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Migration of Sakhalin taimen (*Parahucho perryi*): evidence of freshwater resident life history types

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Abstract Sakhalin taimen (*Parahucho perryi*) range from the Russian Far East mainland along the Sea of Japan coast, and Sakhalin, Kuril, and Hokkaido Islands and are considered to primarily be an anadromous species. We used otolith strontium-to-calcium ratios (Sr/Ca) to determine the chronology of migration between freshwater and saltwater and identify migratory contingents of taimen collected from the Koppi River, Russia. In addition, we examined taimen from the Sarufutsu River, Japan and Tumnin River, Russia that were captured in marine waters. Transects of otolith Sr/Ca for the Sarufutsu River fish were consistent with patterns observed in anadromous salmonids. Two fish from the Tumnin River appeared to be recent migrants to saltwater and one fish was characterized by an otolith Sr/Ca transect consistent with marine migration. Using these transects as benchmarks, all Koppi River taimen were classified as freshwater residents. These findings suggest more work is needed to assess life history variability among locations and the role of freshwater productivity in controlling migratory behavior in taimen.

Keywords Sakhalin taimen · Otolith · Anadromy · Fluvial

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Introduction

Species within the genus *Hucho* and *Parahucho* are among the largest and most endangered salmonids in the world (Zolotukhin et al. 2000). Danube salmon (*Hucho hucho*), Siberian taimen (*H. taimen*) and Sakhalin taimen (*P. perryi*) have experienced significant reductions in range and abundance over the past 30 years (Matveyev et al. 1998; Zolotukhin et al. 2000). Sakhalin taimen (also known as Japanese huchen) *Parahucho perryi*, while the only representative of its genus, is associated with a group of fishes in the genus *Hucho* that are remarkable given their biological characteristics and unusual life histories, including their primitive phylogenetic traits, slow growth, late age at maturity, long generation time,

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broad dietary habits, and threatened status (Holcik et al. 1988; Fukushima et al. 2011). The Sakhalin taimen was originally in the genus *Hucho*, but mitochondrial DNA evidence indicates that the species is distinct phylogenetically and should be categorized into a separate genus (Shed'ko et al. 1996). Sakhalin taimen, in particular, present unique challenges to conservation due to their slow growth, delayed age at maturity, and migratory behavior that potentially increases risk of capture in commercial near-shore ocean fisheries. The range of Sakhalin taimen is restricted to the Russian Far East mainland along the Sea of Japan, and Sakhalin, Kuril, and Hokkaido Islands (Zolotukhin et al. 2000). By-catch records of the Russian commercial fishery suggest a greater than 80% decline in abundance of *P. perryi* across this region during the past 50 years (Zolotukhin et al. 2002; Rand 2006). Populations in Japan are currently found in a few small river basins, and individual river population sizes are estimated to be typically <200 mature individuals (Fukushima 1994). Although less well studied, population sizes in small rivers of mainland Russia, draining to the Sea of Japan, are believed to contain similarly small populations (Zolotukhin et al. 2000).

The Sakhalin taimen, unlike other taimen species, is generally considered to be anadromous (Arai et al. 2004; Edo et al. 2005; Suzuki et al. 2011). Kawamura et al. (1983) described capture of taimen from brackish waters on the eastern shore of Hokkaido Island, Japan. Arai et al. (2004) used otolith microchemistry to demonstrate that taimen collected from a stream on Sakhalin Island had repeatedly migrated to saltwater. Similarly, from Lake Akkeshi on Hokkaido, Japan, Honda et al. (2010) used otolith microchemistry and determined that eight of ten taimen examined were anadromous, and the remaining two samples had migrated to brackish waters. Edo et al. (2005) classified taimen captured near the mouth of the Sarufutsu River, on the island of Hokkaido, as anadromous based on capture in near-shore set-nets, stomach contents containing marine species, and silvery coloration. Suzuki et al. (2011) used otolith microchemistry to confirm that seven taimen captured in set nets near the Sarufutsu River were anadromous. In all these cases, the taimen captured and classified as anadromous ranged from 46.0 to 78.4 cm in length. The taimen examined by Edo et al. (2005) were >8 years old at time of capture. In contrast, Suzuki et al. (2011) found that some fish migrated to sea before age-3. Arai et al. (2004) also examined three juvenile taimen (<13.0 cm fork length) and one adult taimen (70.0 cm fork length) that had not migrated to saltwater based on otolith microchemical profiles. Although the 70.0 cm adult fish examined by Arai et al. (2004) demonstrates that some taimen are not anadromous, it is frequently assumed that large fish automatically equate with anadromy.

Because different contingents, or migratory phenotypes, are likely to be susceptible to different mortality rates given duration of residence in different habitats, it is important to identify the range and degree of migratory behavior within, and among populations to better understand habitat- or life-history related survival. In this study, we used analysis of otolith strontium-to-calcium ratios (Sr/Ca) to identify migratory behavior and reconstruct the chronology of migration between freshwater streams and seawater for Sakhalin taimen that were assumed to be anadromous based on size and coloration or capture in the lower reaches of rivers or in nets set in marine waters adjacent to river mouths. We examined taimen otoliths collected from the Koppi and Tumnin Rivers located in Khabarovsk, Russia and the Sarufutsu River located in Hokkaido, Japan. The Koppi River is currently the focus of conservation efforts aimed at protecting taimen and other fishes and more information is needed to guide these efforts. This study is part of a larger effort to better understand the ecology and genetic population structure of Sakhalin taimen throughout their native range.

Methods

Otolith collection

Otoliths were collected from three rivers draining to the Sea of Japan (Fig. 1). The Koppi and Tumnin Rivers are located in the Khabarovsk Oblast in the Russian Far East and the Sarufutsu River is located on the Soya Peninsula along the northern shore of Hokkaido Island, Japan (Fig. 1). Watershed area differs among the three drainages: Koppi River 7305 km^2, Tumnin River 21 655 km^2, and Sarufutsu River 363 km^2. The Koppi and Tumnin rivers have extensive estuaries, whereas, the Sarufutsu River does not. Each of the rivers support populations of Sakhalin taimen, masu salmon (*Oncorhynchus masou*), chum salmon (*O. keta*), pink salmon (*O.*

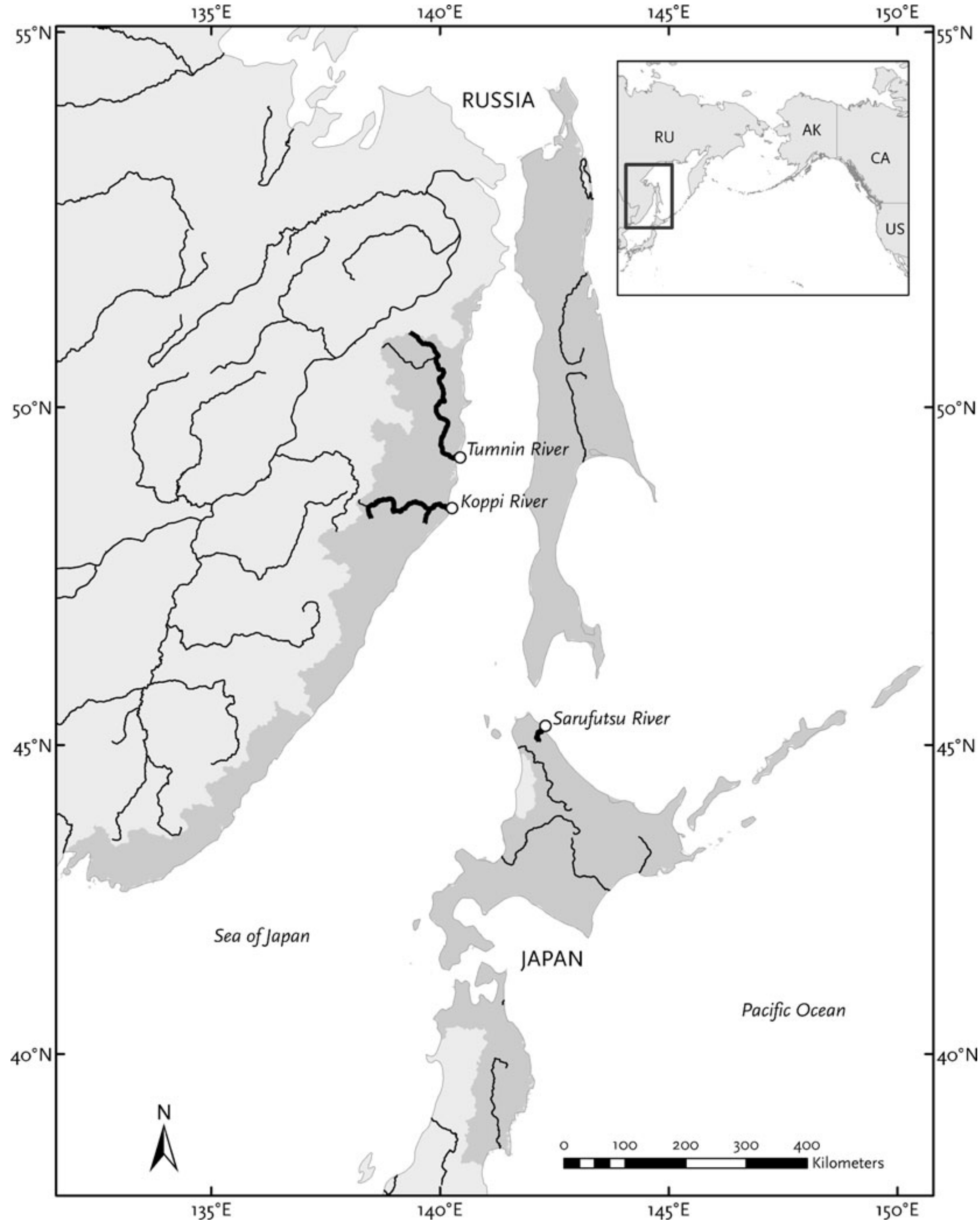

Fig. 1 Location of Tumnin and Koppi Rivers, Russia and Sarufutsu River, Japan. Darker gray shading indicates the historic distribution of Sakhalin taimen

gorbuscha), white spotted char (*Salvelinus leucomaensis*), and other migratory and non-migratory species.

In 2000 and 2001, sagittal otoliths were collected from nine taimen in the Koppi River, three taimen captured just outside the mouth of the Tumnin River, and two taimen captured just outside the mouth of the Sarufutsu River (Fig. 1). Because Sakhalin taimen are a threatened species and lethal sampling was not prudent, all otoliths were collected from mortalities that were incidental to other fisheries or collected from carcasses recovered in freshwater. In addition, sagittal otoliths were collected from a masu salmon and a white spotted char in the Koppi River. The masu salmon and white spotted char were presumed to have migrated to sea (Arai and Tsukamoto 1998; Arai and Morita 2005) and were included for comparison with putative migratory fish. The otoliths from Koppi and Tumnin River fishes were obtained through a joint research project involving the Wild Salmon Center and the Khabarovsk TINRO during 2000–2002. After removal, all otoliths were cleaned and stored in dry vials until analysis.

Otolith preparation and analysis

The chemical composition of otoliths can be used to describe migration in anadromous fishes based on examination of the ratio of strontium (Sr) and calcium (Ca) (Kalish 1990; Secor 1992; Volk et al. 2000). Strontium, an element with binding characteristics similar to Ca, is substituted for Ca in the calcium carbonate matrix of the otolith at levels relative to the concentration of Sr in the environment (Kalish 1990; Zimmerman 2005; Arai 2010). Because Sr/Ca is generally greater (3 to 10 x) in seawater compared to freshwater (Odum 1951) and the concentration of Sr is correlated with salinity (Odum 1951; Ingram and Sloan 1992; Zimmerman 2005), analysis of Sr/Ca ratios across the otolith of a fish can identify growth occurring in freshwater and saltwater.

One sagittal otolith from each fish was embedded in epoxy (Epo-Thin, Buehler Ltd.) and a transverse section was cut through the core using a diamond wafering saw. Otolith mounting and preparation followed the methods of Donohoe and Zimmerman (2010). The transverse section was glued to a cover slip attached to a glass slide on one edge and ground with 2000-grit sandpaper to remove saw marks. The section was then polished with a slurry of 0.05 μm alumina paste. The cover slip was cut with a scribe so that several prepared otoliths could be mounted on a petrographic slide for microprobe analysis. The petrographic slide containing several otoliths was rinsed with deionized water, air dried, and carbon coated. Elemental analysis was conducted with a JEOL JXA-8800 L wavelength dispersive microprobe. A 15 kV, 50 nA, 10 μm-diameter beam was used for all analyses (Zimmerman and Nielsen 2003).

Strontiantite ($SrCO_3$) and calcite ($CaCO_3$) were used as standards for Sr and Ca, respectively. Each element was analyzed simultaneously, and a counting time of 40 s was used to maximize precision (Toole and Nielsen 1992). Strontium was measured using the TAP crystal, and Ca was measured using the PET crystal. A transect of points with a spacing of 10 to 25 μm was measured from the center of the core to the edge of the otolith. To quantify the variability of otolith Sr/Ca among samples, we calculated the coefficient of variation (CV) as the $SD_{Sr/Ca}$ × mean Sr/Ca^{-1} for each fish. We assumed fish that had migrated from freshwater to seawater would have higher CV than fish that remained in freshwater.

After microchemical analysis, the carbon layer was cleaned from the surface of otoliths and each otolith was photographed at a magnification of 45x using a digital camera connected to a dissecting microscope. The otolith was placed on a black background and reflected light was used to accentuate the presumed annuli (Fig. 2). The age of each fish was determined by counting alternating translucent and opaque regions. Under reflected light, annuli correspond to the translucent zone (Kalish et al. 1995). The distance from the center of the core to each annulus was measured along a standardized transect. To describe the relation between age and migration, we plotted annuli over transects of otolith Sr/Ca.

Results

Ages of taimen examined ranged from 3 to 20, and fork lengths at time of capture ranged from 36 to 115 cm (Table 1). Otolith Sr/Ca across a transect of points beginning in the core and ending at the edge of the masu salmon otolith was similar to that of anadromous salmonids reported previously (e.g., Kalish 1990; Arai and Tsukamoto 1998) with a region

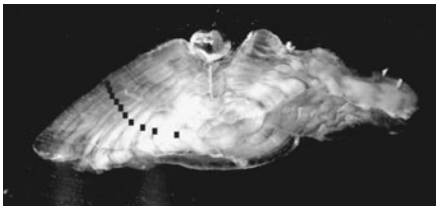

Fig. 2 Micrograph of a transverse section of a taimen otolith to illustrate annuli that are indicated by black squares

Table 1 Fork length (cm), age, minimum, maximum, and mean (± s.d.) and coefficients of variation of otolith strontium-to-calcium ratios (Sr/Ca) for taimen collected from three rivers

River	Fish	Fork length (cm)	Age	Minimum Sr/Ca (x 10^3)	Maximum Sr/Ca (x 10^3)	Mean Sr/Ca ± s.d. (x 10^3)	CV
Koppi	1	100	15	0.98	1.64	1.32±0.12	0.09
Koppi	2	115	8	0.75	1.82	1.24±0.24	0.19
Koppi	3	95	8	0.02	0.25	0.14±0.06	0.43
Koppi	4	62	15	1.21	1.78	1.43±0.13	0.09
Koppi	5	107	15	0.99	1.57	1.21±0.11	0.09
Koppi	6	41	5	0.83	1.84	1.21±0.21	0.17
Koppi	7	43	6	1.04	1.77	1.30±0.17	0.13
Koppi	8	36	3	0.95	1.53	1.21±0.12	0.10
Koppi	9	73	10	1.02	1.77	1.36±0.19	0.14
Tumnin	1	44	7	0.99	1.77	1.43±0.14	0.10
Tumnin	2	47	6	1.12	1.59	1.33±0.11	0.08
Tumnin	3	59	6	1.00	2.53	1.44±0.33	0.23
Sarufutsu	1	92	20	0.79	5.31	2.64±1.14	0.43
Sarufutsu	2	90	17	1.08	3.65	2.47±0.62	0.25

of low Sr/Ca associated with freshwater rearing and increased Sr/Ca ($>2.0\times10^{-3}$) associated with saltwater residence (Fig. 3a). Similarly, the transect of Sr/Ca across the otolith of the white spotted char indicated a period of freshwater residence across the first 2 years

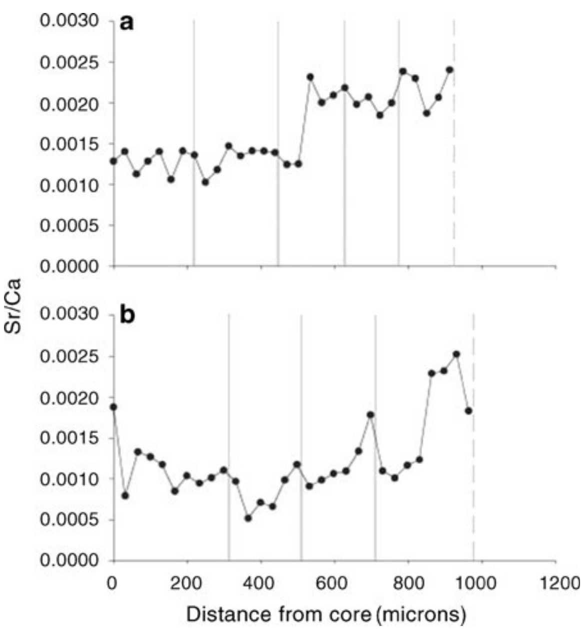

Fig. 3 Transects of otolith Sr/Ca, beginning in the otolith core and ending at the edge of the otolith for (**a**) masu salmon and (**b**) white spotted char captured in the Koppi River, Russia. The dashed line indicates the otolith edge and the solid grey lines indicate location of annuli

of life followed by two peaks of Sr/Ca at ages 2 and 3 (Fig. 3b). Mean Sr/Ca (± s.d.) in the freshwater region (ages 0 and 1) of the masu salmon was 1.30×10^{-3} ($\pm0.14\times10^{-3}$) and mean Sr/Ca was 2.11×10^{-3} ($\pm0.19\times10^{-3}$) in the saltwater growth region. Maximum Sr/Ca measured in the masu salmon otolith was 2.40×10^{-3}. Similarly, for the white spotted char, maximum Sr/Ca was 2.52×10^{-3}. In addition, Sr/Ca in the core region of the white spotted char was high indicating this fish was the progeny of a migratory female (Kalish 1990; Zimmerman and Reeves 2002). The CV of otolith Sr/Ca was 0.26 for the masu salmon and 0.41 for the white spotted char.

Both taimen collected in the mouth of the Sarufutsu River were characterized by otolith transects with regions of elevated Sr/Ca indicating marine residence (Table 1). Sarufutsu taimen 1 was characterized by an otolith transect similar in shape to the masu salmon, with lower Sr/Ca to age 3 followed by higher otolith Sr/Ca through age 20 and maximum Sr/Ca peaked as high as 5.3×10^{-3} (Fig. 4a; Table 1). The otolith transect of Sarufutsu taimen 2 was also characterized by higher Sr/Ca from age 9 to 17 (Fig. 4b), but maximum Sr/Ca peaked at 3.65×10^{-3} (Table 1). Coefficients of variation of otolith Sr/Ca was 0.43 and 0.25 for Sarufutsu River taimen 1 and 2, respectively.

Otolith Sr/Ca transects of taimen captured in the Tumnin River were characterized by two patterns.

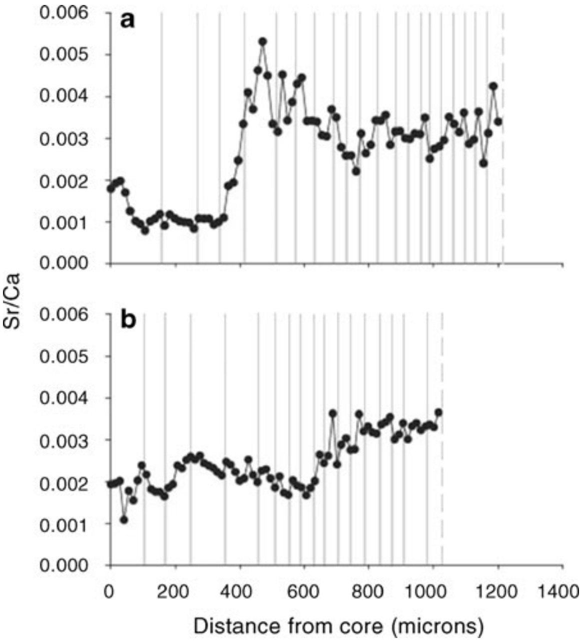

Fig. 4 Transects of otolith Sr/Ca, beginning in the otolith core and ending at the edge of the otolith for taimen (**a**) Sarufutsu 1 and (**b**) Sarufutsu 2 captured at the mouth of the Sarufutsu River, Japan. The dashed line indicates the otolith edge and the solid grey lines indicate location of annuli

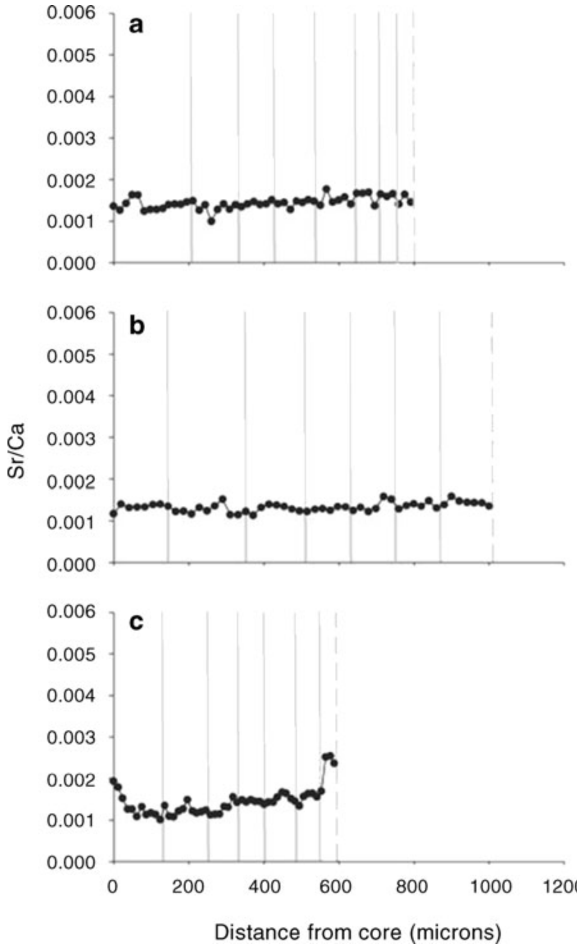

Fig. 5 Transects of otolith Sr/Ca, beginning in the otolith core and ending at the edge of the otolith for taimen (**a**) Tumnin 1, (**b**) Tumnin 2, and (**c**) Tumnin 3 captured adjacent to the mouth of the Tumnin River, Russia. The dashed line indicates the otolith edge and the solid grey lines indicate location of annuli

First, Sr/Ca transects for Tumnin taimen 1 and 2 varied little (Fig. 5a, b) and maximum Sr/Ca was $<1.8\times10^{-3}$ (Table 1). Conversely, Tumnin taimen 3 was characterized by higher Sr/Ca (maximum=2.53×10^{-3}) near the edge of the otolith corresponding to marine residence (Fig. 5c). In addition, Sr/Ca in the core region of Tumnin taimen 3 was high indicating this fish was the progeny of a migratory female (Kalish 1990; Zimmerman and Reeves 2002). Coefficients of variation of otolith Sr/Ca was ≤0.10 for the first two fish (Table 1). Because these fish were captured in saltwater, they were assumed to be recent migrants to seawater. Coefficients of variation of otolith Sr/Ca was for Tumnin taimen 3, and it was classified as a migrant based on increased Sr/Ca, was 0.23.

Otolith Sr/Ca transects for the Koppi River taimen are presented in Fig. 6. The otolith for taimen 3 was optically different from normal otoliths and was, therefore, likely composed of calcium carbonate in the mineral form of vaterite, which typically does not incorporate Sr (Brown and Severin 1999). Because of this, this otolith was excluded from further analysis.

For the remaining Koppi River taimen, maximum otolith Sr/Ca ranged from 1.53×10^{-3} to 1.84×10^{-3} and CV of otolith Sr/Ca ranged from 0.09 to 0.19 (Table 1). Overall, for the Koppi River taimen examined, none of the otolith Sr/Ca values approached the peak values observed in the Sarufutsu River fish. Three Koppi River taimen (2, 7, and 6) were characterized by low variability in otolith Sr/Ca suggesting possible migrations to brackish water (Fig. 6 a, b, c).

For example, two peaks of otolith Sr/Ca were approaching 1.9×10^{-3} at ages 3 and 4 in Koppi River taimen T-1 (Fig. 6a). Similarly, Koppi River taimen 7 and 6 were characterized by peaks approaching 1.85×10^{-3} at ages >2 (Fig. 6 b, c). Koppi River taimen 8, 1, 4, and 5, showed little variation in otolith Sr/Ca (CV≤

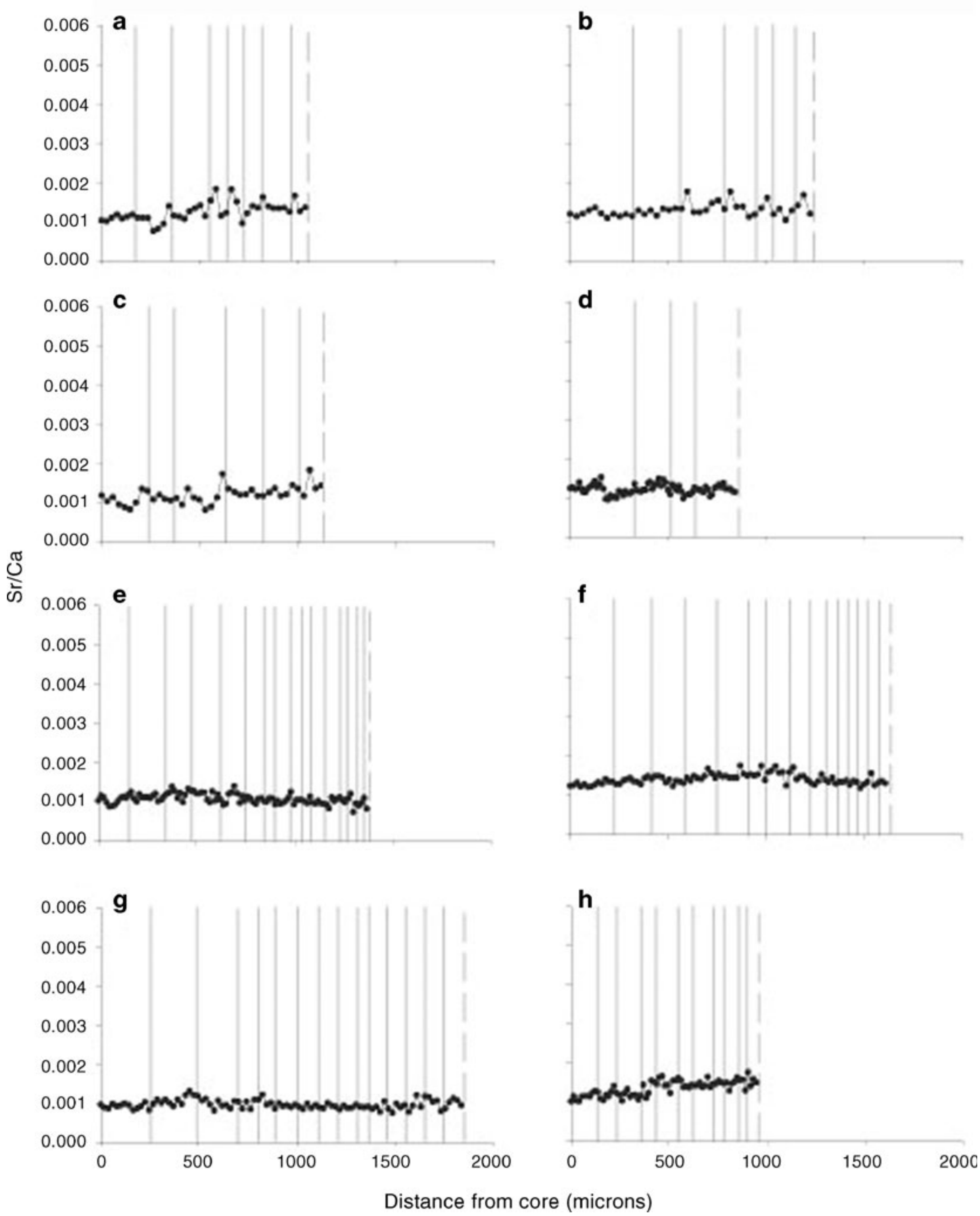

Fig. 6 Transects of otolith Sr/Ca, beginning in the otolith core and ending at the edge of the otolith for taimen captured in the Koppi River, Russia. The dashed line indicates the otolith edge and the solid grey lines indicate location of annuli

0.10), and maximum otolith Sr/Ca was $<1.78\times10^{-3}$ indicating these fish did not migrate to seawater (Fig. 6d, e, f, g; Table 1). Koppi River taimen 9 was characterized by increasing Sr/Ca (Fig. 6h) and a low CV (Table 1).

Discussion

Transects of otolith Sr/Ca indicate that the taimen examined represent a variety of migratory phenotypes. First, the Sarufutsu River taimen exhibited a classic high otolith Sr/Ca signal presumed to indicate residence in high salinity environments. The Tumnin taimen were not characterized by similarly high Sr/Ca values, but one fish had clearly migrated to higher salinity conditions (Tumnin 3). The other two fish appeared to have just migrated to saltwater before capture. The Koppi River taimen were characterized by two patterns of otolith Sr/Ca: (1) little variation with moderate Sr/Ca values (i.e., 1.2×10^{-3} to 1.5×10^{-3}) and (2) transects punctuated by peaks in Sr/Ca> 1.5×10^{-3} (i.e., lower than peaks observed in presumed saltwater migrants; Tumnin 3 and Sarufutsu samples).

We used the Sarufutsu River and Tumnin River samples to guide classification of the Koppi River taimen. Without translocation or rearing studies to determine the relation between salinity and otolith Sr/Ca for taimen, however, it is difficult to determine a threshold value associated with movement into seawater for this study. For example, Zimmerman (2005) raised juvenile salmon, trout, and char in laboratory tanks with salinity increasing in 2 week intervals to validate the relationship between otolith Sr/Ca and increasing salinity. Otolith Sr/Ca differed among species at the same salinity suggesting that it was not appropriate to compare values among species (Zimmerman 2005). Further, Zimmerman (2005) cautioned that otolith Sr/Ca provides only enough resolution to broadly classify otolith material deposited in freshwater, brackish, or saltwater.

Arai (2010) conducted rearing studies using taimen and confirmed that otolith Sr/Ca ratios were correlated with ambient salinity. Arai (2010) found that otolith Sr/Ca was linearly related to ambient salinity and otolith Sr/Ca in freshwater was 1.84×10^{-3}, increased to approximately 3.0×10^{-3} in brackish water (salinity= 11 psu), and was approximately 5.0×10^{-3} in full seawater (salinity=33 psu). Based on those results, a threshold otolith Sr/Ca value of approximately 2.5×10^{-3} could be used to discriminate freshwater and saltwater growth. Using this value, none of the Koppi River fish would be classified as saltwater migrants. We were unable, however, to directly compare our results, nor select a threshold value for the transition to saltwater, from Arai (2010) because of differences in instrumentation between the two studies. Although we analyzed the masu salmon and white spotted char to provide a benchmark for otolith Sr/Ca transects in expected migratory species from the Koppi River, we cannot directly apply thresholds based on these transects to the taimen collected from the Koppi River. They do, however, suggest that otolith Sr/Ca deposited in the Koppi River can be distinguished from otolith Sr/Ca deposited in marine environments.

Because the Tumnin River taimen were captured in set-nets outside the mouth of the river, we expected to find elevated Sr/Ca reflecting migration to seawater. Only one of the samples (Tumnin 3) was characterized by increased Sr/Ca, and these values were only observed in the year of capture, indicating the fish had just migrated to seawater; based on the location of capture, these fish were presumed to have entered seawater, however, only one showed evidence based on a Sr/Ca signature in the otolith. As a result, it is important to recognize that short duration migrations to saltwater (or very recent migrations to saltwater) may not result in increased otolith Sr/Ca. It is, therefore, likely that frequent short migrations to estuarine or near-shore habitats (amphidromous migrations) would not be captured by analysis of transects of otolith Sr/Ca.

Because the temporal resolution of the 10 μm beam used to measure Sr/Ca varies across the otolith transect, it is difficult to compare among years within the same fish. For example, in Koppi River taimen 3, with uniform sampling across annual growth increments, the first year of life contains 13 probe measurement points while later years contain only two or three points (Fig. 6). A point measured in the first year of life is sampling otolith material deposited over a mean of 28 d, whereas at age 10 the same point is sampling material deposited over approximately 180 d. It is likely, therefore, that short-term migrations at later ages are not adequately represented in the life history transects. Further work examining otolith Sr/Ca of fish of known migration history is needed to determine patterns associated with amphidromous species that may make sub- annual migrations between environments of differing salinities.

Yamashiro (1965), as cited in Edo et al. (2005), suggested that smolting of Sakhalin taimen might occur at age 2–3 based on growth rates. Sarufutsu River taimen 1 made an initial migration to sea at age 3 (Fig. 4a). Sarufutsu River taimen 2, however, was characterized by otolith Sr/Ca values as high as 2.5×10^{-3} from its first year of life through age-4 (Fig. 4b). It is not clear if this fish was migrating to brackish water from age-0 to age-8 and then migrated to significantly higher salinity environments at age-9, or if it was rearing in freshwaters of higher Sr content.

Further work is needed to assess the role of freshwater productivity in controlling migratory behavior in Sakhalin taimen while controlling for watershed area and development. Fukushima et al. (2011) found that watersheds of lower topography and extensive wetland and brackish lagoons were important factors in describing the distribution and conservation status of Sakhalin taimen. The eastern shore of Khabarovsk Krai or the western shore of Sakhalin Island would provide an ideal set of streams to test hypotheses concerning the role of freshwater productivity and watershed level controls on life history and productivity of taimen.

Given the conservation status of taimen, this study and others are constrained by small samples sizes. In combination, otolith microchemistry has been used to describe migratory behavior in only a total of 31 Sakhalin taimen: 13 in this study, eight in Arai et al. (2004), and ten in Honda et al. (2010). These results suggest that there are existing gaps in our knowledge of taimen behavior and ecology. It is clear that diadromous migrations are conducted by Sakhalin taimen (Arai et al. 2004; Honda et al. 2010), but it is not clear whether these migrations are annual (as observed in amphidromous species such as Dolly Varden *Salvelinus malma*; Armstrong 1974), or if taimen overwinter in seawater. Further, it is not clear what proportion of Sakhalin taimen are diadromous and how that proportion is controlled by watershed and estuarine productivity. Further work is needed to describe migratory behavior in taimen with an emphasis on determining duration of marine residence, stray rates among streams, use of overwintering habitat, and how migratory behavior is controlled by watershed and estuarine productivity.

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