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Migration history of Sakhalin taimen *Hucho perryi* captured in the Sea of Okhotsk, northern Japan, using otolith Sr:Ca ratios

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Abstract Sakhalin taimen *Hucho perryi* populations have decreased drastically to near extinction. It is urgent to establish an effective conservation strategy based on an understanding of the characteristics of migration and habitat use of this species. We examined the migration history of anadromous Sakhalin taimen captured off the Sarufutsu coast, northern Hokkaido, Japan, using otolith Sr:Ca ratios and also examined the relationship between their otolith Sr:Ca ratios during freshwater and seawater residence in a rearing experiment. Otolith Sr:Ca ratios of some fish from the Sarufutsu coast showed freshwater levels ($0.5–4.0 \times 10^{-3}$) near the core, which thereafter increased to brackish water levels ($4.0–6.0 \times 10^{-3}$), and then to seawater levels ($6.0–10.0 \times 10^{-3}$) in the outermost regions. Those findings indicate that specimens from the Sarufutsu coast migrated to the brackish water region or the sea and spent most of their lives there. The anadromous migration pattern including the timing of downstream migration seems to be flexible among individuals in the species. They migrate between freshwater and seawater or brackish water several times during their lives, showing extensive habitat use. It is essential to secure the continuity among the freshwater, brackish water, and seawater areas for their effective conservation.

Keywords Sakhalin taimen · Migration · Rare species · Anadromous · Otolith Sr:Ca ratios · Conservation

Introduction

Sakhalin taimen *Hucho perryi* is a species of the genus *Hucho*, which is composed of five species [1, 2]. The distribution is limited to the far northeastern part of Asia, from the Primorye region of Siberia to Sakhalin Island, the southern Kurile Islands, and the northern area of Hokkaido [1]. Unlike the other species of the genus, which are strictly freshwater residents, the Sakhalin taimen has been known to perform an anadromous migration [2–4]. The population of this species has decreased remarkably and is close to the extinction level [5]. This situation is thought to result from various human impacts such as indiscriminate fishing, water pollution by development of agricultural land, and habitat destruction. Takami et al. [6] reported that the decrease of Sakhalin taimen was probably the result of the loss of riparian forests and riverine habitats associated with extensive development of agricultural land during the 1960s and 1970s. The construction of dams, barrages, and

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banks also caused habitat destruction including the loss of spawning grounds. In particular, the straightening of the rivers at middle to lower reaches conducted in the last several decades has made the rivers markedly monotonous with a loss of rapids and deep pools, and has damaged the river environment for the reproduction of *H. perryi* [7]. The life history, including migration and habitat use of this species, should be urgently studied for the establishment of an effective conservation strategy.

However, information about the life history of anadromous Sakhalin taimen is still limited with only a few studies being done on ecological aspects of their freshwater phases [8–11], the morphology of anadromous Sakhalin taimen [12], or on the microchemistry of the otoliths and scales [13–15]. Fish otoliths are metabolically inert with the aragonite mineralogy remaining unaltered after deposition [16], so the elemental composition of the otolith reflects to some degree the environment of the water in which the fish lives [17]. The strontium content in otoliths, in particular, varies with fluctuations in ambient salinity, allowing the reconstruction of the anadromous migration history of each fish [18–24]. Regarding Sakhalin taimen, Arai et al. [13] used otolith analysis to report downstream migration of Sakhalin taimen collected at Lake Aynskoye in Sakhalin Island. Honda et al. [14] analyzed otolith Sr:Ca ratios of Sakhalin taimen caught from Lake Akkeshi (brackish water lake) in Hokkaido, Japan, and suggested that the specimens had migrated into brackish waters, but it was unlikely they went into the ocean. The present study is the first report on detailed migration history of anadromous Sakhalin taimen, actually captured in the Sea of Okhotsk, the Sarufutsu coast, northern Hokkaido in Japan. The population of Sakhalin taimen in the Sarufutsu River system is one of the stable populations of this species in Japan [25]. Furthermore, there seems to be a genetic difference among the stocks of several river systems (K. Edo, pers. comm., 2007, 2009). Therefore the life history and migration traits of Sakhalin taimen should be investigated in each river system unit. This knowledge is vital for establishment of an effective conservation strategy for the species whose local population is declining. The Sr:Ca ratio levels of Sakhalin taimen otoliths from fish reared in seawater and freshwater were also examined to verify the affect of salinity on the Sr:Ca ratios in the otoliths of this species.

Materials and methods

Rearing experiment

A total of 12 Sakhalin taimen used for this experiment were provided by the Shibetsu Salmon Museum. We do not report the river lineage from which these specimens were taken to help protect this critically endangered species. They were artificially hatched and kept in freshwater at 16.0–17.0°C under natural light conditions for 6 months before the experiment. The average fork length and body weight at the start of the rearing experiment were 424 ± 41 mm and 838 ± 250 g, respectively. The rearing experiment was carried out in the Shibetsu Salmon Museum. Six of 12 fish remained in a freshwater environment for another 6 months, and the other six were transferred to seawater and also reared for 6 months. The freshwater fish were reared in a 500 L tank supplied with well water at 10–20 L/min. The temperature of the well water was ca. 16.8°C. The seawater rearing was in filtration tank (5,000 L) of the Shibetsu Salmon Museum, which has a water recycling system. The seawater used for the rearing was pumped up from the Shibetsu port and circulated in the system at the rate of 180–240 L/min. The salinity of seawater used for the rearing was 30 psu. The seawater temperature varied from 12 to 17°C, and the average temperature was ca. 15°C during the experiment. Lighting conditions of each rearing tank were based on natural light. Fish of both freshwater and seawater groups were fed 4 days per week with dry pellets (Hokuren). We could not carry out the rearing experiment under a variety of salinities because of the limited numbers of this locally protected fish species.

Wild fish used for the analyses of otolith Sr:Ca ratio

A total of seven wild Sakhalin taimen were used for the analysis of otolith Sr:Ca ratios. All fish were unintentionally caught in a set net fishery mainly targeting pink salmon (*Oncorhynchus gorbuscha*), chum salmon (*Oncorhynchus keta*), and masu salmon (*Oncorhynchus masou*) conducted by the Sarufutsu Fishery Cooperative and Fujimoto Fisheries Company along the Sarufutsu coast of northern Hokkaido, Japan (the Sea of Okhotsk) (Fig. 1).

The fork length, body weight, sex, age, capture date, and number of retained ovulated eggs of each fish are shown in Table 1. Scales were used for the age determination of each fish. Scales were removed from areas above and below the lateral line of the fish body.

Otolith preparation and Sr:Ca ratio analyses

Sagittal otoliths were extracted from each fish and were embedded in epoxy resin (Epofix, Struers, Denmark). They were mounted on glass slides by epoxy bond and ground to expose the core using a grinding machine equipped with a diamond-cup wheel of 13 μm (Discoplan-TS, Struers, Denmark). The ground surface of otolith was polished with 6 and 1 μm diamond paste on a polishing wheel (Planopol-V equipped with PdM-Force, Struers, Denmark), Pt-Pd

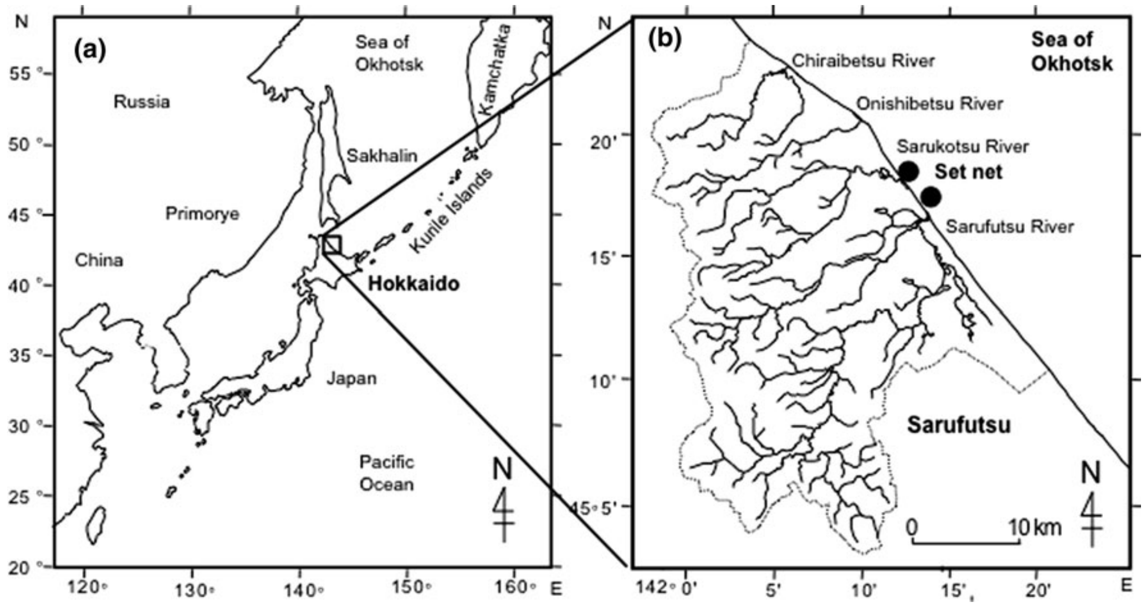

Fig. 1 **a** Map of Hokkaido, northern Japan and Sarufutsu coast. **b** Map of Sarufutsu coast showing the location of the set net

Table 1 Characteristics of the individual Sakhalin taimen captured on the coast of Sarufutsu

Fish no.	Capture date	Sex	Age (years)	Fork length (cm)	Body weight (g)	Number of retained eggs
1	1997.6.26	Male	13	75.2	6,180	0
2	1997.6.26	Female	8	74.4	5,500	2
3	1997.6.26	Female	8	69.4	4,600	0
4	2007.7.4	Male	11	73.2	4,888	0
5	2007.7.4	Male	12	76.5	5,692	0
6	2007.7.4	Female	13	78.6	5,789	0
7	2007.7.4	Female	12	77.6	5,820	0

coated with a high vacuum evaporator (E-1030, Hitachi, Japan) after washing with deionized water.

For life history transect analysis, the profiles of Sr and Ca concentrations were analyzed from the core to the edge along the radius of each otolith using a wave-length dispersive X-ray electron probe microanalyzer (EPMA; JEOL JXA-8900, Jeol, Japan). The accelerating voltage and beam current were 15 kV and 1.2×10^{-8} A, respectively. The electron beam for the rearing experiment samples was focused on a point of 10 μm diameter, and the measurements were spaced at 10 μm intervals. The beam for wild fish was focused on a point of 5 μm diameter, and the measurements were spaced at 5 μm intervals. The outermost 100 μm of the otolith of fish no. 3 (Table 1) was further examined to determine the Sr:Ca ratio deposited under seawater conditions. For this analysis beam diameter and measurement interval were both set to 1 μm with the accelerating voltage and beam current the same as mentioned above.

Two-dimensional X-ray intensity maps of Sr and Ca were also examined by EPMA under the following measurement conditions: accelerating voltage, 15 kV; beam current, 5.0×10^{-7} A; pixel size, 10×10 μm. Calcite ($CaCO_3$) and strontianite ($SrCO_3$) were used as standards.

Results

The rearing experiment showed that there was a remarkable difference in the Sr:Ca ratios between the otoliths of the fish reared in the freshwater and seawater tanks (Fig. 2). The otolith Sr:Ca ratios of individuals reared in freshwater (freshwater sample) maintained lower levels with an average ratio of 1.1×10^{-3} (range 0–3.4×10^{-3}) throughout the rearing period. In contrast, the ratios of individuals transferred into seawater from freshwater (seawater sample) sharply increased to a high level with an average of 5.6×10^{-3} (range 4.0–7.8×10^{-3}) at about 500 μm from the otolith edge. These facts are clearly supported by two-dimensional X-ray intensity maps of Sr content for otoliths of freshwater and seawater samples

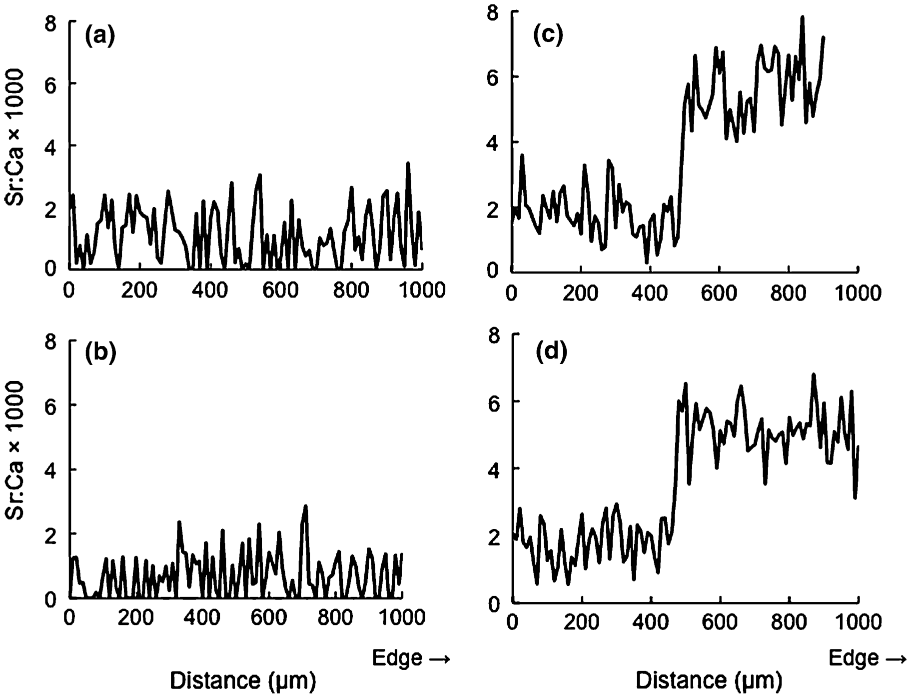

Fig. 2 Profiles of Sr:Ca ratios in the outer edge of the otolith (1,000 µm from the edge) of fish reared in freshwater (**a**, **b**) and seawater (**c**, **d**). The otolith Sr:Ca ratios of individuals reared in freshwater remained low at the edge of otolith. In contrast, the ratios of individuals transferred into seawater from freshwater sharply increased to a high level at about 500 µm from the otolith edge

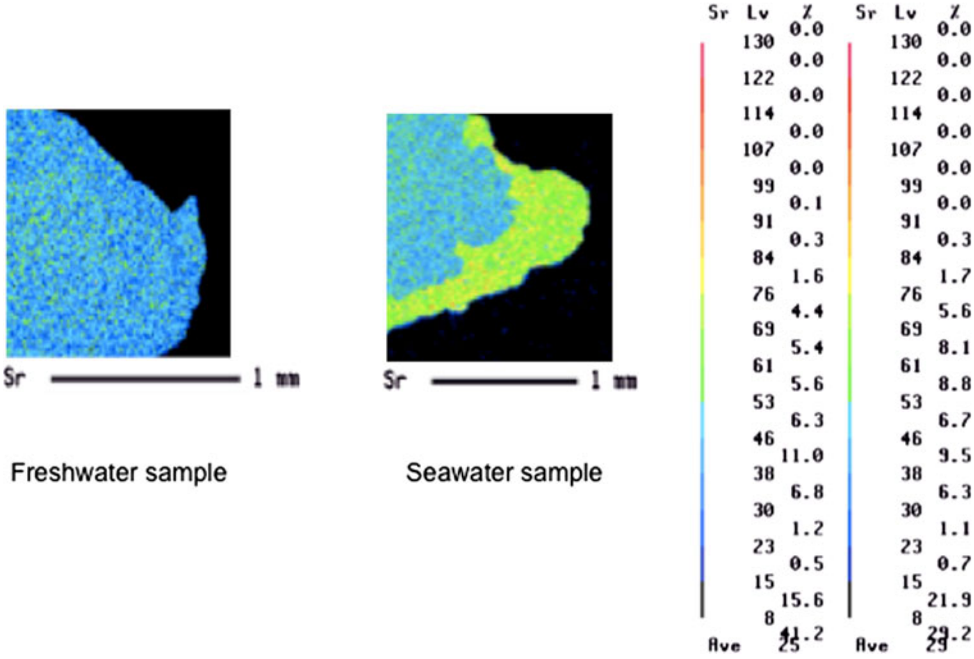

Fig. 3 X-ray intensity maps of the Sr contents in the otoliths of fish reared in freshwater and seawater. Freshwater sample showed low Sr levels (*blue*) consistently. Seawater sample showed low Sr levels (*blue*) at the inside and high Sr levels (*green*) at the outside

(Fig. 3). These results indicated that otolith Sr:Ca ratios of less than 3.0×10^{-3} and more than 5.0×10^{-3} can be correlated with the freshwater and seawater living phases of individual fish, respectively, with ratios between those two levels being associated with the estuarine living phase. Sr:Ca ratio profiles along the otoliths of each fish from the Sarufutsu coast (fish nos. 1–7) are shown in Fig. 4. There appeared to be two or three phases in most of the profiles, with the inner regions closer to the core being lower than the outer levels. Some otoliths went from lower values to higher values (nos. 1, 2, 3, 4, 5). Others went from higher to lower values (nos. 6 and 7). One, no. 6, initially dropped, remained relatively low, and then shifted higher. The inner region with the lower ratio, which extended 1,000 µm from the core in fish nos. 1–4 and 4,000 µm in fish nos. 5 and 7, possibly corresponded to the freshwater living period of the

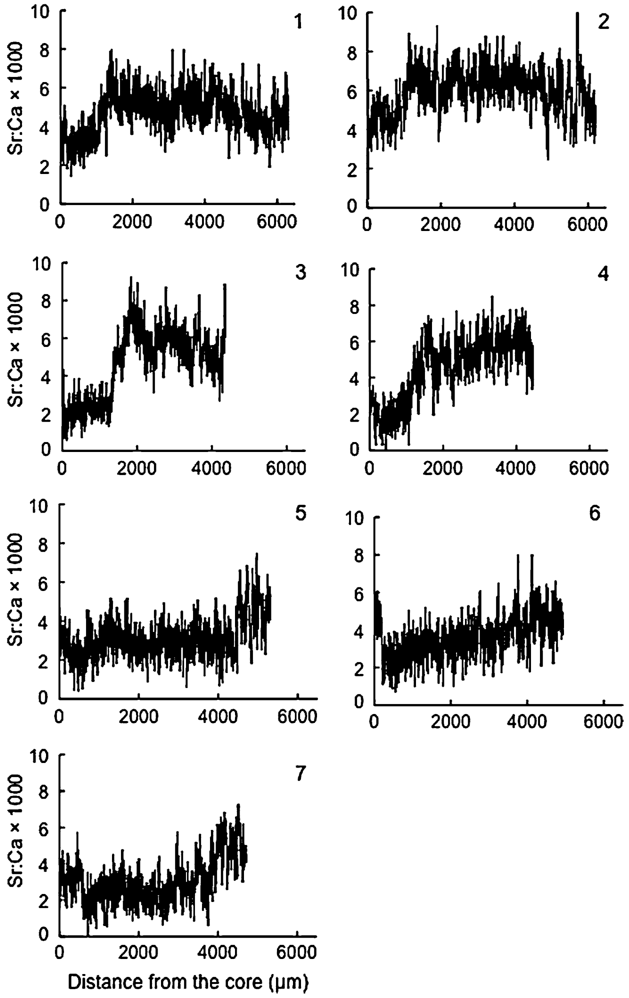

Fig. 4 Profiles of Sr:Ca ratios from the core to the edge of the otoliths of wild fish collected from the Sarufutsu Coast

alevin stage before the downstream migration. The Sr:Ca ratio in the outermost portion of the no. 3 fish otolith (20 μm from the edge) corresponded to the seawater living period and averaged 7.2 × 10^{-3} (range 5.6–10.1 × 10^{-3}), which suggests that the otolith Sr:Ca ratio under natural conditions agreed with the ratio under rearing conditions (Fig. 5).

Discussion

We examined the migration history of anadromous Sakhalin taimen captured along the Sarufutsu coast, northern Hokkaido, Japan, using otolith Sr:Ca ratios. We also examined the relationship between the otolith Sr:Ca ratios of captive fish reared in freshwater and seawater conditions. The otolith Sr:Ca ratios that corresponded to the rearing period in freshwater and seawater were 1.1 × 10^{-3} (range 0–3.4 × 10^{-3}) and 5.6 × 10^{-3} (range 4.0–7.8 × 10^{-3}), respectively. The otolith Sr:Ca ratios of brackish water were estimated to be 3.0–5.0 × 10^{-3}, which agreed with the range reported by Honda et al. [14] and Arai [26]. In these studies, otolith Sr:Ca ratios of some wild fish from the Sarufutsu coast showed low levels (0.5–4.0 × 10^{-3}) near the core, which thereafter greatly increased to 4.0–6.0 × 10^{-3} with the ratios being at higher levels (6.0–10.0 × 10^{-3}) in the outermost regions. Those three levels correspond to the freshwater, brackish water, and seawater living periods, respectively. These findings suggest that specimens from the Sarufutsu coast migrated to the brackish water region or the sea of Okhotsk and spent most of their lives there. On the other hand, Honda et al. [14] analyzed the otolith Sr:Ca ratios of Sakhalin taimen caught from Lake Akkeshi (brackish water lake) in Hokkaido, Japan, and suggested the specimens had migrated into brackish waters, but it was unlikely that they went into the ocean. The variety in the use of the ocean and brackish water habitat between fish from the Sarufutsu coast and Lake Akkeshi seems to be related to the geographical differences in those estuaries. Estuaries along the Sarufutsu coast open directly to the ocean, while Lake Akkeshi is connected to the ocean through Akkeshi Bay. Furthermore, a genetic difference has been suggested between the Sakhalin taimen of the Sarufutsu River system and the Bekanbeushi River system, which flows into Lake Akkeshi (K. Edo, pers. comm., 2007, 2009). Genetic differences might affect the migration pattern and life history traits of those stocks.

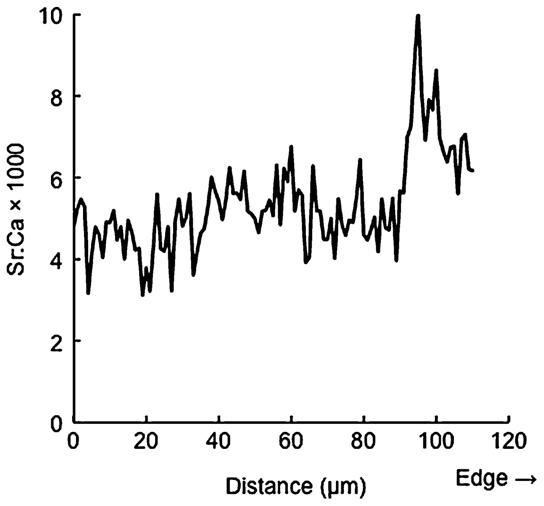

Fig. 5 Profiles of Sr:Ca ratios at the outer the edge of the otolith (110 μm from the edge) of fish no. 3, which was collected from the Sarufutsu Coast

The large difference in the pattern of Sr:Ca ratios of otoliths of wild fish suggests that there are two timings in the initiation of downstream migration in Sakhalin taimen. Fish nos. 1–4 seemed to move down to the estuary or sea before age 3 since their otolith radius of 1,000 μm was smaller than those of reared fish aged 2+ (K. Suzuki, unpubl. data, 2009). On the other hand, fish nos. 5 and 7, in which Sr:Ca ratios increased at an otolith radius of ca. 4,000 µm, possibly performed seaward migration after the age of 4 years. This suggests that the onset of downstream migration in Sakhalin taimen is quite flexible. Sakhalin taimen have been known to begin smoltification in spring at age 2+ and develop a strong salinity tolerance by September of that year (T. Kubo and S. Yamashiro, pers. comm., 1982), which is directly linked with the initiation of downstream migration. Honda et al. [27] suggested that Sakhalin taimen moved downstream in the river in spring and autumn, and the timing was related to individual metabolic efficiency and abundance of food available in the stream. It is possible that the timing of the smoltification and seaward migration of anadromous Sakhalin taimen in the Sarufutsu coast may also be related to various biological factors, such as metabolic efficiency, body growth, distribution of available food, and habitat density in the river. Metcalfe et al. [28] suggested that juvenile Atlantic salmon (*Salmo salar*) with higher metabolic rates had an advantage in terms of social status. The juveniles of higher social status tended to undergo metamorphosis into the migratory smolt stage at a younger age [29, 30], suggesting that the metabolic rate in the juvenile stage likely affects the life history traits of individual fish. Titus et al. [31] also reported that the habitat density of brown trout (*Salmo trutta*) potentially affected the age at first smoltification.

The relatively high Sr:Ca ratios at the center of the core and the subsequent drop that was seen in some of the fish may be related to early life history events such as emergence and exogenous feeding as Saito et al. [32] suggested for chum salmon (*Onchorhyncus keta*). The higher ratio in the otolith core of fish no. 6, which was comparable to the ratios from the sea living period, might be due to the higher ratio of yolk material of the maternal fish, which may have spent more time in the higher salinity environment during the maturation. Kalish [33] reported that otolith Sr:Ca ratios in the primordia of the progeny of anadromous salmonids were greater than those of the progeny of nonanadromous individuals.

In the outer portion of the otoliths with high Sr:Ca ratios in fish nos. 1–4—the portion that appears to correspond to the sea living phase—there were several short drops to ratios less than 5×10^{-3} (Fig. 4). These drops suggest that the fish moved into estuarine environments several times during the sea living period, or even that they might have entered freshwater. Two-dimensional X-ray intensity map of the Sr content for the otoliths of fish no. 3 reveals several rings indicating a shift in the living environment from brackish water to seawater (Fig. 6). The fish seem to migrate between freshwater and seawater or brackish water several times during their lives.

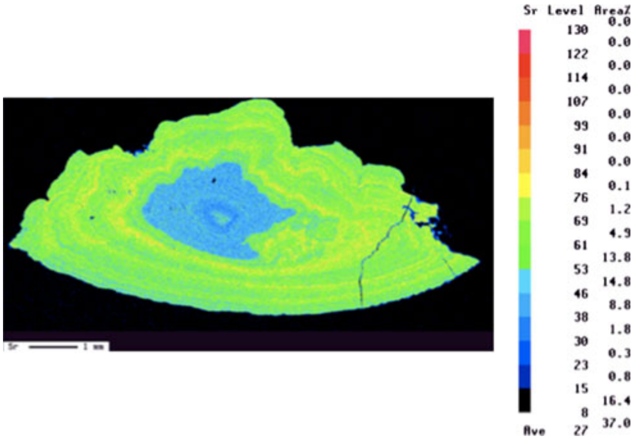

Fig. 6 X-ray intensity maps of Sr contents in the otoliths of fish no. 3 from Sarufutsu Coast. Sr concentration showed low levels (*blue*) at the core of the otolith and then higher levels towards the outside (*green* and *yellow*). Three *yellow concentric rings* in the outer portion of the otolith suggest that the individual moved into a higher salinity region in the estuary or sea

One factor that makes it difficult for Sr:Ca ratios to be used to observe short-term movements in mobile fishes such as Sakhalin taimen is that there is likely to be a lag effect of elemental uptake into otoliths. In black bream (*Acanthopagrus butcheri*), otolith Sr:Ca ratios only increased with increasing ambient Sr concentrations after 20 days of exposure [34]. Fish no. 2, captured in the coastal area on 26 June 1997, had two ovulated ova retained in the abdominal cavity, suggesting that this individual had spawned in the headwaters of a river less than 2 months before being caught, since the spawning of Sakhalin taimen in the coast of Hokkaido only occurs from late April to early May [7, 8]. The Sr:Ca ratios in the outermost portion of the otolith of that individual, however, were about 4×10^{-3}, comparable to the estuarine level. Sakhalin taimen are reported to stay in upstream tributaries for only 5–8 days [35] and subsequently to run downstream over 1–4 days [27]. Such a short stay in a freshwater region may result in relatively high Sr:Ca ratios in the outermost region of otolith with little or no evidence of the short duration in the freshwater environment for spawning being recorded.

The present study confirmed that Sakhalin taimen, captured along the Sarufutsu coast in the Sea of Okhotsk in northern Hokkaido, appeared to utilize estuarine and coastal areas as alternative growing habitats. These results suggest that it is important to preserve the environment of the estuarine and coastal regions for protection of Sakhalin taimen. Furthermore the anadromous migration pattern including the timing of downstream migration may be flexible among individuals in Sakhalin taimen. The connectivity between headwaters of rivers that are used as spawning grounds of Sakhalin taimen and the estuarine and coastal sea that may be used as important growth habitats is vital for the protection of the species. Kawaguchi et al. [36] reported that improvements in inadequately constructed weirs could restore the migration and spawning of Sakhalin taimen in the Sarukotsu River in Sarufutsu, which is the area of the present study. Considering the establishment of the conservation strategy of Sakhalin taimen, it is essential to maintain the connectivity of the river for the conservation of the migration route as well as the spawning and growth habitats. In the future, detailed trace element analyses of otoliths together with other approaches such as biotelemetry, which can directly monitor the movement and behavior of individual fish, would offer the key to understanding the unknown life history and help to establish an effective conservation strategy for this endangered species.

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