onagawa and Kitsunezaki) during the summer season of 2001 (*asterisk* significant differences among nursery grounds, $P < 0.05$)
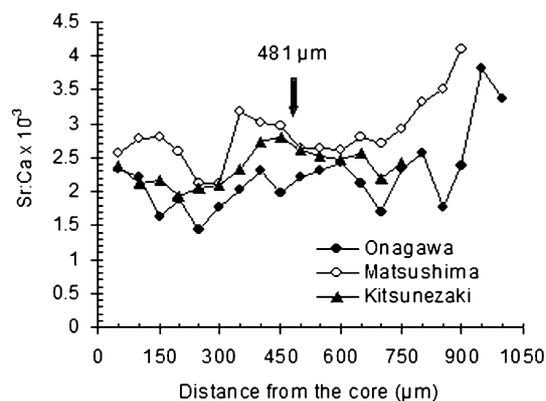
Fig. 12 *Sebastes inermis*. Profiles of Sr:Ca concentration ratios of YOY collected in *Zostera* (Matsushima) and *Sargassum* beds (Onagawa and Kitsunezaki) during the summer season of 2001. Sr:Ca ratios were measured with a wavelength dispersive electron microprobe. Each point represents the average for every 50 μm ($n = 3$) from the core to the otolith edge. The *arrow* denotes the combined average radius at settlement in the three habitats (i.e. 481 μm)

rising water temperature in seagrass beds as summer progresses can affect fish growth directly or indirectly: directly because temperatures higher than an optimum become a limiting factor for growth and indirectly because the rising water temperature produces a loss of habitat and, consequently, the limitation of food (Love et al. 1991; Jenkins et al. 1996; Plaza et al. 2002). However, irrespective of the causal factors linked to the shift from opaque to hyaline otolith deposition, the first hyaline zone in the present study seemed to be a quantifiable event.

The width and length of the otolith nucleus were reliable macroscopic indices of the timing of formation of the FHZ. YOY could be accurately classified to nursery grounds by the nucleus dimensions. In addition, we were able to classify adult fish with unknown juvenile history. Furthermore, the percentage of adults assigned to the *Zostera* or *Sargassum* beds by the 2000 and 2001 classification functions showed minor variations in both years. Certainly variations in the predictions could be expected, because nucleus dimensions of adults did not encompass the same year classes from which the classification functions were derived. Despite this consideration, nucleus dimensions showed a good temporal stability. On the other hand, the discriminatory power of the nucleus dimensions was also evident through the histograms of the adult fish collected. The proportion of the modes followed a similar tendency to that in the LDFA. This fact suggests that even in absence of an ideal classification procedure, the mere distribution of nucleus dimensions of adult fish, based on the same year classes, may be used as a proxy for distinguishing the proportion of adults that grew in *Zostera* or *Sargassum* beds, at least when distinct separation occurs, such as in Ayukawa.

The significant inverse relationship between the age at FHZ formation and birth date suggests that the timing of formation of the FHZ is also an indicator of parturition date (i.e. early extruded YOY will have a longer period of opaque deposition in their otoliths than later extruded ones). Thus, by using an inverse regression of birth date on either age at FHZ formation or nucleus dimensions as explanatory variables, we can estimate (at a population level) the proportion of larval cohorts extruded earlier and later within a given year class from otoliths from either YOY or adult fish. This approach, analogous to hatch date analysis (Methot 1983; Campana and Jones 1992), would allow testing of how the proportion of survivors varies among years and whether environmental cues may be linked to differences in the relative survival of larval cohorts, so as to identify factors potentially influencing recruitment success.

It is important to emphasize that both the trace elements and nucleus dimensions for each fish were considered as independent observations representative of the whole population, despite the fact that samplings were restricted to a short period. This species has a parturition season of about two and a half months in the winter (Plaza et al. 2004). After parturition, larvae and/or small juveniles remain planktonic for about 3 months, and then they settle every spring (during a short settlement season of one and a half months) in distinctive settlement groups, synchronized to the new and full moon phases, into the *Zostera* and/or *Sargassum* beds (Plaza et al. 2003). In addition, this species seems to synchronize parturition with settlement, because YOY extruded earlier in the parturition season tend to settle earlier than those extruded later, although they settle at similar sizes (Plaza et al. 2003). Yet, the collections made in summer (when the settlement season is over) would encompass most YOY supplied by a discrete settlement. Consequently, YOY caught may be representative of the population of settled YOY (i.e. the survivors) in a given nursery ground. Moreover, the discriminatory capacity of both trace elements and nucleus dimensions is independent of variations in collecting date, birth date, and settlement dates, because the size of YOY was used as a covariate and because nucleus dimensions seemed to be environmentally mediated at a fixed time. Furthermore, we showed that nucleus dimensions were related to birth date in the *Zostera* bed (i.e. wider nucleus dimensions would correspond to those YOY that were extruded earlier, e.g. January). Consequently, a more accurate classification could be expected in years when birth dates are opposite in trend, or completely overlapping. At present, however, the differences found in birth date distributions between the *Zostera* and *Sargassum* beds cannot be explained. This is perhaps linked to: (1) the reproductive strategy of the adult population, (2) habitat selection, and/or (3) merely coincidental.

In summary, both nucleus dimensions and trace element composition of otoliths offer great potential for use as natural tags to classify YOY *S. inermis* to their nursery grounds. The advantages of using nucleus dimensions of otoliths as natural tag are various: it is a

very quick method, because otoliths of *S. inermis* are very thin, so the width and length of the nucleus can be measured with high precision without polishing with an image analysis system. Consequently, a great number of otoliths can be analyzed to reduce variance. Furthermore, the cost is very low compared with other approaches (e.g. marking and recapture, or otolith microchemistry). With further comparison of nucleus dimensions among additional sites in Sendai Bay, it will be possible to create more precise algorithms for answering questions on habitat use by YOY and the contribution of nursery grounds to the adult stock. LDFA using otolith dimensions has made clear that fish growing in the Matsushima *Zostera* bed are crucial to sustaining the adult population of *S. inermis* in Sendai Bay. In addition, since the changes from hyaline to opaque otolith deposition and vice versa are distinctive in temperate fishes, the time of formation of the FHZ and its macroscopic indicators (i.e. width and length of the nucleus) may be important for answering questions on habitat selection and the contribution of nursery grounds to the adult stock in species simultaneously using *Zostera* and rocky shore habitats as their nursery grounds. Therefore, further attempts to estimate the timing of formation of the FHZ among related species are encouraged. Likewise, the high accuracy of classification given by trace element analysis suggests that this approach is a reliable tool for classifying adult *S. inermis* with unknown history in order to disclose their nursery grounds. This approach may be possible if the otolith core (i.e. portion that was laid down when the YOY inhabited a nursery ground) is extracted and dissolved for analysis by HR-ICPMS. Consequently, the contribution of each nursery ground to the adult population might be assessed.

References

Adams SM (1976) The ecology of eelgrass, *Zostera marina* (L.), fish communities. I. Structural analysis. J Exp Mar Biol Ecol 22:269–291

Arai T, Tsukamoto K (1998) Application of otolith Sr:Ca ratios to estimate the migratory history of masu salmon, *Oncorhynchus masou*. Ichthyol Res 45:309–313

Beckman DW, Wilson CA (1995) Seasonal timing of opaque zone formation in fish otoliths. In: Secor DH, Dean JM, Campana SE (eds) Recent developments in fish otolith research. University of South Carolina, Columbia, pp 27–44

Bell JD, Westoby M (1988) Variations in seagrass height and density over a wide spatial scale: effects on common fish and decapods. J Exp Mar Biol Ecol 104:275–295

Campana SE (1999) Chemistry and composition of fish otoliths: pathway, mechanisms and applications. Mar Ecol Prog Ser 188:263–297

Campana SE, Jones CM (1992) Analysis of otolith microstructure data. In: Stevenson KD, Campana SE (eds) Otolith microstructure examination and analysis. Can Spec Publ Fish Aquat Sci 117:73–100

Campana SE, Jones CM (1998) Radiocarbon from nuclear testing applied to age validation of black drum, *Pogonias cromis*. Fish Bull (Wash DC) 96:185–192

Campana SE, Fowler AJ, Jones CM (1994) Otolith elemental fingerprinting for stock identification of Atlantic cod (*Gadus morhua*) using laser ablation ICPMS. Can J Fish Aquat Sci 51:1942–1950

Casselman JM (1987) Determination of age and growth. In: Weatherly AH, Gill HS (eds) The biology of fish growth. Academic, Orlando, Fla., pp 209–2042

Dove SG, Gillanders BM, Kingsford MJ (1996) An investigation on chronological differences in the deposition of trace metals in the otolith of two temperate reef fishes. J Exp Mar Biol Ecol 205:15–33

Edmond JS, Fletcher WJ (1997) Stock discrimination of pilchards *Sardinops sagax*, by stable isotope ratio analysis of otolith carbonate. Mar Ecol Prog Ser 152:241–247

Edmond JS, Moran MJ, Caputi N, Morita M (1989) Trace element analysis of fish sagittae as an aid to stock identifiction: pink snapper (*Chrysophyris auratus*) in Western Australia waters. Can J Fish Aquat Sci 46:50–54

Frederick JL (1997) Evaluation of fluorescent elastomer injection as method for marking small fish. Bull Mar Sci 61:399–408

Gillanders BM, Kingsford MJ (1996) Element in otoliths may elucidate the contribution of estuarine recruitment to sustaining coastal reef populations of a temperate reef fish. Mar Ecol Prog Ser 141:13–20

Haines BG, Modde T (1996) Evaluation of marking techniques to estimate population size and fish year survival of Colorado squawfish. N Am J Fish Manag 16:905–912

Hatanaka HM, Iizuka K (1962) Studies of the fish community in the *Zostera* area. III. Efficiency and production of *Sebastes inermis*. Nippon Suisan Gakkaishi 28:305–313

Jenkins GP, Wheatley MJ, Poore AGB (1996) Spatial variation in recruitment, growth and feeding of postsettlement King George whiting, *Sillaginoides punctata*, associated with seagrass beds Port Philip Bay, Australia. Can J Fish Aquat Sci 53:350–359

Jerald A Jr (1983) Age determination. In: Neilson LA, Johnson DL (eds) Fisheries techniques. American Fisheries Society, Bethesda, Md., USA, pp 301–324

Kai Y, Nakabo T (2002) Morphological differences among three color morphotypes of *Sebastes inermis*. Ichthyol Res 49:260–266

Kai Y, Nakayama K, Nakabo T (2002) Genetic differences among three color morphotypes of the black rockfish, *Sebastes inermis*, inferred from mtDNA and AFLP analyses. Mol Ecol 11:2591–2598

Kalish JM (1989) Otolith microchemistry: validation of the effects of physiology, age and environment on otolith composition. J Exp Mar Biol Ecol 132:151–178

Kalish JM (1990) Use of otolith microchemistry to distinguish the progeny of sympatric anadromous and non-anadromous salmonids. Fish Bull (Wash DC) 88:657–666

Kalish JM, Johnson JM, Smith DC, Morison AK, Robertson SG (1997) Use of the bomb radiocarbon chronometer for age validation in the blue grenadier *Macruronus novazelandie*. Mar Biol 128:557–563

Letcher BH, Terrick TD (1998) Thermal marking of Atlantic salmon otoliths. N Am J Fish Manag 18:406–410

Levin PS, Hay ME (1996) Responses of temperate reef fishes to alterations in algal structure and species composition. Mar Ecol Prog Ser 134:37–47

Love MS, Westphal W, Collins RA (1985) Distributional patterns of fishes captured aboard commercial passenger fishing vessels along the northern Channel Island, California. Fish Bull (Wash DC) 83:243–252

Love MS, Carr MH, Haldorson LJ (1991) The ecology of substrate-associated juveniles of the genus *Sebastes*. Environ Biol Fishes 30:225–243

Methot RD Jr (1983) Seasonal variation in survival on larvae of northern anchovy, *Engraulis mordax*, estimated from age distribution of juveniles. Fish Bull (Wash DC) 81:741–750

Miller TJ, Crowder LB, Rice JA, Marschall EA (1988) Larval size and recruitment mechanisms in fishes: toward a conceptual framework. Can J Fish Aquat Sci 45:1657–1670

Milton DA, Chenery SR, Farmer MJ, Blaber SJM (1997) Identifying the spawning estuaries of the tropical shad terubok, *Tenualosa toil*, using otolith microchemistry. Mar Ecol Prog Ser 153:283–291

Moser HG, Boehlert GW (1991) Ecology of pelagic larvae and juveniles of genus *Sebastes*. Environ Biol Fishes 30:203–224

Munk KM, Smoker WW, Beard DR, Mattson RW (1993) A hatchery water-heating system and its application to 100 percent thermal marking of incubating salmon. Prog Fish-Cult 55:284–288

Neilson JD, Geen GH (1985) Effects of feeding regimes and diel temperature cycles on otolith increment formation in juvenile chinook salmon, *Oncorhynchus tshawytscha*. Fish Bull (Wash DC) 83:91–101

Nishimura A, Yamada J (1988) Geographical differences in early growth of walleye pollack, *Theragra chalcogramma*, estimated by back-calculation of otolith daily growth increments. Mar Biol 97:459–465

Oxenford HA, Hunte W, Deane R, Campana SE (1994) Otolith age validation and growth-rate variations in flyingfish (*Hirundichthys affinis*) from the eastern Caribbean. Mar Biol 118:585–592

Pearson DE (1996) Timing of hyaline-zone formation as related to sex, location, and year of capture in otoliths of the widow rockfish, *Sebastes entomelas*. Fish Bull (Wash DC) 94:190–197

Plaza G, Katayama S, Omori M (2001) Otolith microstructure of the black rockfish, *Sebastes inermis*. Mar Biol 139:797–805

Plaza G, Katayama S, Omori M (2002) Abundance and early life history traits of young-of-the-year *Sebastes inermis* in a *Zostera marina* bed. Fish Sci (Tokyo) 68:1254–1264

Plaza G, Katayama S, Omori M (2003) Timing of parturition, planktonic duration, and settlement patterns of the black rockfish, *Sebastes inermis*. Environ Biol Fishes 68:229–239

Plaza G, Katayama S, Omori M (2004) Spatio-temporal patterns of parturition of the black rockfish *Sebastes inermis* in Sendai Bay, northern Japan. Fish Sci (Tokyo) 70:256–263

Rooker JR, Secor DH, Zdanowicz VS, Itoh T (2001) Discrimination of northern bluefin tuna from nursery areas in the Pacific Ocean using otolith chemistry. Mar Ecol Prog Ser 218:275–282

Rooker JR, Secor DH, Zdanowicz VS, Demetrio G, Relini LO (2003) Identification of Atlantic blue tuna (*Thunnus thynnus*) stocks from putative nurseries using otolith chemistry. Fish Oceanogr 12:75–84

Schaffelke B, Klumpp DW (1996) Biomass and productivity of a tropical seagrass community in north-west Australia (Exmouth Gulf). In: Kuo J, Walker DI, Kirkman H (eds) Seagrass biology. pp 13–20

Schramm HL (1989) Formation of annuli in otoliths of bluegills. Trans Am Fish Soc 118:546–555

Secor DH (1992) Application of otolith microchemistry analysis to investigate anadromy in Chesapeake Bay striped bass *Morone saxatilis*. Fish Bull (Wash DC) 90:798–806

Secor DH, Henderson-Arzapalo A, Piccoli PM (1995) Can otolith microchemistry chart pattern of migration and habitat utilization in anadromous fishes? J Exp Mar Biol Ecol 192:15–33

Smith PE (1985) Year-class strength and survival of 0-group clupeids. Can J Fish Aquat Sci 42[Suppl 1]:69–82

Sokal RR, Rohlf FJ (1995) Biometry, 3rd edn. Freeman, San Francisco

Stone M (1977) Asymptotics for and against cross-validation. Biometrika 64:29–35

Thorrold SR, Jones CM, Swart PK, Targett TE (1998a) Accurate classification of juvenile weakfish *Cynoscion regalis* to estuarine nursery areas based on chemical signatures in otoliths. Mar Ecol Prog Ser 173:253–265

Thorrold SR, Campana SE, McLaren JW, Lam JWH (1998b) Trace element signatures in otoliths record natal river of juvenile American shad (*Alosa sapidissima*). Limnol Oceanogr 43:1826–1835

Thresher RE, Proctor CH, Gunn JS, Harrowfield IR (1994) An evaluation of electron probe microanalysis of otoliths for stock delineation and identification of the nursery areas in a southern temperate groundfish, *Nemadactylus macropterus* (Cheilodactylidae). Fish Bull (Wash DC) 92:817–840

Volk EC, Schroder SL, Fresh KL (1990) Inducement of unique otolith banding patterns as a practical means to mass-mark juvenile Pacific salmon. Am Fish Soc Symp 7:203–215

William T, Bedford BC (1974) The use of otoliths for age determination. In: Bagenal TB (ed) The ageing of fish. Unwin, London, pp 114–123

Yamashita Y, Otake T, Yamada H (2000) Relative contributions from exposed inshore and estuarine nursery grounds to the recruitment of stone flounder, *Platichthys bicoloratus*, estimated using otolith Sr:Ca ratios. Fish Oceanogr 9:316–327

Yokogawa K, Igushi M, Yamada K (1992) Age, growth, and condition factor of black rockfish, *Sebastes inermis*, in southern coastal water of the Harima Sea (in Japanese). Suisanzoshoku 40:235–240

Zar JH (1998) Biostatistical analysis, 4th edn. Prentice Hall, Englewood Cliffs, N.J., USA