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Otolith microstructure of the black rockfish, *Sebastes inermis*

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Abstract Otolith microstructure was examined and described with regards to the early life history events of black rockfish, *Sebastes inermis*, collected in a temperate seagrass bed in Matsushima Bay, eastern Japan. The extrusion check was validated upon examining newly extruded reared larvae. Tetracycline treatments and a thermal marking experiment showed that the otolith increments were produced daily. Four zones were identified, from a clear central zone (CZ), which evolved a clear extrusion check to a translucent zone, visible only in large juveniles. Extrusion dates were distributed from late December to early March, with peaks in January and February. Only the CZ and a planktonic zone (PZ) were observed in newly immigrated juveniles. The PZ showed mean values significantly longer in the January cohort (92 days) than the February cohort (76 days), and the increment width varied significantly over time and between cohorts as well. The PZ was delimited by a clear prominent transition check (TCh) accompanied by an abrupt shift in increment width (the post-settlement zone). The TCh, which was formed at 24.5 mm of mean back-calculated total length, seemed to be linked to the settlement of juveniles in *Zostera marina* belts. The usefulness of thermal marking and those factors that seem to influence the occurrence of the otolith zones are discussed.

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Introduction

The daily increment patterns of otoliths have permitted great progress in studies on the growth dynamics of fishes. In addition to age and growth, fish otoliths may also record life history events such as metamorphosis, settlement and migration (Victor 1982, 1986a,b; Kingsford and Milicich 1987; Thorrold and Williams 1989; Jones 1992; Hare and Cowen 1994; Sponaugle and Cowen 1994; Hamer and Jenkins 1996; Jenkins et al. 1996; Linkowski 1996; Wilson and McCormick 1997). The potential of otolith microstructure as an efficient mono-specific recording code remains largely unrealized (Campana and Neilson 1985). Hence, suitable descriptions of otolith microstructure are of tremendous importance for further studies of the growth dynamics of any particular species. Likewise, validation of the periodicity of otolith increment formation is a prerequisite for otolith studies of any species and sometimes for different spawning stocks or local populations (Geffen 1992).

The black rockfish *Sebastes inermis* is a commercially important species in coastal waters from southern Hokkaido to Kyushu, Japan, and along the southern Korean Peninsula (Utaga and Taniuchi 1999). Planktonic *S. inermis* larvae and small juveniles recruit to shallow *Zostera* beds, and as juveniles increase in size and as the biomass of *Z. marina* becomes unfavorable, they move to different habitats (Harada 1962; Hatanaka and Iizuka 1962a,b; Love 1991). Yet there is a total lack of studies on the otolith microstructure of this species. As a consequence, the dynamics of their early life history remain largely unexplored.

The present study had two purposes: to describe the otolith microstructure of juvenile *S. inermis* in relation to the occurrence of early life history events, and to validate the daily periodicity of increment deposition. The results of a preliminary attempt to use thermal marking as a method to validate the daily increments are evaluated, and the ecological and behavioral factors that seem to affect the otolith microstructure are discussed.

Materials and methods

Study area and sampling methods

Matsushima Bay is a shallow, enclosed embayment (< 2.5 m depth), located between 32°20′N and 38°20′N on the northwestern coast of Japan and connected by a narrow entrance to the Pacific Ocean. The submerged vegetation is mainly *Z. marina*, which grows on sandy and muddy bottoms and forms continuous or patchy covers. Sampling procedures were carried out from March to November in 1998 and 1999 at five stations (A, A′, B, C, D), forming part of a research program to study the early life history of *S. inermis* in this nursery ground. Fish were collected in the daytime by drawing a hand-trawl net (150 m with 5 mm mesh) two to three times from a small motor boat, at the beginning and end of each month. Juveniles were preserved in 95% ethanol solution for posterior otolith examination. Total length (TL) was measured to the nearest 0.01 mm without correcting for shrinkage. Temperature during the study period ranged from 6. 5–25.4°C and from 6.7–29°C, in 1998 and 1999, respectively. A total of 2903 individuals were collected during 1998 and 1999; and in early and late March no fish were collected, except for 8 individuals which were collected in late March 1998. For the purposes of this paper the information on TL was pooled monthly, among stations and years.

Validation of the extrusion check in newly extruded larvae

Pregnant females were reared under a continuous flow of seawater in a 200-l fiberglass-reinforced plastic tank in January 1999. Newly extruded larvae were sufficiently fed twice a day with rotifers. Temperature was not controlled, and ranged from 8.5°C to 10°C. Larvae were sampled at 5-day intervals and preserved in 95% alcohol solution.

Validation of the daily periodicity of increment deposition

Juvenile *S. inermis* of about 20–30 mm TL were collected in seagrass (*Z. marina*) beds on 12 April 1999. Two control lots were reared separately under the same artificially regulated light cycle of 12 h light:12 h dark. Water temperature was not regulated, and ranged from 15°C to 20°C during the experiments. Fishes were fed sufficiently with newly hatched *Artemia* nauplii twice a day. Five specimens from control lot 1 were sampled every day during a week of acclimatization to look for any unpredictable changes in otolith microstructure induced by the rearing conditions. After the standard acclimatization, some juveniles were sacrificed to evaluate the growth conditions during the subsequent experiments, and the remaining fishes were kept under the same light and feeding conditions. Individuals taken from control lot 2 were reared under two tetracycline immersion treatments (TC) and one thermal marking experiment as follows.

In the first experiment, juveniles were immersed in seawater containing 400 mg TC l^{-1} for 24 h, with constant aeration but without feeding. After this treatment, they were fed sufficiently with *Artemia* nauplii twice a day for 6 days, and then the TC treatment was repeated.

In the second experiment, juveniles were immersed in seawater containing 800 mg TC l^{-1} for 10 h. After the treatment, the same feeding regime as in the first experiment was used for 30 days. Fish were sampled 10, 20 and 30 days after immersion.

Replicated thermal marking experiments were conducted under the same light and feeding regimes as the TC experiments. Water temperature was kept at about 19°C for the first 3 days and was then dropped to 15°C and maintained there for the rest of the period. Five fish were killed every day during the first 3 days at 15°C, and five every day at 5, 10, 15, 20 and 30 days after the temperature reduction.

Otolith microstructure

In rearing experiments, both lapilli and sagittae of reared larvae were easy to observe without polishing, after fixation on a slide glass with a small drop of transparent fingernail. However, to disclose the entire sequence of increments, otoliths required major preparation. Sagittae taken from juveniles < 55 mm TL were mounted in epoxy resin on slide glasses and polished from both sides. Because of the marked concavity in sagittae of juveniles > 55 mm TL, otoliths were embedded in polyethylene plastic, which was sectioned after polymerization to remove excess of medium, and then polished in a frontal plane. Lapilli were easier to prepare in the overall length range analyzed; these were mounted in epoxy resin on slide glasses and ground from one side. All otoliths were ground until the nucleus became clearly visible, with 800–2000 grit lapping films and 4000 grit grinding paste. Otoliths taken from reared juveniles did not need to be grounded.

Counting and measuring of increments was carried out using an image analysis system (Quantimet 600, Leica), at magnifications of 400–1000×. All counts were made twice across the area of distinctive increments; otolith radii and increment widths were measured to the nearest 0.1 μm from the core along the posterior otolith axis, consistently the best line for increment counting in both otoliths. Hence, radii from both sagittal and frontal planes of sagittae could be used without further corrections. Otolith increments from the TC treatment were examined and photographed through a UV-light microscope according to Campana and Neilson (1982).

Results

The general appearance of the otolith microstructure of *S. inermis* was similar in both sagittae and lapilli, except for the exclusive occurrence of accessory primordia in sagittae. There were no significant differences in the mean number of increments between lapilli and sagittae, nor were there such differences between the left and right otoliths of each pair (two-factor ANOVA, Table 1). In both, lapilli and sagittae the increments were clearly discernible in their marginal areas, even in whole otoliths. However, near the core, increment widths were wider and easier to read in sagittae than in lapilli, due to the larger size. Hence, only the sagittae were used in this study, except for TC experiments.

Table 1 *S. inermis*. Two-factor ANOVA to test the significance of increment counts between otolith types (sagittae and lapilli) and between otolith positions (left and right of each pair). Mean counts and standard deviation are given for each case. Newly settled juveniles collected in April 1998 were used

	Sagittae		Lapilli	
	Left	Right	Left	Right
Mean ($n = 20$)	92.70	91.95	92.50	93.05
SD	6.77	5.59	6.09	6.23
ANOVA				
Source of variation	*df*	MS	*F*	*P*
Type	1	4.05	0.11	0.73
Position	1	0.2	0.06	0.94
Position×Type	1	8.45	0.23	0.63
Error	76	36.48		

Validation of extrusion check

Mean body size and sagittal radius from all larvae just after extrusion were 6.68 ± 0.35 mm TL and 26.5 ± 1.6 μm, respectively. Sagittae at extrusion always had a faint check surrounded by 6–10 rings (Fig. 1). These rings never completely encircled the otolith perimeter, although they were visible by light microscopy even at 400× magnification. Five and ten days after extrusion, otoliths always showed the second check at a mean radius of 26.1 ± 1.4 μm (Fig. 2). This radius was not significantly different from that of newly extruded larvae (*t*-test, $P > 0.05$). This confirmed the second check as the extrusion check (EC).

Validation of daily periodicity of ring deposition

Juvenile *S. inermis* grew well and survived with low mortality in the rearing experiments; only 3.47% of the fish died after acclimatization for about 1 month in rearing experiments. Significant differences in TL were obtained between the beginning (fishes not reared in control lot 1) and the end of control lot 1, TC and thermal experiments (ANOVA, Tukey test, $P < 0.05$; Table 2). Consequently, otolith increments were clearly discernible.

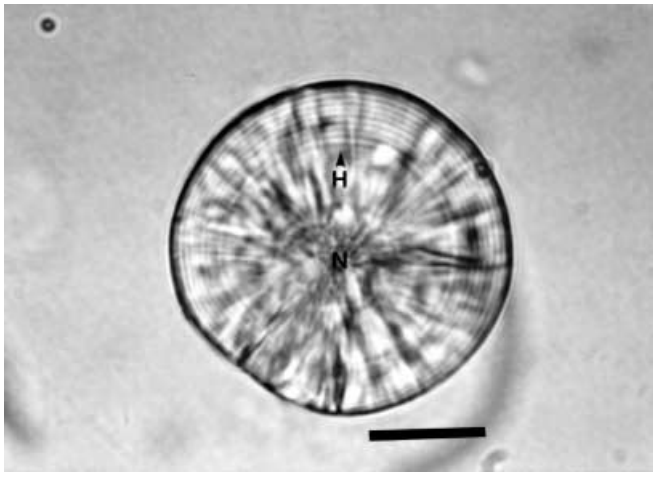

Fig. 1 *S. inermis*. Photograph at 1000× magnification of sagitta from a newly extruded larvae [*H* first check (hatch check); *N* nucleus]. Scale bar = 25 μm

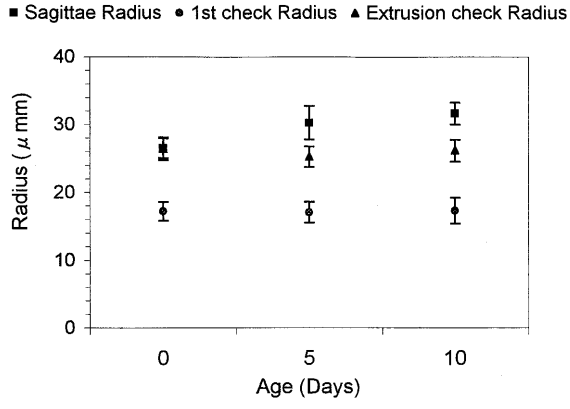

Fig. 2 *S. inermis*. Results of validation of extrusion check in newly extruded larvae reared for 10 days

Table 2 *Sebastes inermis*. Total length at the end of the rearing experiments (*lot 1S* TL of juveniles sacrificed after the standard acclimatization in control lot 1). ANOVA, Tukey test $*P < 0.05$

Treatment	*n*	Total length (mean ± SD)	Successfully marked fish (%)
400 mg TC	16	38.13 ± 3.01	60
800 mg TC	25	41.66 ± 3.23	100
Thermal	42	39.41 ± 2.00	100
Control lot 1	32	42.65 ± 2.01	
Lot 1S	23	28.48 ± 1.78*	

Because the fluorescent band in all TC treatments was much more distinguishable in lapilli than in sagittae, only the lapilli were considered in the analysis.

In the first immersion experiment (400 mg TC l^{-1}), 60% of the fish examined had a fluorescent ring, although the fluorescence was weak. All specimens immersed in 800 mg TC l^{-1} had a suitable fluorescent mark (Fig. 3A; Table 2).

An abrupt reduction in water temperature induced the formation of a continuous dark zone in all sagittae examined, changing the transparency of otoliths from hyaline to opaque deposition. Daily monitoring of otoliths for 3 days after the thermal treatment proved that the drop in water temperature did not disrupt the otolith growth or the deposition of daily increments (Fig. 3B,C).

Because of the higher percentage of mark formation in the 800 mm TC l^{-1} immersion and thermal marking experiments, only data obtained from these two experiments were used for the validation of daily periodicity of ring deposition. Regression coefficients of the number of increments against the number of days elapsed were not significantly different from 1.0 at the 95% confidence level in either experiment (Table 3). Data pooled from both experiments also showed a regression coefficient not different from 1.0 and homogeneous variance (Barlet's test; Zar 1999). These results suggest that otolith increments in *S. inermis* were formed daily (Fig. 4; Table 3).

Otolith microstructure of juveniles collected in seagrass beds

Four zones differing in microstructure were accumulated outward from the center of sagittae. In the following subsections each zone will be described in relation to the occurrence of some early life history events.

Central zone (CZ)

The nucleus was visible as a dark cavity with a radius of 8.7 ± 0.3 μm surrounded by two checks distant from the

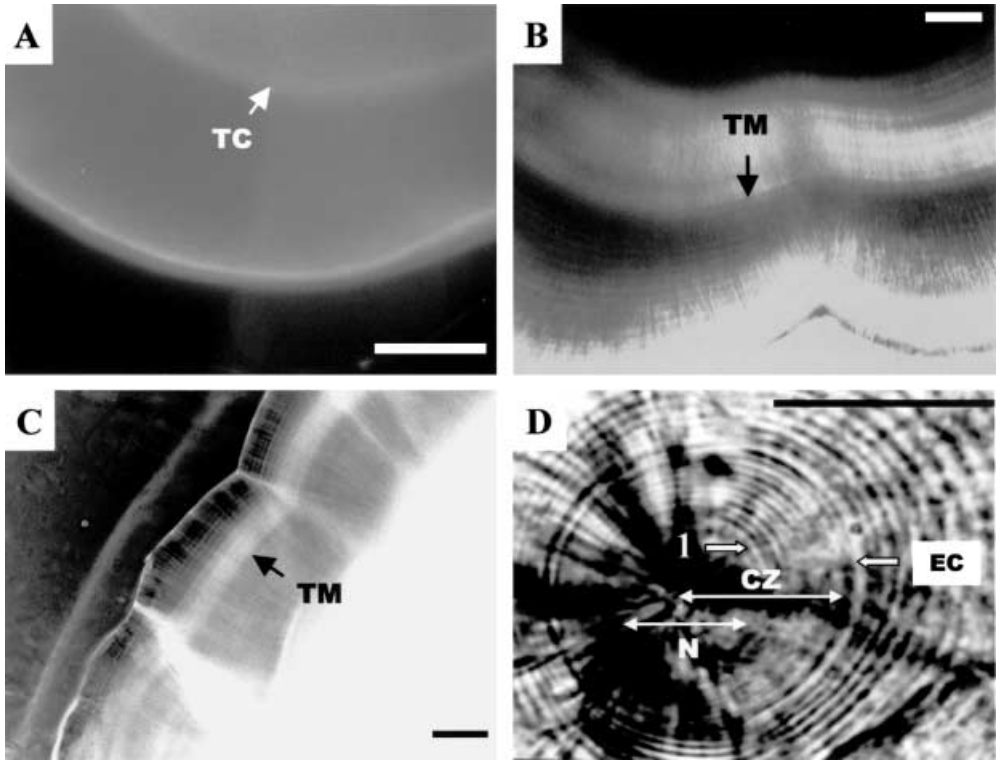

Fig. 3A–D *S. inermis*. Light microscopic image. **A** Lapillus showing the fluorescent band (*TC*) observed in tetracycline immersion treatments. Normal (**B**) and inverted image (**C**) of an intact sagitta illustrating the marks (*TM*) produced in thermal marking procedures. **D** Central zone (*CZ*) of sagitta from a 35 mm TL juvenile (*N* nucleus; *1* first check; *EC* extrusion check). B–D: sagittal planes; scale bars = 30 μm

Table 3 *Sebastes inermis*. Regression of increment counts on elapsed days for tetracycline (*TC*) and thermal marking experiments. $*P > 0.05$ is the probability level of the *t*-test of deviation from 1 ring day^{-1} (slope = 1)

Experiment	*n*	*b*	SE	r^2
TC (800 mg l^{-1})	25	1.019*	0.010	0.97
Thermal marking	42	0.995*	0.005	0.99

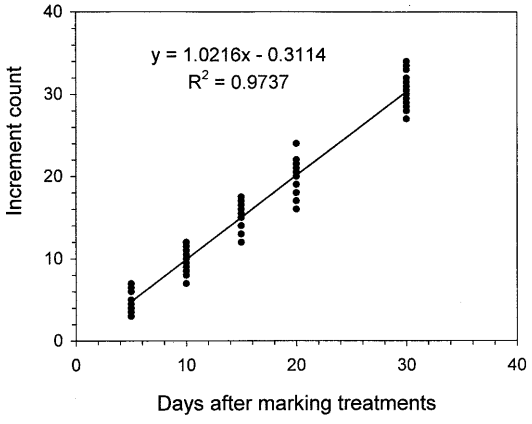

Fig. 4 *S. inermis*. Linear regression of increment number against days after treatment. Data for 800 mg TC l^{-1} (lapillus) and thermal treatments (saggita) combined

nucleus. The first check is a faint transitional line bordering the innermost amorphous features. The EC is the outer border of the CZ and is clearer than the first. A few unreliable rings were visible between both checks. The average radius of the first check was 16.0 ± 1.4 μm, and that of the EC was 24.7 ± 1.5 μm (range 21.0–27.9 μm; Fig. 3D). Validations of EC allow us to back-calculate extrusion dates from the calendar day of sampling. Extrusion dates were distributed from late December to early March, with the higher frequencies in January and February (Fig. 5a). However, because birth date distribution was not corrected for mortally effects, the proportion of each cohort cannot be linked to survival.

Planktonic zone (PZ)

This zone extends from the EC to a clear transition check (TCh) (Fig. 6A,B). The TCh marks the boundary between the PZ and post-settlement zone (PSZ). An estimate of the number of days during which the PZ was formed was calculated by counting the increments from the EC to TCh. The duration of PZ formation ranged from 76 to 113 days (mean = 92 ± 8 days) and from 52 to 94 days (mean = 76 ± 9 days) in January and February cohorts, respectively (Fig. 5b); the mean PZ duration was significantly greater in the former (ANOVA, $F_{1,327} = 151.76$, $P < 0.001$). Otolith microstructure in the PZ also differed between cohorts, December and March cohorts were not analyzed, due to their small contribution to the whole. Figure 7 shows a 10 day average of increment widths over time for January and February otoliths from 15 juveniles for each cohort collected during the early summer. To reduce the masking effect

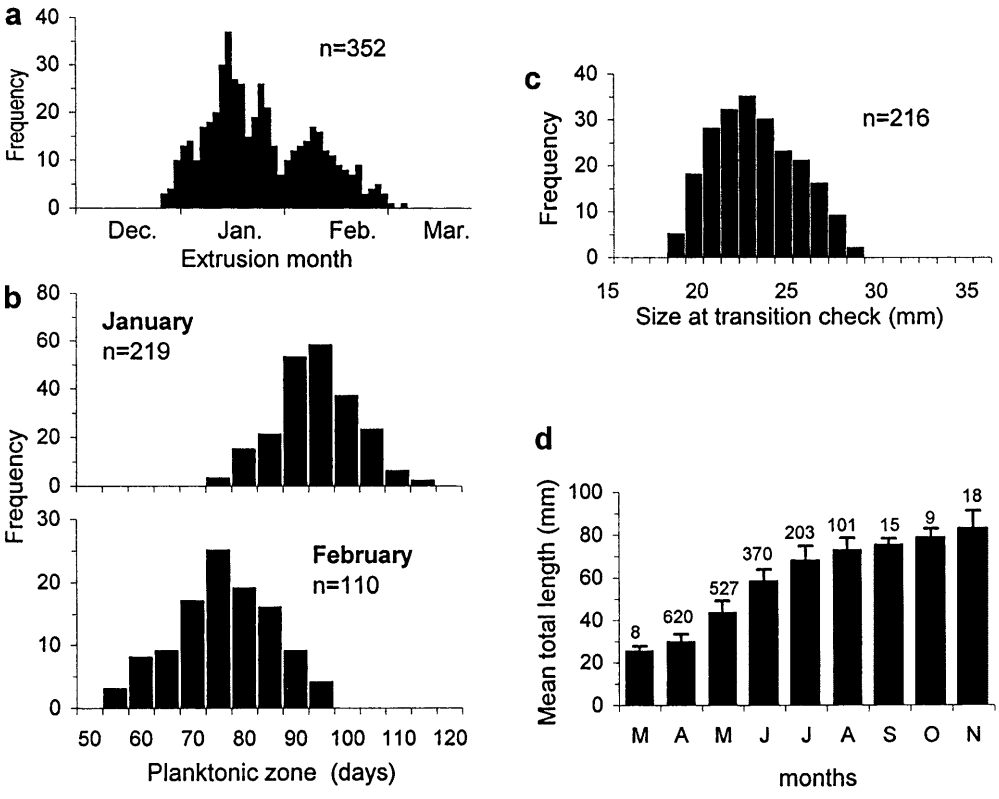

Fig. 5a–d *S. inermis*. Frequency distributions of juveniles collected in Matsushima seagrass beds during 1998 and 1999 according to birth month (**a**), planktonic zone, for the January and February cohorts (**b**) and back-calculated size at transition check (**c**). **d** Seasonal fluctuation of mean total length pooled by birth month. *Error bars* and *numerals above bars* denote standard deviation and numbers of fish measured, respectively

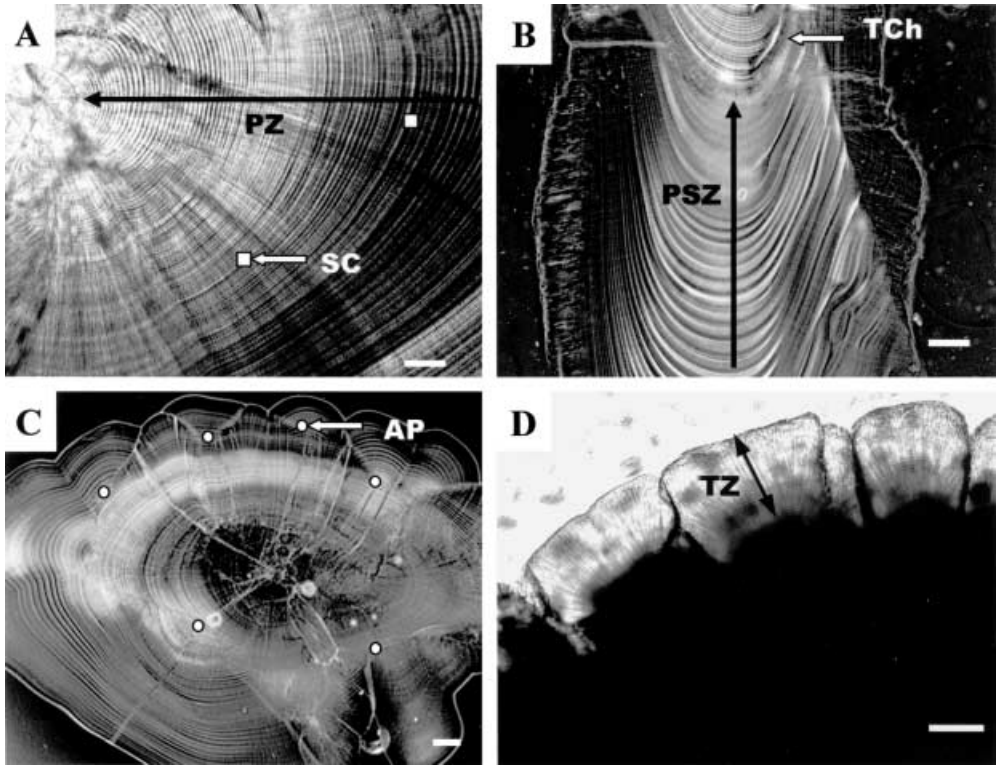

Fig. 6A–D *S. inermis*. Light microscopic image of the sagittae of juveniles in Matsushima seagrass beds. **A,B** January-cohort sagittae (*PZ* planktonic zone; *SC* sporadic checks; *PSZ* post-settlement zone; *TCh* transition check). **C,D** Saggitae from a 42 mm TL juvenile collected in mid-May (*AP* accessory primordium; *TZ* translucent zone). A,C,D: sagittal planes; B: frontal plane; scale bars = 30 μm

on the increment width profile produced by the variability in PZ duration, only individuals with PZ formation periods ranging from 70 to 80 days were used. The January cohort showed increments very distinctive during the first 20 days, increasing gradually in width from 2.7 ± 0.7 μm near the EC to 4.1 ± 0.8 μm at the end of this period. Then increment width decreased gradually, to increase again to 5.1 ± 1.1 μm around the outer

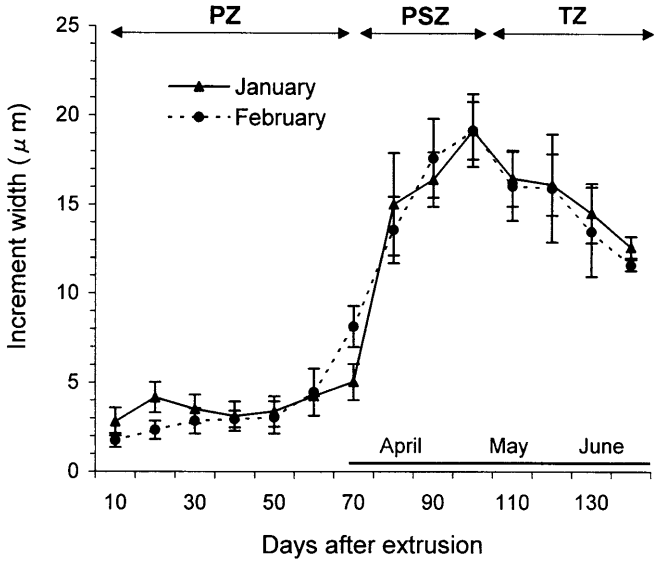

Fig. 7 *S. inermis*. Increment width of sagittae in January and February cohorts of 30 juveniles caught in early summer in Matsushima seagrass beds in 1998. Captions over the graph denote the otolith zones described in the text

boundary of the PZ. The regularity of the otolith increments after the decrease in width was disrupted by areas of less resolution and by the presence of sporadic checks (SC) (Fig. 6A). On the other hand, increments from February otoliths were very distinct throughout the PZ, increasing gradually from 1.7 ± 0.3 μm near the CZ to 8.1 ± 1.1 μm near the TCh. A repeated-measures ANOVA for the first 30 days of the PZ detected significant differences in increment widths over time ($F_{29,812} = 42.57$, $P < 0.001$) and between January and February cohorts ($F_{1,28} = 98.71$, $P < 0.001$).

Post-settlement zone (PSZ)

This zone was not found in otoliths of newly settled juveniles collected in late March, at a mean TL of 21.59 ± 1.12 mm (Fig. 5d). However, as the season and the growth of juveniles progressed, it became clearly visible. Increment widths after the TCh were clearly broader than those in the PZ (16.8 ± 1.6 μm; Fig. 6B). Additionally, eight to ten new growth centers – accessory primordia (AP) – were visible in sagittal planes (Fig. 6C). APs were distributed over dorsal, ventral, anterior and posterior regions, and new APs grew adjacent to previously formed APs. Increments emanating from APs had a higher contrast and were wider than the increments formed before. Ventral and posterior APs seemed to be formed earlier than the others, although only a chronological examination of APs would show the pattern of formation of these new growth centers. APs began to occur at 20 mm TL and were not found in fish > 35 mm TL.

A linear regression equation of fish size (TL) on sagittal radius (R) of juveniles collected in seagrass beds was significant ($P < 0.0001$; $TL = 0.036R + 8.11$; SE (slope) $= 0.642$, SE(intercept) $= 0.0004$; $r^2 = 0.95$, $n = 215$, $F = 3252.12$). As a consequence, fish size at the TCh could be back-calculated. There was an evident overestimation of the size at extrusion by the regression above; 8.11 mm TL in contrast to the 6.63 mm TL obtained from newly reared larvae. Hence, we used the biological intercept method as being less intercept-dependent than the Frasser–Lee (Francis 1990) and regression methods (Campana 1990; Campana and Jones 1992). The following function was applied:

$$L_a = L_c + (O - O_c)(L_c - 6.63)(O_c - O_i)^{-1}$$

where, as above, "6.63" corresponds to the fish size (mean TL) taken from the larval rearing experiment, as described in "Materials and methods", and O_i, to the otolith size at this biological intercept.

Back-calculated size at the TCh ranged from 18 to 30 mm TL, with a mean of 23.15 ± 3.49 mm TL (Fig. 5c). There were no significant differences between the mean back-calculated size at the TCh and the mean size of newly settled juveniles collected in late March (ANOVA, $F_{1,222} = 0.72$, $P = 0.39$).

Translucent zone (TZ)

The TZ was found only in fish collected after mid-June (56.5 ± 5.6 mm TL). In this growth zone, increments were narrower and less resolvable than those at the outer boundary of the PSZ (Figs. 6D, 7). Increment width in this layer decreased gradually from 16.5 ± 1.4 μm just after the PSZ to 12.1 ± 0.9 μm in late June (Fig. 7). The occurrence of this layer was also synchronized with the slower increase in mean TL observed in large juveniles after June (Fig. 5d). The TZ extended over the remaining juvenile period in seagrass beds (summer and autumn), and the resolution of the increments gradually decreased.

Discussion

Validation of daily periodicity of ring deposition

Otolith increments of various *Sebastes* species are deposited daily (Boehlert 1981; Yoklavich and Boehlert 1987; Laidig et al. 1991; Kokita and Omori 1998). We have shown that this is also true of the black rockfish, *S. inermis*. Although the method of marking the otoliths of small fishes by immersing them in tetracycline solution has been widely used, the incorporation rates are lower for juveniles than for larvae (Geffen 1992). This fact was also evident in our experiments, since only the immersions with 800 mg l^{-1} produced suitable fluorescent marks. These were clearer in the lapilli, possibly due to their smaller size than in the sagittae. On the other hand, we obtained interesting results from our thermal marking

experiment. Thermal marking is a relatively new method that has been successfully applied in mass marking studies, especially with hatchery-reared salmon. It is based on the otolith's ability to record an abrupt change in temperature, which stops fish growth and produces a dark mark on the otolith (Munk et al. 1993; Akinicheva and Rogatnykh 1996; Letcher and Terrick 1998). Until now, thermal marking has been used to produce strong dark and hyaline checks (by dropping and raising temperature). Although previous studies suggested that a reduction in water temperature reduces the otolith accretion rate and changes the chemical composition (Volk et al. 1990; Munk et al. 1993; Akinicheva and Rogatnykh 1996; Letcher and Terrick 1998), no report has described the growth pattern of otoliths after temperature reduction when conditions are kept constant. In the present study, although the otolith transparency changed from hyaline to opaque, corresponding to an abrupt drop in water temperature, we saw no decrease in otolith growth, and the clear, dark mark produced allowed us to validate the ring formation in *S. inermis*. The advantages of thermal marking were: reduction of fish handling, the ability to simultaneously treat a large number of fish, and less variation in estimating daily otolith increments. In contrast, the well-known phenomenon that constant temperature can reduce otolith-increment contrast (Campana and Neilson 1985) is a potential disadvantage, but this was not observed in our study. A further possible disadvantage of thermal marking might be its dependence on the propensity of some species to register environmental shifts in their otoliths, particularly those indicating variation in temperature and feeding conditions. An example of this predicament is that settlement marks occur in certain species (Victor 1982; Sponaugle and Cowen 1994; Wilson and McCormick 1997), but are absent in others (Kingsford and Milicich 1987). Hence, although our experiments may open the way for new thermal manipulation procedures, an evaluation of this method in another species should be performed. Additionally, the optimal reduction in temperature to produce a suitable mark without disrupting growth should be evaluated as well.

Interpretation of otolith zones

Observation of the otoliths of newly extruded larvae confirmed that the second clear check was the EC. This check always involved some faint rings outside the first check. A similar structure has been described for *S. thompsoni* (Kokita and Omori 1998), *S. jordani* (Laidig and Ralston 1995) and *Sebastes* spp. (Penney and Evans 1985). However, the EC radius in these species ranged from 14 to 18 μm, which is far smaller than that of *S. inermis* (21–28 μm). Considering the viviparous nature of *Sebastes* species, the existence of a pre-extrusion ring is not surprising. The embryos develop within egg envelopes during most of gestation and hatch several days before parturition (Wourms 1991). So the faint rings might be formed before extrusion but after hatching. Although such pre-extrusion rings were clearly visible in otoliths of newly extruded larvae, the daily nature of them and the time of their formation cannot be asseverated.

Otolith microstructure of the PZ appears to constitute the planktonic period for this species. This affirmation was supported by the fact that only the first two zones occurred in newly settled juveniles (collected in late March) and that the mean size of these settlers did not differ significantly from the mean back-calculated size at the TCh. The widespread distributions of the PZ formation period found here (one individual had a planktonic period more than two standard deviations from the mean) suggest that delay metamorphosis is occurring. This characteristic has been well documented in other demersal fishes (Victor 1986a,b; Sponaugle and Cowen 1994; McCormick 1999) and in many invertebrate larvae (Richmond 1985; Pechenik et al. 1996; Gebauer et al. 1998; Moss 1999; Chicharro and Chicharro 2000). Delayed metamorphosis has been viewed as a larval adaptation to expand their bio-geographical range and to increase their chance of finding a suitable habitat to settle (Sponaugle and Cowen 1994). If settlement occurs at a relatively fixed time, it is reasonable that individuals extruded earlier have longer planktonic periods, as our data suggest. What role might delayed metamorphosis be playing in the black rockfish, *S. inermis*, which migrates early every spring into its nearshore nursery ground? This question is interesting considering that similar ontogenetic movements have also been described for other rockfishes, and seem to constitute an adaptive strategy of using nearshore areas with minimal offshore dispersal (Moser and Boehlert 1991). Furthermore, the decrease in increment resolution and width suggests a reduction in growth for individuals extruded earlier, if, in fact, we assume that growth increment is an instantaneous measure of growth rate. Changing environmental conditions between cohort times might be responsible of the growth differences; however, delayed metamorphosis with reduced larval growth cannot be disregarded, as was revealed by Victor (1986b) for *Thalasomma bisfasciatuus* (a coral reef fish).

The same reasons given above to link the PZ with the planktonic period also suggest that PSZ was formed by the shift of environmental conditions associated with the immigration into seagrass belts. The increment pattern in this zone seemed to be synchronized with the increase in size of juveniles in the seagrass beds. Newly immigrated juveniles were about 21 mm TL at 70 days old (collected at late March); after immigration they seemed to double in body size after approximately 1 month in the seagrass belt. Furthermore, the fact that the first AP was formed in the innermost portion th PSZ implies that the APs were formed during the seagrass phase. APs appear with or after metamorphosis from larvae to juveniles in many fish species (Campana and Neilson 1985; Gartner 1991; Linkowski 1991; Sogard 1991; Hare and Cowen 1994). Some hypotheses have been proposed to

explain the origin of APs, e.g. habitat shift (Campana 1984; Sogard 1991), ontogenetic dietary shift (Marks and Conover 1993) and/or physiological changes associated with metamorphosis (Hare and Cowen 1994). Both the dietary shift and habitat shift hypotheses for the occurrence of these structures seem reasonable. *S. inermis* changes its feeding habits with the availability of new types of prey in the seagrass habitats (Harada 1962; Hatanaka and Iizuka 1962a). Hare and Cowen (1994) suggested that otolith growth changed with the transition from larvae to juveniles in response to a shift in sacculus cavity shape. The fact that these structures were not found in the lapilli of *S. inermis* and that the physiological changes from larvae to juveniles in this species occur between 17 and 35 mm TL (Suzuki and Aida 1999) might support Hare and Cowen's hypothesis. Because of the importance of APs as an ontogenetic marker, it might be interesting to confirm their formation in *S. inermis* from other habitats.

The TZ showed narrower increments than the PSZ. This might indicate a decrease in growth as the season progressed, because the hyaline zone is formed during a period of slow growth (Pearson 1996). Future attempts should be made to clarify why the hyaline zone forms in juvenile *S. inermis*.

Finally, this study has shown the tremendous importance of otolith microstructure in examining the early life events of *S. inermis*. Studies of growth pattern, growth history, habitat shifts and settlement dynamics can now be conducted to reveal the early survival process of this species.

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