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<td>Arai, Takaomi; Hirata, Takafumi; Takagi, Yasuaki</td>
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Application of laser ablation ICPMS to trace the environmental history of chum salmon

*Oncorhynchus keta*

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Running title: Trace element in otolith of *Oncorhynchus keta*
Abstract

Trace element levels in otoliths of chum salmon *Oncorhynchus keta* were examined by means of laser ablation inductivity coupled plasma mass spectrometry (LA-ICPMS). A close linear relationship in the Sr:Ca ratios between EPMA (X-ray analysis with an electron microprobe) and LA-ICPMS analyses was found \((p<0.0001)\), suggesting that the latter technique could be used to separate the marine and freshwater life phases. Mg:Ca, Cr:Ca, Zn:Ca and Ba:Ca ratios in either the core region or the oceanic growth zone of the otoliths varied among sites. These differences suggest that elemental compositions may reflect environmental variability among spawning (breeding) or habitat sites. Thus, those element ratios demonstrate the potential to be used to distinguish between fish spawning (breeding) sites and habitats for this species of salmon.

*Key words: Oncorhynchus keta; otolith microchemistry; trace element; laser ablation technique; migration*

1. Introduction

The chum salmon *Oncorhynchus keta* is one of seven species of North Pacific *Oncorhynchus* and is the second most abundant species of Pacific salmon. *O. keta* has the widest natural geographical distribution, ranging from Korea and Japan northward to the Arctic coasts of Russia and North America and then southward to Oregon (Salo, 1991). The fish is anadromous, and the young chum salmon generally go to sea immediately from the ground (Miller and Brannon 1982). After the migration in ocean waters for 1 to 5 years, they return to their natal river in fall, being of age 2 to 6 years (Nagasawa and Torisawa 1991). Such restricted homing behavior will lead to geographically distinct populations. While 50 years of ocean research has greatly advanced our understanding of the distribution and many aspects of the biology of chum salmon, the behaviour and habitat use of individual salmon on the high seas remain poorly understood. Information on individual environmental migratory histories would provide basic knowledge for both fish migration studies and fisheries management, allowing effective and sustainable use of the chum salmon resources.

The measurement of trace metals in fish otoliths has the potential to greatly increase the understanding
of movements and environmental history of individual fishes and to aid in distinguishing population-specific differences for the purpose of stock discrimination. Strontium (Sr) is one of the elements whose concentration in water changes in relation to the salinity (e.g. Kalish, 1990). The Sr content in otoliths is higher for sea migrants than for freshwater residents (Arai 2002), and Sr is known to alternate with calcium in bony tissues in proportion to the concentrations in the surrounding water (Arai 2002). Furthermore, Sr is permanently incorporated in this way during growth, so that the difference in Sr between growth zones can be used to reconstruct individual life histories of salmonid fishes such as Atlantic salmon *Salmo salar* and rainbow trout *Oncorhynchus mykiss* (Kalish, 1990), sockeye salmon *O. nerka* (Rieman et al., 1994), masu salmon *O. masou* (Arai & Tsukamoto, 1998), chum salmon *O. keta* (Arai & Miyazaki, 2002), brown trout *Salmo trutta* (Arai et al., 2002), Sakhalin taimen *Hucho perryi* (Arai et al., 2004), white-spotted char *Salvelinus leucomaenis* (Arai & Morita, 2005), and dolly varden *Salvelinus malma* (Morita et al., 2005). Thus, techniques that sample specific loci offer the greatest potential for gaining insight into the life history and movements of salmonid fishes. However, most studies have used X-ray analysis with an electron microprobe (EPMA), a technique that is highly restricted to detecting the most abundant elements (Gunn et al., 1992; Thresher et al., 1994).

Laser ablation inductivity coupled plasma mass spectrometry (LA-ICPMS) is a recently developed technique for point sampling that combines the benefits of sampling specific loci with the sensitivity of the ICPMS (Nesbitt et al., 1998; Iizuka & Hirata, 2004; Apinya & Hirata, 2004). This technique uses a narrow laser beam to probe solid samples. The technique combines the low detection limits and wide dynamic range. Geffen et al. (1998) showed that the uptake of mercury and lead into sand goby *Pomatoschistus minutus* and sole *Solea solea* otoliths was related to the water concentrations of these metals. Bath et al. (2000) conducted experiments with the spot *Leiostomus xanthurus* that demonstrated a positive relationship between the Sr:Ca and Ba:Ca ratios in the otolith and in the water. This method has been used to discriminate among different stocks of several fishes by examining the elemental composition of their otolith.

The objectives of the present study are to examine the trace element composition in the core region and ocean growth zone in the otoliths of the chum salmon *Oncorhynchus keta*, and to ascertain the appropriateness of otolith trace element composition for discriminating among spawning (breeding) habitats.
2. Materials and methods

2.1. Sample collection and otolith preparation

Specimens of *Oncorhynchus keta* were collected from breeding localities in Japanese rivers, i.e. the Otsuchi and Tsugaruishi rivers, Iwate Prefecture in Hoshu Island and the Yurappu River, Hokkaido Island, during 2003 and 2004 (Fig. 1). An additional five specimens of chum salmon collected in Otsuchi Bay in 2000 were also examined using solution ICPMS analysis so the results could be compared with those of LA-ICPMS analysis. A total of 25 specimens (15 from the Otsuchi, 5 from the Tsugaruishi and 5 from the Yurappu) were used in the present study.

Sagittal otoliths for LA-ICPMS analysis were extracted from each fish, embedded in epoxy resin (Struers, Epofix), and mounted on glass slides. The otoliths were then ground to expose the core using a grinding machine equipped with a diamond cup wheel (Struers, Discoplan-TS). They were then polished further with OP-S suspension (colloidal silica suspension for final polishing) on an automated polishing wheel (Struers, RotoPol-35) equipped with a semi-automatic specimen mover (Struers, PdM-Force-20). Finally, they were cleaned and rinsed with deionized water prior to being examined (Fig. 2A).

2.2. Otolith microchemical analyses

The concentrations of seven isotopes, $^{24}\text{Mg}$, $^{43}\text{Ca}$, $^{52}\text{Cr}$, $^{55}\text{Mn}$, $^{64}\text{Zn}$, $^{86}\text{Sr}$ and $^{138}\text{Ba}$, were measured in a series of ablations across the life history transect from the core (hatch) to the edge (death) by laser ablation inductively coupled plasma mass spectrometry (LA-ICPMS). The LA-ICPMS system used was a Thermo Electron PlasmaQuad 2 Omega ICPMS coupled with a GeoLas 200CQ laser ablation system operating at DUV wavelength ($\lambda = 193$ nm). In order to improve the precision of the measurement, both the He-flushing technique (Eggins et al., 1998) and the stabilizer device (Apinya and Hirata, 2004) were applied. The details of the instrument-running conditions of both the ICPMS and the laser unit are given in Table 1. The calibration of the ICPMS was examined using the certified reference material, either NIST SRM610 or NIST SRM612, distributed by the National Institute of Standards and Technology. The
ablation pit size used in this study was a diameter of 31.5 µm, and the single spot laser ablation was carried out in the otolith core region at 50 µm intervals (5 ablations in each specimen), and then across the transverse axis of the otolith at either 100 µm or 200 µm intervals (7-21 ablations in each specimen) (Fig. 2B). In the Otsuchi Bay specimens, four of ten specimens were only analyzed in the otolith core region. Background levels and standards (NIST 610, NIST 612) were examined before and after each series, with each series comprising a maximum of three otoliths. Calcium was used as an internal standard to improve the reliability of the abundance measurement (Campana et al., 1994). The calcium concentration was normalized to be 400,000 µg/g based on the stoichiometry of calcium carbonate, and the concentrations of other elements were calculated by internal correction using Ca. The limits of detection (µg/g) achieved in this study were as follows: $^{24}$Mg 3.4-5.6, $^{43}$Ca 111-498, $^{52}$Cr 2.5-4.3, $^{55}$Mn 1.3-3.8, $^{64}$Zn 0.7-2.1, $^{86}$Sr 4.8-6.3, and $^{138}$Ba 0.05-0.2. These were taken to represent three standard deviations for the mean blank (background) count of each isotope. All elemental data were expressed as molar ratios to Ca.

Following the LA-ICPMS analysis, we examined the otolith strontium (Sr) and calcium (Ca) concentrations in the Otsuchi River otoliths with wavelength dispersive X-ray spectrometry using an electron microprobe (EPMA). For electron microprobe analyses, all otoliths were Pt-Pd coated by a high vacuum evaporator. Life-history transect analyses of Sr and Ca concentrations, were performed along a line down the longest axis of each otolith from the core to the edge using a wavelength dispersive X-ray electron microprobe (JEOL JXA-8900R), as described in Arai et al. (1997, 2004). Calcite (CaCO$_3$) and strontianite (SrCO$_3$) were used as standards, and the accelerating voltage and beam current were 15 kV and 1.2 x $10^{-8}$ A, respectively. The electron beam was focused on a point 30 µm in diameter, with measurements spaced at 100 µm intervals.

For solution ICPMS analysis, otoliths were mechanically cleaned of attached tissue, and then washed in an ultrasonic bath and rinsed with deionized water. After cleaning, the otolith samples were dried at 80 °C for 12 h.

In preparation for instrumental analysis, each whole otolith was weighed to the nearest 0.001 g and placed in a PTFE (Teflon) vessel. Otoliths were then digested using microwaves to a transparent solution using a concentrated nitric acid. The resultant solutions were diluted with doubly deionized water and transferred to acid-washed sample tubes. Elemental concentrations were determined using inductively coupled plasma mass spectrometry (ICPMS) (Agilent 7500c). Internal standards were added to all samples,
and the standards were calibrated as described in Arai et al. (2002). The concentrations of all elements are reported as µg/g on a dry weight basis. The limits of detection for each of the elements ranged from 0.01 to 0.001 µg/g, and all of the element concentrations in the otolith were above the detection limits.

2. 3. Statistical analyses

Differences between data were tested using the Mann Whitney U-test. Differences among data were tested using analysis of variance (ANOVA), and afterwards using Scheffe’s multiple range tests for combinations of two data. The significance of the correlation coefficient and regression slope were tested by Fisher’s Z-transformation and by analysis of covariance (ANCOVA) (Sokal & Rohlf, 1995).

3. Results

Based on previous studies on Sr:Ca ratios in *Oncorhynchus keta* by EPMA, the Sr:Ca ratios in the otoliths of fishes differ are different when the fish are in freshwater habitats than when they are in seawater habitats (Arai & Miyazaki 2002). Thus, the Sr:Ca ratios of otoliths help in determining the movement between different habitats with differing salinity regimes (Fig. 3). Close linear relationships were apparent between the Sr:Ca ratios in otoliths determined in EPMA analyses and those determined in LA-ICPMS analyses (Fig. 4, p<0.0001). The slope of the relationship between these Sr:Ca ratios did not differ significantly from 1 (p>0.5).

Mg, Cr, Mn, Zn, Sr and Ba concentrations (mean ± SD) in otoliths (5 specimens from Otsuchi Bay) according to solution ICPMS analysis were 26.2 ± 4.2 µg/g, 1.1 ± 0.8 µg/g, 1.1 ± 0.3 µg/g, 36.3 ± 6.0 µg/g, 3430 ± 292 µg/g and 14.0 ± 7.0 µg/g, respectively (Table 2). These element concentrations in otoliths (76 spots in 6 specimens from Otsuchi Bay) by LA-ICPMS analysis were 17.0 ± 7.8 µg/g, 22.1 ± 25.6 µg/g, 6.6 ± 6.8 µg/g, 43.9 ± 29.8 µg/g, 2217 ± 302 µg/g and 8.8 ± 3.6 µg/g, respectively (Table 2). All of the element concentrations determined in several spots by LA-ICPMS analysis overlapped with those determined by solution ICPMS, although the former analysis examined only 7-21 spots along the life history transect. However, significant differences in the element concentrations between the two instrumental analyses were found in Cr, Mn and Sr (p<0.05), although these differences were not large. No significant differences were
apparent in Mg, Zn and Ba (p > 0.05).

The element ratios in the core region varied among sites (Fig. 5). The Mg:Ca ratio in the Otsuchi River was significantly lower than in the Tsugaruishi and Yurappu Rivers (p<0.0001), while no significant difference in the ratio occurred between the Tsugaruishi and Yurappu Rivers (p>0.5). In the Cr:Ca ratio, significant differences occurred between the Otsuchi and Yurappu Rivers and between the Tsugaruishi and Yurappu Rivers, while no significant difference in the ratio occurred between the Otsuchi and Tsugaruishi Rivers (p>0.05). Although a significant difference in the Ba:Ca ratio occurred between the Otsuchi and Tsugaruishi Rivers (p<0.005), any differences between the Otsuchi and Yurappu Rivers and between the Tsugaruishi and Yurappu Rivers were not significant (p>0.5). No significant difference was apparent in the Mn:Ca, Zn:Ca and Sr:Ca ratios among the sites (p>0.5).

The mean element ratios in the ocean growth zones of the otoliths also varied among sites (Fig. 6). The Mg:Ca ratio in the Otsuchi River was significantly lower than those in Tsugaruishi and Yurappu Rivers (p<0.0001), while no significant difference in the ratio occurred between the Tsugaruishi and Yurappu Rivers (p>0.5). Significant differences occurred in the Cr:Ca ratio between the Otsuchi and Yurappu Rivers and between the Tsugaruishi and Yurappu Rivers, while no significant difference in the ratio occurred between the Otsuchi and Tsugaruishi Rivers (p>0.5). Zn:Ca ratio in the Tsugaruishi River was significantly lower than those in the Otsuchi and Yurappu Rivers (p<0.05-0.001), while no significant difference in the ratio was observed between the Otsuchi and Yurappu Rivers (p>0.5). No significant difference was observed in the Mn:Ca, Sr:Ca and Ba:Ca ratios among the sites (p>0.5).

4. Discussion

This is the first description of ontogenic changes in the otolith element ratios in chum salmon Oncorhynchus keta, although several previous studies estimated the migratory history of salmonid fishes including chum salmon by examining the Sr:Ca ratio in the otolith using EPMA (Kalish, 1990; Riemann et al., 1994; Arai & Tsukamoto, 1998; Arai & Miyazaki, 2002; Arai et al., 2002; Arai et al., 2004; Arai & Morita, 2005; Morita et al., 2005). These results showed that the patterns in the otolith Sr:Ca ratios of salmonid fishes reflected similar changes in ambient environmental salinity that were related to life history. In the present study, a close linear relationship in the Sr:Ca ratios determined by the EPMA and LA-ICPMS analyses was found, suggesting that the latter technique could be used to distinguish between the marine
and freshwater life phases (Fig. 4).

The Mg:Ca, Cr:Ca, Zn:Ca and Ba:Ca ratios in either the core region or the ocean growth zone of the otoliths varied among sites, but the Mn:Ca and Sr:Ca ratios did not differ among sites (Figs. 5, 6). These differences suggest that elemental compositions may reflect environmental variability among spawning (breeding) or habitat sites. Kalish (1990) reported that sea-farmed rainbow trout passed a chemical signal in the form of elevated Sr to the nuclei of the otoliths of their progeny. This indicates that the Sr:Ca ratio in otolith primordia can be used to distinguish the progeny of sympatric anadromous and freshwater-resident salmonids. Rieman et al. (1994) also found that the Sr:Ca values in the otolith primordial of known anadromous sockeye progeny were significantly higher than those from known freshwater-resident females. The Sr:Ca ratio in the otolith cores of chum salmon (about 4 x 10^{-3}; Fig. 5) in the present study was higher than that reported previously for freshwater-resident salmon (<2 x 10^{-3}) such as sockeye salmon Oncorhynchus nerka, rainbow trout O. mykiss and chinook salmon O. tshawytscha (Rieman et al., 1994; Volk et al., 2000). Higher Sr:Ca values appear to be associated with the species that enter freshwater with well-developed eggs and spawn (breeding) quickly, such as O. keta, while the lower values are associated with fish that enter rivers with incompletely developed eggs and reside in freshwater from several to many months before spawning, such as freshwater-resident salmon, e. g. sockeye salmon, rainbow trout and chinook salmon. Recent laboratory experiments revealed a clear relationship between the concentration of trace metals in otoliths and the environment (Geffen et al., 1998; Bath et al., 2000; Milton and Chenery, 2001). Milton and Chenery (2001) found that the Sr:Ca, Ba:Ca and Cu:Ca ratios in the otoliths of barramundi Lates calcarifer were positively correlated with the water ratios. Several other factors may also influence the deposition rates of trace elements in otoliths. Kalish (1991) suggested that differences in otolith microchemistry may be determined by the presence of calcium-binding proteins in the blood plasma. The concentrations of metal ions in the endolymph that are available for precipitation onto the otolith surface are hypothesized to be a function of the concentration of these Ca-binding proteins and the degree of discrimination of the proteins against other metal ions for Ca. Studies showing a relationship between the Sr:Ca ratio and fish growth rates support the concept that otolith microchemistry can have physiological effects (Sadovy & Severin, 1992). Regardless of the mechanism, the present data suggest that the fish could be separated among sites on the basis of their elemental composition. Thus, those element ratios demonstrate the potential to help distinguish between the fish spawning (breeding) sites and habitats of
chum salmon.

The Sr concentration in seawater is relatively constant at approximately 8mg/l throughout the world’s oceans, which is higher than that in freshwater (0.02-0.11 mg/l) (Angino et al., 1966; Rosenthal et al., 1970; Rosenthal, 1981). The otolith Sr:Ca ratios found in the present study are consistent with the migration patterns of chum salmon. Mg is also a common constituent of seawater, with concentrations in riverine waters generally at least 2 orders of magnitude lower than in oceanic waters (Spaargaren, 1991). Mg might also show a pattern similar to that of Sr if indeed otolith Mg:Ca ratios reflect concentrations in the ambient water. This hypothesis was not demonstrated convincingly by an analysis of differences among sites. These results indicate that the Mg level in the otolith might not be directly related to the concentration in the water.

Cr, Mn and Ba follow biologically active or nutrient-type distributions in seawater. These concentrations are relatively high in river water compared to seawater (Broecker and Peng, 1982). In the present study, the largest ratio differences were appeared in the Cr:Ca ratios of fish from the different watersheds and these differences existed for both core and ocean growth zone (Fig. 5, 6). These differences suggest that Cr may more reflect environmental variability between habitats. Several other physiological factors may also more influence the deposition rates of Cr in otoliths, and further study is needed to clarify the uptake mechanism into the otolith. The Cr, Zn and Ba element ratios demonstrate the potential of element ratios to be used to distinguish the fish habitats in ambient water environment.

Acknowledgements

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References


Table captions

Table 1  Operating conditions of the LA-ICPMS system used in this study.

Table 2  Element concentrations (μg/g) as determined by solution ICPMS and LA-ICPMS analyses in otoliths of chum salmon Oncorhynchus keta collected from Otsuchi Bay, northern Japan.

Figure captions

Fig. 1. Map showing the collection site of salmon hatchery of chum salmon Oncorhynchus keta in Japan.

Fig. 2. Otolith microstructure of juvenile chum salmon with reflected light observations. A; pre-analysis image by LA-ICPMS, B; post-analysis image by LA-ICPMS

Fig. 3. Typical change in the Sr:Ca ratio by LA-ICPMS analysis along line history transect from core (0
μm) to the edge in the sagittal plane of the otolith. Arrow indicates the transition point from freshwater growth to seawater growth as determined from the Sr:Ca ratio fluctuation pattern along the life history transect.

Fig. 4. Regression of Sr:Ca ratio data from EPMA and LA-ICPMS analyses.

Fig. 5. *Oncorhynchus keta*. Element ratios (± SD) in the core region of otoliths.

Fig. 6. *Oncorhynchus keta*. Element ratios (± SD) in the ocean growth zone of otoliths.
Table 1 Operating conditions of the LA-ICPMS system used in this study

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<td>Instrumentation</td>
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Table 2: Element concentrations (μg/g) examined by solution ICPMS and LA-ICPMS analyses in otoliths of chum salmon Oncorhynchus keta collected from Otsuchi Bay, northern Japan.

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<td>Mg</td>
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<td>Cr</td>
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<td>Mn</td>
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<tr>
<td>Zn</td>
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<tr>
<td>Sr</td>
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<tr>
<td>Ba</td>
<td>14.0 ± 7.0</td>
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Sr:Ca ratios x 1000 vs. Distance from the core (μm)

Arai et al. Fig. 3
$y = 0.95x + 0.08$
$r = 0.880$

Arai et al. Fig. 4
Fig. 5

Element ratios in otolith core

Mg:Ca  Cr:Ca  Mn:Ca  Zn:Ca  Sr:Ca  Ba:Ca

Otsuchi River (Iwate)  Tsugaruishi River (Iwate)  Yurappu River (Hokkaido)

Arai et al. Fig. 5
Element ratios in otolith of ocean growth zone

- Mg:Ca
- Cr:Ca
- Mn:Ca
- Zn:Ca
- Sr:Ca
- Ba:Ca

Locations:
- Otsuchi River (Iwate)
- Tsugaruishi River (Iwate)
- Yurappu River (Hokkaido)

Arai et al. Fig. 6