Application of otolith Sr : Ca ratios to estimate the migratory history of masu salmon, *Oncorhynchus masou*

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After emergence from river redds, masu salmon (*Oncorhynchus masou*) generally spend one or two years in freshwater, prior to seaward migration. After remaining in ocean waters for 1 year, they return to their natal river in spring, being of age 2.5 to 3.5 years (Kubo, 1980). However, some non-migrating individuals remain in freshwater as a fluvial form. Decision of sea-run or freshwater residence is made depending on individual growth history, sex and ambient environmental conditions (Utoh, 1976, 1977; Kubo, 1980). Thus, their migratory pattern in the life cycle is variable and complicated. Information on individual migratory histories would provide basic knowledge for both fish migration studies and fisheries management, allowing effective and sustainable use of the masu salmon resources.

Wave-length dispersive electron microprobe analysis of strontium (Sr) to calcium (Ca) ratios in otoliths has recently been focused as a method for distinguishing between freshwater and marine migratory phases in diadromous fishes such as brown trout, *Salmo trutta*, Atlantic salmon, *Salmo salar*, and rainbow trout, *Oncorhynchus mykiss* (Kalish, 1990), striped bass, *Morone saxatillis* (Secor, 1992; Secor et al., 1995) and sockeye salmon, *Oncorhynchus nerka* (Rieman et al., 1994). Accordingly, this technique might also be applied to solving the complicated problem of the migratory pattern in masu salmon.

The objective of this study was 1) to describe the ontogenic changes in otolith Sr : Ca ratios of *O. masou*, and 2) to examine the usefulness of this technique in reconstructing the migratory history of the species.

Materials and Methods

For comparisons of otolith Sr : Ca ratios of *Oncorhynchus masou* with different migratory patterns, field and hatchery individuals with known migratory history were collected (Table 1). Adult specimens collected at the mouth of the Kaji River, Niigata Prefecture, Japan, between 16 May–30 August 1994, maintained in a freshwater pond without food for 1–6 months at the Niigata Prefectural Inland Water Fisheries Experimental Station (specimens hereafter referred to as Upstream Migrants). They were sacrificed between 2 October–15 November 1994, after egg collection in autumn. Apparently discernible two size groups of masu salmon were collected in coastal water off Niigata Prefecture; small juvenile smolts (FL<30 cm, BW<400 g) just after seaward migration, collected by set net of the Murakami coast on 21

Table 1. Sampling location, sampling date, fish length and weight, longitudinal otolith length and number of otoliths used for microchemistry analysis

Fish group	Sampling location	Sampling date	Fork length (cm)	Body weight (g)	Longitudinal otolith length (μm)	Number analyzed
Upstream Migrant	Kaji River	October 2–November 15, 1994	42.3–61.0	580–2400	4760–5210	6
Coastal Pre-adult	Sea of Japan (Sanpoku)	May 1, 15 1991	49.5, 63.7	1890, 3750	4480, 4820	2
Coastal Juvenile	Sea of Japan (Murakami)	May 21, 1993	26.5, 28.4	258, 327	2540, 3210	2
Freshwater Resident	Hatchery	June 25, 1997	30.5–31.7	398–409	3420–3660	3
Unknown	Arakawa River	March 23, 1997	39.3	818	4520	1

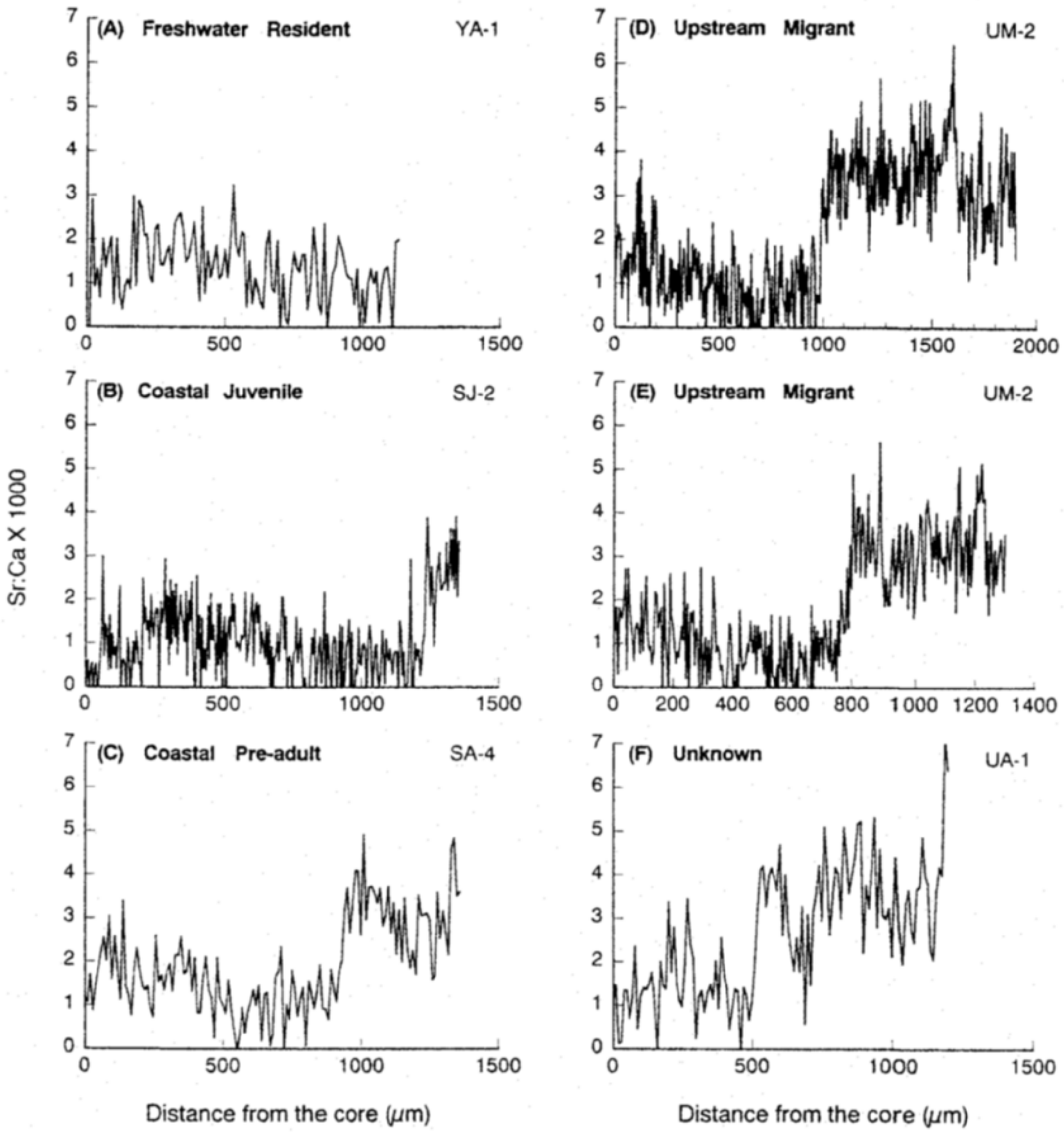

Fig. 1. *Oncorhynchus masou.* Transects of otolith Sr:Ca ratios measured with a wavelength dispersive electron microprobe from the core to the edge. Each point represents all data for every 5 μm (B, D, E) and 10 μm (A, C, F). The number at the upper right indicates fish number. A, B, C and D were analyzed in posterior axis and E and F were in dorsal axis.

May, 1993 (Coastal Juvenile), and large pre-adults (FL>40 cm, BW>1500 g) just before upstream migration for autumn spawning, which were collected by set net off the Sanpoku coast on 1–15 May, 1991 (Coastal Pre-adult). Parrs which had never experienced sea water were obtained from hatchery stock at the Niigata Prefectural Inland Water Fisheries Experimental Station, on 25 June, 1997 (Freshwater Resident).

To determine whether or not the technique could reveal the migratory history of an unknown specimen, an additional *O. masou* individual was obtained from the Arakawa River, Niigata prefecture on 23 May, 1997 (Unknown). The body size of the latter was intermediate to the above specimens and its migratory history difficult to judge from morphological characteristics and collection data.

Sagittal otoliths were extracted from each individual and the longitudinal otolith lengths measured. Otoliths were then embedded in epoxy resin (Strues, Epofix), mounted on glass slides and ground to expose the core. After polishing with 6 μm and 1 μm diamond paste on a polishing wheel (Strues, Planopol-V), they were cleaned in an ultrasonic bath, rinsed with deionized water and carbon coating by high vacuum evaporation.

Electron microprobe analyses were carried out on the total of 14 specimens (Table 1). Sr and Ca concentrations were measured along the posterior or dorsal axis from the otolith core using a wave-length dispersive X-ray electron microprobe (JEOL JXA-733). Calcite ($CaCO_3$) and Strontianite ($SrCO_3$) were used as standards. The accelerating voltage and beam current were 15 kV and 7 nA, respectively. The electron beam was focused on a point about 5 μm or 10 μm in diameter, with spacing measurements at 5 μm or 10 μm intervals. Each datum point represents the average of three measurements (each counting time: 4.0 seconds). Furthermore, to confirm within-sample variability, transects analysis along the posterior and dorsal axis within a sample were also carried out.

Results

Typical life-history transects representative of several migratory types of *Oncorhynchus masou* are shown in Figure 1. Freshwater Resident maintained a low level of Sr : Ca ratios, averaging 1.35×10^{-3} from the core to the edge (Fig 1A). Coastal Juvenile and Pre-adult also showed low Sr : Ca ratios phase from the core to the point 820–1200 μm (phase-L), averaging 1.38×10^{-3} and 1.04×10^{-3}, respectively. Thereafter, the ratios increased sharply, averaging 3.31×10^{-3} and 3.69×10^{-3}, respectively, and were maintained at the higher levels until the outermost regions (phase-H, Fig. 1B, C). Upstream Migrants exhibited a similar pattern (average 1.04×10^{-3} for phase-L; 3.43×10^{-3} for phase-H) (Fig. 1D), but had a longer phase-H (700–900 μm) than the Coastal Juvenile and Pre-adult (300–600 μm). Some of the Upstream Migrants (3 of 6 specimens) showed a slight decrease in Sr : Ca ratios at the otolith edge (average $< 2 \times 10^{-3}$). The radius at which phase-L changed to phase-H was termed the transition point (TP), which was considered as a timing of downstream migration of the individual. The mean TP for Upstream Migrants was 950 μm (range 850–1020 μm, 5 specimens) in the posterior axis of the otolith, Sr : Ca ratios increasing drastically at TP about three times (mean increase: 2.90×10^{-3}, range 2.61×10^{-3}–3.32×10^{-3}).

Tests of within-sample variability in Sr : Ca ratios showed similar changes in both posterior and dorsal axes (Fig. 1 D, E), although due to the shorter length of the otolith radius in dorsal axis, the transition point from phase-L to phase-H shifted 200 μm toward the core in the latter. Therefore, both transects were applicable to the life-history analysis.

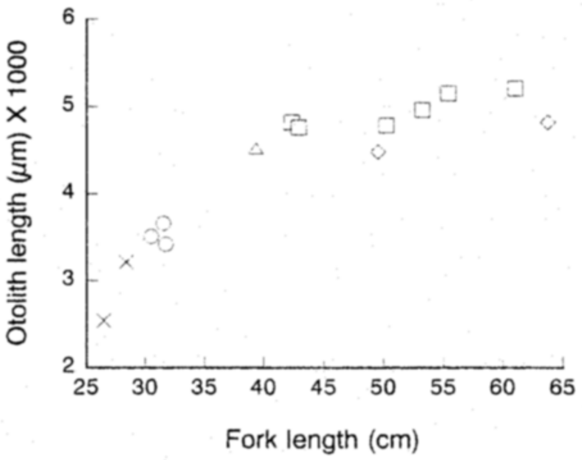

Fig. 2. *Oncorhynchus masou.* Relationship between fork length and otolith length used for microchemistry analysis. Freshwater Resident from hatchery (○); Coastal Juvenile from Murakami (×); Coastal Pre-adult from Sanpoku (◇); Upstream Migrant from Kaji River (□); Unknown from Arakawa River (△).

The Unknown specimen had a TP from phase-L (1.31×10^{-3}) to phase-H (3.56×10^{-3}) with an increase of 3.44×10^{-3}, which occurred some 500 μm from the core in the dorsal axis (Fig. 1F). The fish also had a longer phase-H (700 μm) than the Coastal Juvenile and Pre-adult, similar to that found in the Upstream Migrants.

A roughly curvilinear relationship found between the fork length and otolith length (Fig. 2), suggested that otoliths of masu salmon grow with increasing body length, but with increasingly lowered growth rates in fish over 40 cm FL.

Discussion

This study confirmed that otolith Sr : Ca ratios in masu salmon reflected changes in ambient environmental conditions and could indicate habitat transitions. All Coastal Juvenile and Pre-adult and Upstream Migrant fish had a TP some 1000 μm from the otolith core, and thereafter maintaining constantly high Sr : Ca ratios toward the edge. Therefore, those specimens had experienced the marine environment following downstream migration. Overall, the Sr : Ca ratio patterns observed in the masu salmon otoliths were consistent with those seen in other salmonids; viz., *Salmo trutta*, *S. salar* and *Oncorhynchus mykiss* (Kalish, 1990), and *O. nerka* (Rieman et al., 1994). These considerations strongly suggested that otolith Sr : Ca ratios analysis was available to the estimation of the individual migratory history in masu salmon.

The occurrence of a TP in the Unknown specimen from the Arakawa River (Fig. 1F) indicated that this specimen had also spent a previous period in a marine environment.

Although this study is the first description of ontogenic changes in otolith Sr : Ca ratios in masu salmon showing its potential for estimating migratory history, Tsukamoto et al. (1989) has previously estimated the migratory history of individual masu salmon by examining oxygen stable isotopic ratios ($^{18}O/^{16}O$) in the otolith by mass spectrometry. The patterns in the otolith $^{18}O/^{16}O$ ratios of masu salmon reflected similar changes in ambient environmental salinity, related to life history. However, comparing the analytical procedure for both methods, the latter method was more complex compared with the nondestructive analysis performed in the present study. Furthermore, recent progress in otolith Sr : Ca ratio techniques have demonstrated the considerable potential the method for reconstructing seasonal life history traits and ontogenic development of diadromous fishes: e.g. freshwater eels, *Anguilla anguilla* and *A. rostrata* (Casselman, 1982), freshwater gobies, *Stenogobius genivittatus* and *Awaous stamineus* (Radke et al., 1988), striped bass, *Morone saxatillis* (Secor, 1992; Secor et al., 1995) and arctic charr, *Salvelinus alpinus* (Radke et al., 1996). Therefore, the otolith Sr : Ca ratios technique by EPMA has a great potential as a convenient powerful tool to reconstruct the individual migratory history as well as a life history traits and ontogenic development.

In spite of the five Upstream Migrants having been maintained in a freshwater pond for 6 months, a clear phase-L (low Sr : Ca ratios) was not observed the near the otolith edge in these specimens, though a slight decrease was detected in three specimens. This might be due to the extremely slow or no otolith growth following upstream migration in masu salmon (Fig. 2). Because active feeding regime does not take place during maturation in freshwater following upstream migration (Kubo, 1980). Accordingly, information of low freshwater Sr : Ca ratios would not be incorporated into the edge of otolith. Furthermore, another factor possibly influencing relatively high otolith Sr : Ca ratios might be ontogenic variations in Sr : Ca ratios in masu salmon related to gonad maturation and spawning. Booke (1964) and Elliot et al. (1979) suggested that during the period leading up to spawning, salmonids were characterized by a gradual increase in levels of both plasma Ca and plasma protein, both being involved in the synthesis of gonadal tissue. Kalish (1989) suggested that seasonal variations in Sr : Ca ratios in otolith of blue grenadier, *Macruronus novaezelandiae*, could have resulted from high plasma protein levels during gonad maturation and spawning. Increases in Ca-binding plasma protein due to a decrease in free Ca could result in an increase in the Sr : Ca ratios in the endolymph and, accordingly, a corresponding increase in the otolith Sr : Ca ratios during period. However, further studies are required on the growth patterns of otolith and Sr metabolism in otoliths in order to understand the minute ontogenic changes in otolith Sr : Ca ratios.

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