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Investigation on the plausible coastal use of masu salmon parr collected from rivers in the western Shiretoko Peninsula

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Our study investigated if the masu salmon *Oncorhynchus masou* parr, inhabiting rivers in the western Shiretoko Peninsula, utilizes coastal habitats before smolting using otolith microchemistry analysis. The otolith Sr/Ca ratios were observed to increase temporarily in four of ten individuals in the specimens collected from the Rusha and Ponbetsu rivers, suggesting the potential utilization of coastal habitats by the masu salmon parr. The same was observed in one individual collected from the Ishihama-sawa River in the Omoe Peninsula, indicating the utilization of coastal saltwater before smolting. In steep rivers, such as those in the Shiretoko and Omoe peninsulas, it is plausible that the accidental displacement of masu salmon parr to coastal waters, followed by their return to the river, is a frequent occurrence.

Keywords salmonids, nomads, early migrating type, otolith microchemistry

Introduction

The life histories of salmonids are diverse, and their partial migration is a particularly striking. Some individuals migrate to the sea and return to their natal rivers to spawn, whereas others remain there until their spawning events (Jonsson & Jonsson 1993; Ferguson et al. 2019). Typical salmonid migration occurs through the freshwater juvenile stage as parr, followed by their downstream migration to the sea after smoltification in the spring, accompanied by silvery body coloration, physiological changes, and hormonal alterations. In addition to this typical migration pattern, salmonids also known to descend to estuaries and the sea before smoltification (Koski 2009; Simmons et al. 2013; Birnie-Gauvin et al. 2019). In Atlantic salmon *Salmo salar* and brown trout *Salmo trutta*, a large proportion of the population may descend without smoltification (Birnie-Gauvin et al. 2019). In the coho salmon *Oncorhynchus kisutch* (Koski 2009) and brown trout (Taal et al. 2018), non-smoltified parr and juveniles have been reported to use in estuarine and coastal waters and migrate back to rivers after a short coastal residence. This type of life history is called "nomad" (Koski 2009). Previously, most of the nomad-like individuals that descended to the sea without smoltification at the age of 0+ years were thought to be die as they were not fully adapted to seawater; however, recent studies have shown their survival and return as spawning adults (Bennett et al. 2015; Flitcroft et al. 2016; Wynne et al. 2023).

Partial migration, a population composing both anadromous migrants and freshwater residents, is observed in the masu salmon *Oncorhynchus masou*, salmonid species endemic to Asia (Morita 2018). Anadromous individuals spend more than a year in the river before smoltification and then migrate to the sea to grow. In contrast, resident individuals remain in the river without migrating until spawning. In addition to this typical migratory dimorphism, the temporal use of coastal waters by masu salmon parr, such as the nomads reported in

coho salmon, has been observed. Kubo (1976) documented frequent observations of masu salmon fry descend into brackish waters. Such movement is likely to occur after rainfall-induced flooding. For instance, on July 14, 1946, the author caught several small parr in the brackish water at the mouth of the Oshironai River. The author reported their subsequent possible movement upstream and return to their natural (freshwater) habitats. In addition, it was reported that juvenile masu salmon stocked into the lower reaches of a river were recaptured in another river several months later, providing evidence that they moved up via coastal waters into another river before the smolting season (Ando & Kawamura 2003). Matsubayashi et al. (2017) examined the profile of the otolith Sr stable isotope ratio ($^{87}Sr/^{86}Sr$) in three adult masu salmon collected from the Churui River on the east coast of the Shiretoko Peninsula and identified that one descended to the sea as a juvenile, returned to the river after a certain period, and later migrated back to the sea. This migration pattern was consistent with the nomadic life history of coho salmon. The otolith Sr/Ca ratio profiles of masu salmon juveniles collected from two steep rivers in Hokkaido suggested that they descended to the sea during their juvenile stages (Kuroki et al. 2020).

However, existing knowledge on the coastal water use by masu salmon at the parr stage is limited, and its widespread occurrence remains unclear. The importance of life history diversity for the stability and long-term viability of fish populations is increasingly acknowledged (Schindler et al. 2010; Cordoleani et al. 2021). Understanding the entire life history of masu salmon, including rare life history types, is critical to the conservation of this species. In this study, we collected masu salmon parr from freshwater rivers with steep mouths. We analyzed otolith Sr/Ca ratios to determine the extent to which individuals had experienced coastal brackish waters before smolting.

Materials and methods

1. Study rivers

Ten masu salmon parr were collected in September 2022 from the Rusha River (44.198°N, 145.195°E) and Ponbetsu River (44.194°N, 145.187°E), flowing along the west coast of the Shiretoko Peninsula facing the Sea of Okhotsk (Table 1). Sampling was conducted using dip nets 30 cm in diameter. We attempted to collect masu salmon parr from the Ponputa River (44.188°N, 145.174°E), located 1.26 km southwest of the Ponbetsu River. However, no masu salmon could be collected. All these rivers were steep, and salmonids were observed in the river mouths. All collected specimens were parr and exhibited juvenile morphology during freshwater life stages. Fork length (FL, mm) and body weight (g) were measured in the field, and sex was determined by visual observation of the gonads. Sagittal otoliths were extracted from each individual and preserved in 99 % ethanol to analyze their age and migration history.

2. Otolith analyses

The age of masu salmon parr was estimated by counting the opaque bands of otoliths under a stereomicroscope, following the procedure described by Morita & Nagasawa (2010). The otoliths were embedded in epoxy resin (EpoFix, Struers), and grounded to expose the core, and the further polished by a colloidal silica suspension liquid (OP-S, Struers). After cleaning with distilled water and drying, the otoliths were coated with Pt-Rd using an ion sputterer (E-1030, Hitachi). Electron microprobe analysis was performed from the otolith core to the edge using an electron probe microanalyzer (JXA-8230, JEOL) to investigate the changes in strontium (Sr) and calcium (Ca) concentrations along the growth stages. The measurement conditions were at an acceleration voltage of 15 kV and a current value of 12 nA. The electron beam was focused on a point 1 μm in diameter and measurement interval was 1 μm from the core to the edge. The concentration conversions (weight %) of the X-ray intensities for both elements were obtained by using strontium titanate ($SrTiO^3$) and calcium silicate ($CaSiO^3$) as standards and the concentration ratio of Sr to Ca × 1000 was represented as the Sr/Ca ratio (hereafter referred to as the Sr/Ca ratio).

In addition to the above specimens, otoliths from one masu salmon parr collected from the Ishihama-sawa River, located on the Omoe Peninsula in southern Iwate Prefecture and

Table1. Masu salmon *Oncorhynchus masou* used for otolith analyses

No.	Collection date	Collection site	Folk length (mm)	Body weight (g)	Sex	Maturation	Age
1	2 September 2022	Rusha River	110	16.1	Female	Immature	0+
2	2 September 2022	Rusha River	111	17.2	Female	Immature	0+
3	2 September 2022	Rusha River	102	14.2	Female	Immature	0+
4	2 September 2022	Rusha River	101	13.4	Female	Immature	0+
5	2 September 2022	Rusha River	112	19.1	Male	Immature	0+
6	2 September 2022	Rusha River	102	16.8	Male	Mature	0+
7	2 September 2022	Rusha River	109	16.9	Female	Immature	0+
8	2 September 2022	Rusha River	105	17.5	Male	Mature	0+
9	2 September 2022	Rusha River	114	18.7	Female	Immature	0+
10	2 September 2022	Ponbetsu River	169	62.9	Male	Mature	1+
11	8 September 2022	Ishihama-sawa River	143	39.4	Male	Mature	1+
12	16 July 2022	Pacific coast, eastern Hokkaido	480	n.d.	Female	Mature	2+

one adult masu salmon collected from the sea along the Pacific coast near Kushiro City in eastern Hokkaido were included in the Sr/Ca ratio analysis (Table 1). Similar to the Rusha and Ponbetsu rivers, the Ishihama-sawa River is steep. Otolith samples were prepared following the same process as describe above. The measurement conditions for otolith analysis of parr collected from the Ishihama-sawa River were the same. The same acceleration voltage and current value were used for a larger otolith of adult collected from the sea, except that the electron beam was focused on a point 10 μm in diameter and measurement interval was 10 μm from the core to edge.

Three specimens (Nos. 6, 9, and 11) were also analyzed for two-dimensional mapping of otolith Sr/Ca ratios. The measurement conditions were an acceleration voltage of 15 kV and a current of 50 nA. The electron beam was focused on each point for 100 ms with a pixel size of 2 μm × 2 μm.

The presence of sea entry for each individual was estimated from the profile of the moving average of five Sr/Ca ratio measurements based on the studies of Arai & Tsukamoto (1998) and Kasugai et al. (2014). Arai & Tsukamoto (1998) showed that the mean otolith Sr/Ca ratios of hatchery freshwater-reared masu salmon remained low at 1.35, while those of juvenile and immature individuals collected by set net along the coast of Niigata Prefecture and adult fish returning to the Kaji River during the freshwater life stage ranged 1.04–1.38, whereas those of individuals during the oceanic migration period ranged 3.31–3.69. Kasugai et al. (2014) reported that the mean otolith Sr/Ca ratios of masu salmon collected from Lake Kusshoro, Hokkaido, and its tributaries were 0.29–0.93, indicating that they were freshwater lake residents. Therefore, we considered that the otolith Sr/Ca ratios of ≥3 could enter brackish or coastal waters.

Results

Masu salmon parr (n = 10) was collected from the Rusha and Ponbetsu rivers on the west coast of the Shiretoko Peninsula with FL ranged 101–169 mm and weighing 13.4–62.9 g. All six females were immature. Four males, one was immature and three were mature (Table 1). Nine individuals (101–114 mm FL) were 0+ years old; the largest (169 mm FL) mature male was 1+ years old.

The otolith Sr/Ca ratios of six individuals (Nos. 3, 4, 6, 7, 8, and 10) were <3 for most of the measurement values, although the values were slightly higher at ~200–300 μm from the otolith core (Figs. 1, 2a). The otolith Sr/Ca ratios of four individuals (Nos. 1, 2, 5, and 9) were temporarily high values (3.00–4.14) up to ~300 μm from the otolith core and then decreased (Figs. 1, 2b). Among them, two individuals (Nos. 1 and 9) showed high values (3.00–3.44) again at ~500 μm from the otolith core. These results suggest that some individuals collected from the Rusha River used the coastal water shortly after emergence without smoltification.

The otolith Sr/Ca ratios of an individual collected from the Ishihama-sawa River (No. 11) averaged 3.98 (range: 2.90–4.99) at ~90 to 130 μm from the otolith core (Figs. 1, 2c). The peak value averaged 4.22 (range: 3.29–5.39) at 220–370 μm from the core, followed by a decrease. After that, the values remained low (<3) for a while, then increased from at ~850 μm, averaging 3.11 (range: 2.35–3.85) between 900 and 1240 μm, and slightly decreased around the otolith edge. This profile indicates that this parr might have migrated between freshwater and coastal areas several times before being collected from thew lower reaches of the Ishihama-sawa River.

The otolith Sr/Ca ratios of the individual (No. 12) collected from the Pacific coast of eastern Hokkaido (Fig. 1) remained low, averaging 0.46 (range: 0.00–1.36) from the otolith core to 940 μm, suggesting that the fish had consistently remained in freshwater during its freshwater parr stage. The Sr/Ca ratios increased sharply at 950–1200 μm and retained high values with an average of 3.68 (range: 2.30–4.98) from 1030 μm to the edge (1500 μm), though temporarily low values (<3) were observed. This profile indicates that the fish spent most of this period at sea before being collected from the coastal area.

Discussion

We observed that the otolith Sr/Ca ratios of some individuals collected from the Rusha River during the parr stage were similar to those of anadromous individuals collected along the Pacific coast during the oceanic period, suggesting that they might have used the coastal area soon after emergence. However, it was difficult to determine the presence or absence of seawater accretion experience based only on the otolith Sr/Ca ratios, as it was still unclear whether many of the Rusha River individuals were exposed to the seawater environment. Thus, further research is required based on observing and collecting the masu salmon parr in the coastal areas. The high Sr/Ca ratios around the otolith core of an individual collected in the Ponbetsu River might reflect factors other than environmental salinity, such as the influence of parental fish origin due to egg yolk absorption (Volk et al. 2000). However, no spawning of masu salmon has been observed in the Ponbetsu River in recent years. No fish of age 0+ years were also observed during this study. Therefore, it is possible that this individual entered the Ponbetsu River via the sea at an early stage of age 0+ years. An individual collected from the Ishihama-sawa River repeatedly showed high otolith Sr/Ca ratios, suggesting that this fish likely used coastal areas without smoltification; though this fish was identified as a precocious male resident.

On the Shiretoko Peninsula, several salmonids have been reported to temporarily use coastal areas during the juvenile stage while migrating to and from rivers other than their natal rivers in the surrounding waters. For example, juveniles of Sakhalin taimen *Parahucho perryi* (152–200 mm FL) have been caught in the Rusha River. However, it was considered that they entered the Rusha River from other rivers via the sea because this river is not a spawning river for this species (Komiyama & Takahashi 1988). In addition, chum salmon *Oncorhycnhus keta* fry released from the Uebetsu River on the east coast of the Shiretoko Peninsula after being otolith-marked with alizarin complexone were reported to have been collected from the neighboring rivers, such as Kotanuka and Sakimui rivers, in the same year (Kasugai & Saneyoshi 2013, 2014, 2015). Southern Asian Dolly Varden *Salvelinus curilus*, which is widespread in the Shiretoko Peninsula, has high Sr/Ca ratios detected near the otolith core, suggesting that they have been using coastal waters since their juvenile stage and

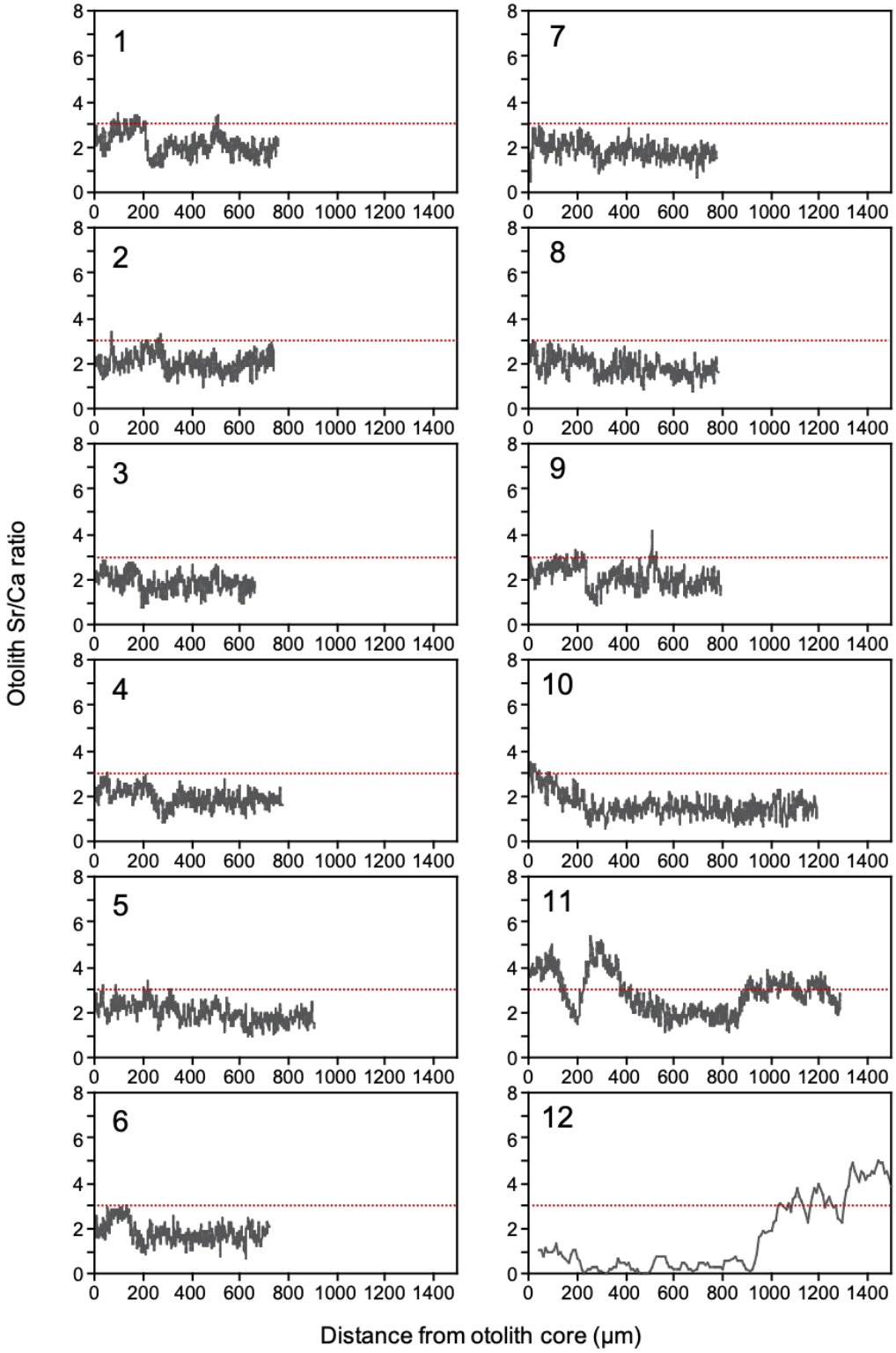

Fig. 1. Profiles of Sr/Ca ratios from otolith core to edge. The numbers in each figure are the individual numbers in Table 1. Red dashed lines indicate the position of the otolith Sr/Ca ratio of 3

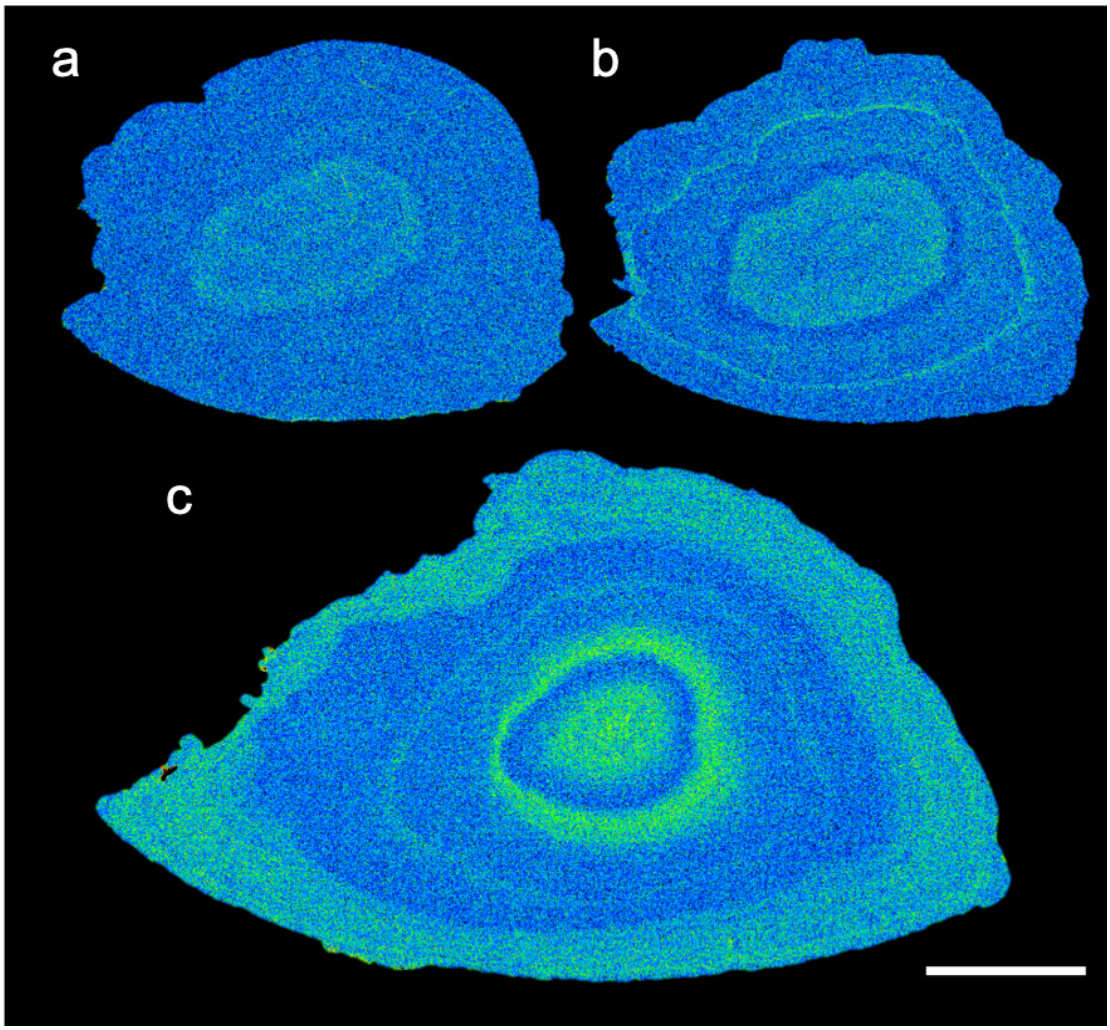

Fig. 2. Two-dimensional mapping of otolith Sr/Ca ratios. a: mature male (102 mm FL) collected from the Rusha River (No. 6); b: immature female (114 mm FL) collected from the Rusha River (No. 9); c: mature male (143 mm FL) collected from the Ishihama-sawa River (No. 11). Mappings of otolith Sr/Ca ratios are shown in blue for low values and in green for high values. The scale bar is 500 μm

they are experienced in seawater environments, even when their body color is not transformed to smolting (Umatani et al. 2018). In addition, a juvenile (230 mm FL) white-spotted charr *Salvelinus leucomaenis*, which does not have any spawning rivers in the core area of the Shiretoko Peninsula, was captured below the weir in the Matsunori River on the east coast of the Shiretoko Peninsula (Morita & Sahashi, unpublished, 6 November 2017), suggesting that the individual entered the Matsunori River via the sea from other rivers outside of the Shiretoko Peninsula.

Such coastal use by salmonids during the parr stage may contribute to the expansion of the distribution of the species (Taal et al. 2018) and could be a factor in the recent expansion of the masu salmon distribution along the Shiretoko Peninsula (Sahashi et al. 2018). Although many masu salmon migrated to most rivers in the Shiretoko Peninsula (Sano 1969), their numbers declined, and they were considered near extinct in the Rusha River in the 1980s (Komiyama & Takahashi 1988).

However, the masu salmon populations have recovered since the 2000s, independent

of hatchery releases and dam improvements (Sahashi et al. 2018). In the Orumappu River on the east coast of the Shiretoko Peninsula, the previously extinct masu salmon population has subsequently recovered, and the entry of masu salmon parr was first observed in the process (Morita 2022). Salmonids can return to their natal river, imprinting it at the smolt stage (Hasler & Scholz 1983). If the rivers of hatching and smoltification differ, the imprinted rivers differ. Although it is believed that the straying of adults contributes to the expansion of the salmonid distribution, the dispersal of juveniles between rivers before smoltification may be more effective in doing so in a shorter period of time.

In recent years, the migration of juvenile salmonids between the sea and rivers has been recognized as one of the life history diversities of salmonids, and their role in population resilience has been recognized (Flitcroft et al. 2016; Birnie-Gauvin et al. 2019; Kuroki et al. 2020; Wynne et al. 2023). Although rearing experiments have confirmed that the masu salmon parr has a certain level of saltwater tolerance at the parr stage (Ouchi 1983; Ban et al. 1987; Ishiguro et al. 2000; Nagakura et al. 2000; Hayashi et al. 2021), information on the use of coastal waters by the species before smoltification in the field is lacking. Further studies are needed to understand the diversity of the life history of this species, including its seaward migration at the age of 0+ years before smoltification.

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知床半島西岸の河川で採集されたヤマメの沿岸域利用の可能性について：黒木 真理・後藤 暁彦・佐橋 玄記・野別 貴博・森田 健太郎

知床半島西部の河川に生息するサケ科魚類のヤマメ*Oncorhynchus masou*に沿岸域を利用する個体がどの程度存在するのか把握するため，耳石微量元素分析を用いて調べた．その結果，知床半島西岸を流れるルシャ川とポンベツ川で採集されたパーマークを有するヤマメ10個体のうち，4個体で耳石Sr/Ca比が一時的に高値を示し，スモルト化を伴わずに沿岸域を利用している可能性が示唆された．また，岩手県南部の重茂半島の石浜沢川で採集された1個体のヤマメについても同様に沿岸域を利用しているものと考えられた．知床半島や重茂半島のような急峻な河川では，浮上後まもないヤマメ稚魚が偶発的に沿岸まで流され，その後再び河川に移動するということは頻繁に起こっていると推察される．