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Effect of salinity on strontium:calcium ratios in the otoliths of Sakhalin taimen, *Hucho perryi*

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Abstract In order to determine the salinity effect on otolith strontium (Sr):calcium (Ca) ratios, the Sr and Ca contents of otoliths of Sakhalin taimen, *Hucho perryi*, reared for 60 days in four salinities (freshwater, one-third seawater, two-thirds seawater, and full seawater) were examined. The Ca and Sr contents and the resultant Sr:Ca ratios in the rearing water increased linearly with salinity. Otolith Sr:Ca ratios were also found to be positively correlated with salinity. However, the Sr:Ca did not correspond to temperature fluctuations during the experiment. These results indicate that Sr:Ca ratios in otoliths can be used to reconstruct the migratory history of the fish by differentiating when the fish migrated between freshwater and seawater.

Keywords *Hucho perryi* · Migration · Otolith · Salinity · Sr:Ca ratios

Introduction

Otoliths are calcium carbonate concretions with a small amount of organic matter located in the inner ears of teleost fishes. Their function is for hearing and balance in the fishes. The elemental composition of otoliths may reflect that of the surrounding water [1]. Changes in the strontium (Sr):calcium (Ca) ratios in otoliths have received increasing attention because they provide a method of reconstructing the migratory history of the fish. Sr is a Ca analogue sharing a similar crystal ionic radius and can substitute for Ca in the aragonite lattice of otoliths [2]. The Sr concentration in seawater is approximately 100-fold higher than that in freshwater [1], and thus Sr appears to be a good tracer of the environmental conditions encountered, notably salinity variation under either natural or controlled conditions. In the Salmonidae family, otolith Sr or Sr:Ca ratio analyses have been widely used to reconstruct the environmental history due to the occurrence of anadromy [3–9].

A salmonid, Sakhalin taimen, *Hucho perryi* Brevort, is restrictedly distributed in Hokkaido, Sakhalin, southern Kuries, and eastern Siberia [10]. The species is iteroparous and is the largest salmonid in the western Pacific Ocean, attaining a weight of up to 25–60 kg and living up to 16 years [11–13]. This species, now very rare, is seriously endangered and close to extinction (Critically Endangered, IUCN 2009) due to overfishing, spawning ground loss, and water pollution. Thus, information on individual migratory histories might provide a knowledge base for conservation biology as well as fish migration studies, allowing effective and sustainable management of this endangered species.

Recent studies have indicated that based on variations in the Sr:Ca ratios in otoliths, the migratory history of Sakhalin taimen can be reconstructed [14]. In spite of the general acceptance of the use of otolith Sr:Ca ratios to describe the migration in salmonid species including Sakhalin taimen, the method has been little validated through controlled rearing studies under laboratory conditions. In the absence of controlled rearing studies with Sakhalin taimen, Arai et al. [14] relied upon results from other species. When the migratory history is studied using otolith Sr and Sr:Ca ratios, it is indispensable to assess the applicability of the salinity response.

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In this study, the effect of salinity on the incorporation of Sr and Ca, and the resultant Sr:Ca ratio, into the otoliths of the hatchery-reared Sakhalin taimen was examined. The results provide further understanding of the migration of the species as well as of the mechanisms by which the elements are incorporated into teleost fish otoliths.

Materials and methods

Sakhalin taimen ($n = 80$) was obtained from a local hatchery (Huchen Farm in Ajigasawa, Aomori Prefecture) and transported to the International Coastal Research Center, The University of Tokyo. The mean total length of the juveniles was 206.3 ± 14.7 mm (mean ± SD). After collection and transportation, all fish were allowed to acclimate in freshwater for 5 days prior to the initiation of the experiment. To examine the relationship between Sr and Ca concentrations in the otoliths and various ambient salinity conditions, four groups of 15 fishes were each reared for 60 days in either freshwater, one-third seawater, two-thirds seawater, or full seawater, and half of the water for all groups was changed every 10 days during November 2004 and January 2005. The fishes were fed once daily and unconsumed food was removed. Survival to completion of the experiment was 93–100%. Temperature was not controlled in the experiment. The rearing water salinity and temperature were monitored every 30 min for the three seawater groups, but not for the freshwater group, using salinity and temperature data loggers (Compact CT, Alec Electronics, Kobe, Japan). The salinity and temperature were measured daily by refractometer and water temperature meter, respectively, for the freshwater group. The rearing water was filtered using a 0.45-μm filter and diluted with Milli-Q water before the Sr and Ca concentrations were measured by an inductively coupled plasma mass spectrometer (ICP-MS) (Agilent-7500cs, Agilent Technology, Tokyo, Japan) according to Arai and Hirata [15].

Sagittal otoliths were excised from each fish, embedded in epoxy resin (Epofix, Struers, Copenhagen, Denmark), and mounted on glass slides. The otoliths were then ground to expose the core, using a grinding machine equipped with a diamond cup-wheel (Discoplan-TS, Struers, Copenhagen, Denmark), and polished further with 6 and 1 μm diamond paste on an automated polishing wheel (Planopol-V, Struers, Copenhagen, Denmark). Finally, they were cleaned in an ultrasonic bath and rinsed with deionized water prior to examination.

For electron microprobe analyses, all otoliths were platinum-palladium coated by a high vacuum evaporator. All specimens were used for "life-history transect" analysis of the Sr and Ca concentrations, which were measured 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, Jeol, Tokyo, Japan), as described in Arai et al. [14]. Data are expressed as either Sr:Ca ratio $\times 10^{-3}$ or Sr:Ca ratio $\times 10^{3}$.

Regressions of Sr and Ca concentrations in the water against ambient salinity were used to test the relationship between these variables. Regressions of Sr:Ca values against ambient salinity were calculated using Sr:Ca values measured on the otolith edges from the point at which the Sr:Ca abruptly increased (Fig. 1) and the commencement of the experiment was estimated based on the number of otolith increments for each fish.

Differences among data were tested by an analysis of variance (ANOVA) and subsequently by Scheffe's multiple range test for the combination of two data. Significance of the correlation coefficient and regression slope were tested by Fisher's Z-transformation [16].

Results

The rearing water salinities were constant for each seawater group (Fig. 2). The mean (±SD) salinities of the

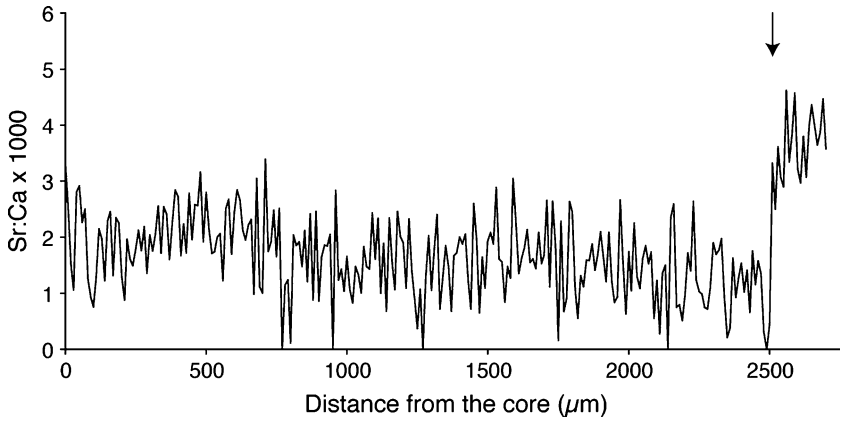

Fig. 1 Transects of otolith Sr:Ca ratios measured with a wavelength dispersive electron microprobe from the core to the edge in a specimen. Each point represents all data for the respective 10 μm intervals. *Arrow* indicates the point of abrupt increase in otolith Sr:Ca ratios, and mean Sr:Ca ratios for each fish were calculated between the point and edge of the otolith

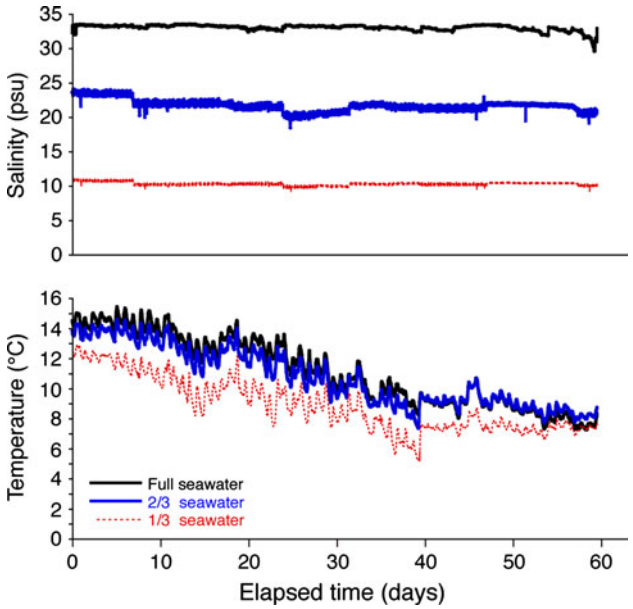

Fig. 2 Fluctuations of rearing water salinity (*upper*) and temperature (*lower*) for three seawater groups (one-third seawater, two-thirds seawater, full seawater) during the experimental period between November 2004 and January 2005

one-third seawater, two-thirds seawater, and full seawater were 10.75 ± 1.98, 21.67 ± 0.84 and 33.00 ± 0.44 psu, respectively. The salinity of the freshwater group showed 0 psu throughout the experimental period. The rearing water temperatures decreased gradually for each seawater group ranging from 12.88 to 15.01°C in November 2004 to 5.20 to 7.33°C in January 2005 (Fig. 2).

The mean Sr and Ca concentrations in the rearing waters ranged from 1.168 nmol g^{-1} (freshwater) to 75.82 nmol g^{-1} (full seawater) and from 411.9 nmol g^{-1} (freshwater) to 7,639 nmol g^{-1} (full seawater), respectively. Both the Sr and Ca concentrations in the rearing water differed significantly among the four experimental salinities ($p < 0.005$), and they both significantly increased with salinity ($p < 0.0001$) (Fig. 3a, b). The mean Sr:Ca ratios in the rearing waters ranged from 2.84×10^{-3} (freshwater) to 9.94×10^{-3} (full seawater). The Sr:Ca ratios in the rearing water increased with salinity (Fig. 3c). There were significant differences between the freshwater and three seawater groups and between the one-third seawater and other seawater groups ($p < 0.005$), while no significant difference was found between the two-thirds and full seawater groups ($p > 0.05$).

The mean (±SD) Sr:Ca ratio in the otoliths significantly increased from $1.84 \times 10^{-3} \pm 0.10 \times 10^{-3}$ in freshwater to $4.98 \times 10^{-3} \pm 0.17 \times 10^{-3}$ in full seawater ($p < 0.001$) (Fig. 4). The relationship between

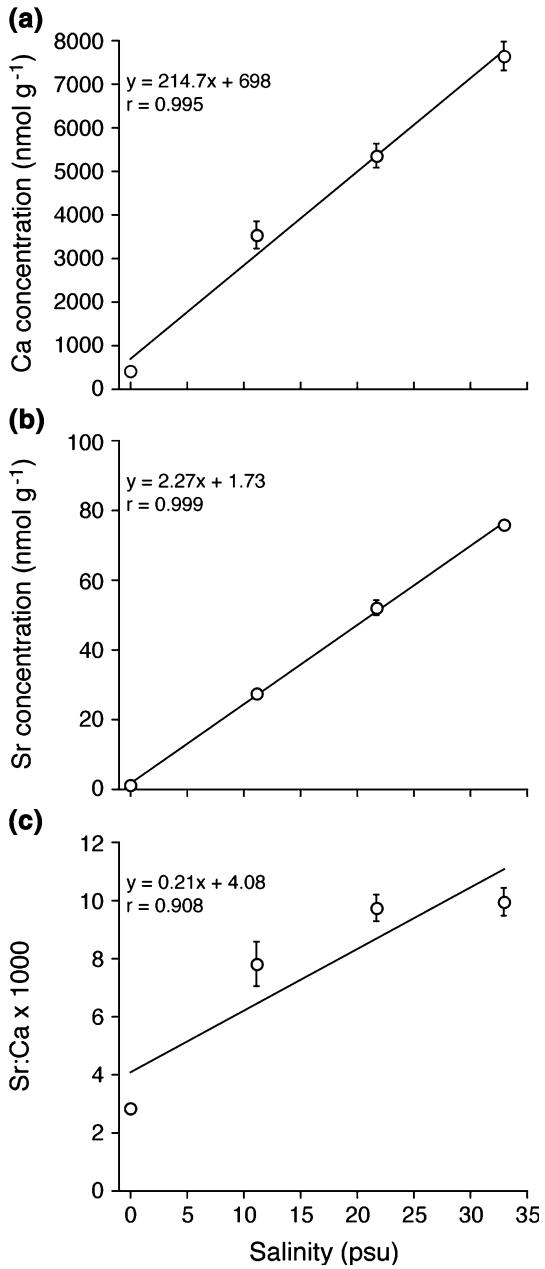

Fig. 3 The relationship between salinity and Ca (**a**) and Sr (**b**) concentrations, and the Sr:Ca concentration ratio (**c**) in rearing waters

Sr:Ca ratio in otoliths and salinity was significant related ($p < 0.0001$), and was fitted by linear regression (Fig. 4). Thus, the Sr:Ca ratio in otoliths was affected by salinity.

In order to evaluate the other factors such as temperature and measurement conditions on otolith Sr:Ca incorporations, the Sr:Ca ratios were measured during the experimental period (Fig. 5). The results during the experiment were quite consistent and stable for each seawater group.

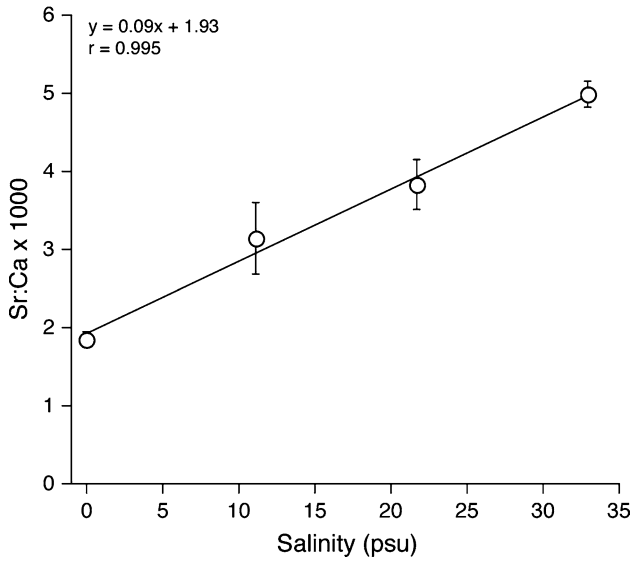

Fig. 4 The relationship between salinity and Sr:Ca ratio in otolith

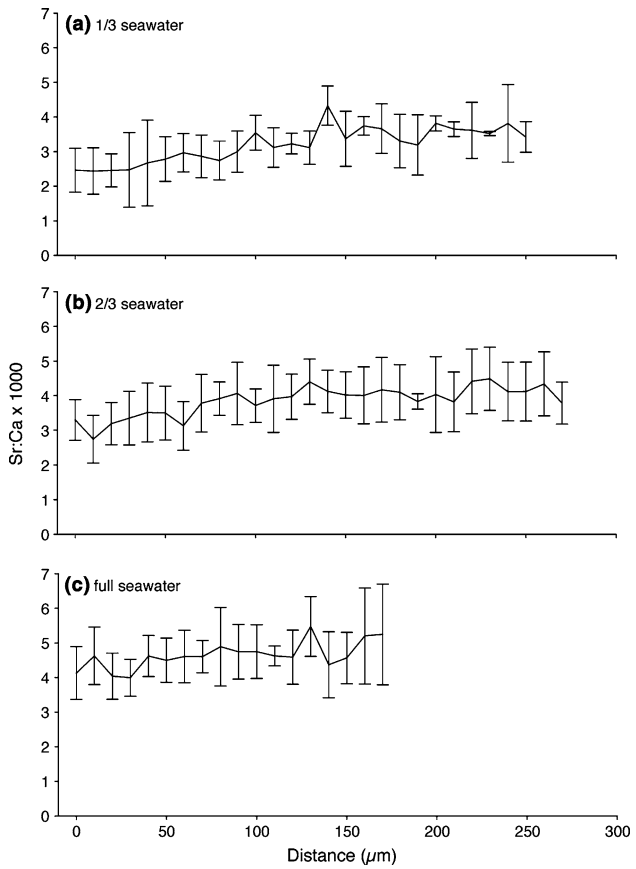

Fig. 5 Transects of otolith Sr:Ca ratios measured with a wavelength dispersive electron microprobe from the points of abrupt increase in otolith Sr:Ca ratios to the edge in each salinity group [one-third seawater (**a**), two-thirds seawater (**b**), full seawater (**c**)] during the experimental period. Each point represents all data for the respective 10 μm intervals

Discussion

In the present study, the use of otolith Sr and Sr:Ca ratios to examine the migration history of Sakhalin taimen among freshwater, brackish water, and seawater habitats was supported. Otolith Sr and Sr:Ca ratios were linearly related to salinity and hence ambient Sr:Ca ratios (Figs. 3c, 4), although these slopes did not coincide. The discrepancy might be due to the kinetics of Ca and Sr incorporation from ambient water to otolith mediated via blood and endolymph. The relationship between otolith Sr:Ca ratio and ambient water salinity has been examined for many species. However, the response of otolith Sr:Ca to salinity is inconsistent among species. A linear regression of otolith Sr:Ca ratio on salinity was reported for the Japanese eel *Anguilla japonica* [17]. However, the regression of otolith Sr:Ca ratio on salinity differed significantly between fish reared in freshwater and those reared in seawater, and the otolith Sr:Ca ratio did not increase with increasing salinity for the gray mullet *Mugil cephalus* [18]. This suggests that the relationship between otolith Sr:Ca ratio and salinity may be species-specific. Thus, a validation study between otolith Sr:Ca ratio and salinity should be carried out for each species before migration history analyses are performed.

The intercept of the regression of otolith Sr:Ca ratio on salinity was approximately 2 when the salinity was 0 psu in the present study (Fig. 4). This result suggests that using an otolith Sr:Ca ratio of 2 to discriminate between freshwater and seawater habitat use in the Sakhalin taimen is reliable. Based on this result, the salinity history of the species may be reconstructed from otolith Sr:Ca ratios.

Water temperature is also reported to be a factor affecting otolith Sr:Ca incorporation. An inverse relationship was found between temperature and otolith Sr:Ca ratio in the Atlantic herring *Clupea harengus* [19, 20] and the Atlantic cod *Gadus morpha* [21]. However, otolith Sr:Ca ratios increased linearly with increasing ambient temperature in the goldfish [22]. Further, otolith Sr:Ca ratio showed no temperature relationship in the bearded rock cod *Pseudophycis barbatus* [3]. The present study also found that the otolith Sr:Ca ratio had no temperature relationship in Sakhalin taimen. The fluctuation patterns for all experimental groups during the experiment were constant (Fig. 5) in spite of the fact that those ambient temperatures decreased gradually (Fig. 3). The results suggest that temperature did not affect otolith Sr:Ca incorporation in Sakhalin taimen.

By using otolith Sr:Ca analyses, Arai et al. [14] found that Sakhalin taimen from Sakhalin Island had an anadromous life history. All samples collected from the field had a transition point from a low Sr:Ca ratio to a high Sr:Ca ratio and thereafter maintained constantly high Sr:Ca ratios

up to the edge. The mean high Sr:Ca ratio was $6.36 \pm 0.90 \times 10^{-3}$ (SD) with a range of $5.50–7.79 \times 10^{-3}$. The Sr:Ca values corresponding to the marine life period found in the previous study were higher than those found in the full seawater group in the present study (4.98×10^{-3}). Such a difference has also been found between freshwater rainbow trout and anadromous rainbow trout (steelhead) *Oncorhynchus mykiss* [23]. The reason for this discrepancy is unclear, but it may be related to population-specific differences in Sr incorporation at higher salinity or to ontogenic-related changes in osmoregulatory capabilities. The hatchery population of Sakhalin taimen used in the present study was a freshwater resident population and therefore may have exhibited different osmotic capabilities and associated otolith incorporation processes than would an anadromous population. In brook char *Salvelinus fontinalis*, freshwater resident and anadromous populations showed physiological differences related to saltwater adaptability [24]. Resident char did not show the same Na^+/K^+ ATPase activity observed in anadromous char. Anadromous char were characterized by higher plasma osmolality and Cl and Na concentrations. Further, Sakhalin taimen typically does not migrate to sea during the juvenile stage. In the present study, however, the fishes were subjected to seawater when at a much smaller size than that observed in wild Sakhalin taimen populations. It was not feasible to transfer these fish to seawater at the timing of smolting. Further studies of the effects of growth stage and osmoregulation on the incorporation of Ca and Sr in otoliths should be carried out to achieve a more detailed understanding of the life history of Sakhalin taimen using otolith microchemistry.

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