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Use of Otolith Microanalysis to Determine Estuarine Migrations of Japanese Sea Bass *Lateolabrax japonicus* Distributed in Ariake Sea

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Japanese sea bass *Lateolabrax japonicus* is a common euryhaline marine fish distributed in eastern Asian coastal waters, of which life history can be characterized by occasional or seasonal entry to freshwater habitats. Otolith microanalysis for Sr:Ca ratio by a wave-length dispersive electron microprobe was utilized to determine life history trajectory of the Japanese sea bass distributed in Ariake Sea. Laboratory-raised sea bass juveniles which were maintained at various constant salinity regimes and serially decreasing-increasing salinity conditions showed that Sr:Ca ratio was significantly lower in fishes exposed to freshwater than in those to brackish and sea water over salinity 10 ppt. Sr:Ca ratio chronology of a yearling collected in the Chikugo River estuary of Ariake Sea located in western Japan, revealed that fish had immigrated to freshwater habitat during the early life stage and moved between freshwater and estuarine areas. An adult sea bass captured around a spawning ground was confirmed to migrate seasonally into freshwater area by Sr:Ca ratio chronology. These findings validated that otolith microanalysis is a useful tool to determine life history trajectory for Japanese sea bass from very early life through adult stages.

Key words: otolith, Sr:Ca ratio, Japanese sea bass, estuary migration, Ariake Sea

Japanese sea bass (Percichthyidae: *Lateolabrax japonicus*), are large top predators in estuarine and coastal ecosystems in Japan. Populations of sea bass occur over a wide latitudinal range and contribute to important coastal fisheries. Sea bass spawn in inshore marine habitats[1,2] and larvae are transported into embayments and estuarine nursery habitats. As advanced-stage juveniles and adults, sea bass exhibit seasonal immigration and emigration between estuarine and deeper coastal waters.[3]

Based upon field surveys of larvae and juveniles in the Chikugo River estuary (Fukuoka Prefecture, Kyushu), Matsumiya *et al.*[4,5] proposed that the life cycle of Ariake Sea *L. japonicus* (named Ariake sea bass, in the present paper) has an obligate freshwater juvenile stage. Annual field surveys revealed that up-estuary dispersal with increasing size occurred during March and April for late stage larvae and very early stage juveniles. Early stage juveniles (>17 mm, SL) were predominately found in freshwater habitats of the Chikugo River.[4] High densities of zooplankton prey occurring at the estuarine turbidity maximum and in freshwater regions of the River which could provide good nursery conditions.[6,7] The Chikugo River estuary has a high tidal amplitude (c.a. 6 meters at spring tide) and we speculate that larvae and juveniles can only retain themselves in riverine habitats by invading regions upriver to the saltfront. In addition, local fishermen have observed adult Ariake sea bass in freshwater regions of the Chikugo River during the summer months.

To investigate freshwater dependency of Ariake sea bass, we evaluated whether otolith microanalysis of Sr:Ca patterns in otoliths could be used to reconstruct ontogenetic and seasonal patterns of migration between freshwater, estuarine, and marine habitats. Strontium concentration in seawater is one order of magnitude higher than in freshwater and varies in direct proportion to salinity in estuarine environments.[8,9] Therefore, strontium levels in otoliths of fish exposed to seawater should be substantially higher than those exposed to freshwater. And, as a fish migrates along a salinity gradient, the Sr:Ca ratio in its otoliths should record the rate of movement among salinity zones. The otolith microchemistry method has been calibrated for striped bass (Moronidae: *Morone saxatilis*),[10,11] a species closely related to *L. japonicus*.[12] To determine whether salinity could be predicted from otolith strontium, otolith microanalysis was performed on hatchery produced sea bass juveniles exposed to conditions of constant or varying salinity. Otolith chronologies of Sr:Ca were also constructed for a yearling collected in the Chikugo River estuary and an adult captured from an inshore spawning ground.

Materials and Methods

Sea bass juveniles hatched on 8 February and reared at the Chiba Prefectural Tokyo Bay Sea-farming Center were transported to the Kyoto University Fisheries Research Station (Maizuru, Kyoto) on 26 April and 12 May 1996. Two experiments were conducted during May-July 1996 for

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juveniles aged 101–156 days after hatch. In Experiment 1, juveniles (30.5±0.8 mm SL) were immersed in alizarin complexone (ALC) solution (60 mg per *l*) to mark their otoliths according to Kuwada and Tsukamoto.[13] They were then exposed to four salinities (0, 10, 20, and 30 ppt) at 17°C for a 14d period (20 May to 4 June). Salinity levels were established by diluting ambient marine water (ca. 30 ppt) to the desired salinity level. Strontium and Calcium contents of the experimental waters were analysed by atomic absorption spectrometry, average Sr:Ca ratio being 0.0233, 0.0178, 0.0173, and 0.0171 at salinity 0, 10, 20, and 30 ppt, respectively. At the end of the experiment, juveniles were sacrificed and their sagittal otoliths removed. In Experiment 2, juveniles (30.5±0.8 mm SL) were again marked with ALC and then exposed to a 49d cycle of salinity: 30 ppt-20 ppt-10 ppt-0 ppt-10 ppt-20 ppt-30 ppt. Salinity levels were changed every 7 days (20 May to 9 July).

Otoliths were cleaned in 10% hypochlorite solution and rinsed with deionized water. They were then embedded in epoxy (Spurr), sectioned in transverse plane, mounted, and polished as described in Secor *et al.*[14] Otolith sections were carbon-coated in a high vacuum evaporator. X-ray intensities for Sr and Ca elements in the otolith matrix were quantified using a JEOL JXA-840A wave-length dispersive electron microprobe (Central Facility for Microanalysis, University of Maryland, College Park, MD 20742) with Calcite and Strontianite as standards. Standardizing strontium concentration to calcium levels reduced biases associated with machine drift and permitted comparison to other species.[10] Analytical methods for measuring molar weights of Sr and Ca followed those described by Secor.[15] Two types of analyses were performed. A point probe estimate was the mean three discrete point (area=5 μm^2) measurements taken near the edge of the otolith section. Transect probes were series of point measurements of Sr and Ca taken across the microstructures of otoliths at 13.5 μm to 15 μm intervals. ALC marks in the otolith were used to insure that only portions of the otolith corresponding to the experimental period were analyzed.

One yearling (22 cm, TL) and one adult (69 cm, TL) Ariake sea bass were collected in December 1995 in the Chikugo River estuary and around the spawning area, respectively. Sagittae were removed and prepared for transect probes.

Results

Otolith Sr:Ca of sea bass was significantly influenced by salinity in Experiment 1 (ANOVA; d.f.=14; $P=0.0012$) with 79% of the total variance of otolith Sr:Ca explained by salinity. Multiple range tests showed that the 0 ppt treatment resulted in significantly lower Sr:Ca ratios than the 10, 20, or 30 ppt treatments (Fig. 1). Although there was an increasing trend in Sr:Ca with salinity, differences among 10, 20, and 30 ppt treatments were not significant. A regression predicting salinity from otolith Sr:Ca ratio was best fitted with a linear slope (Fig. 2). Otoliths from two individuals exposed to a cycle of decreasing then increasing salinity (Experiment 2), showed a corresponding trend in Sr:Ca ratio in their otoliths (Fig. 3). The cycles were asymmetric with the nadir displaced towards the

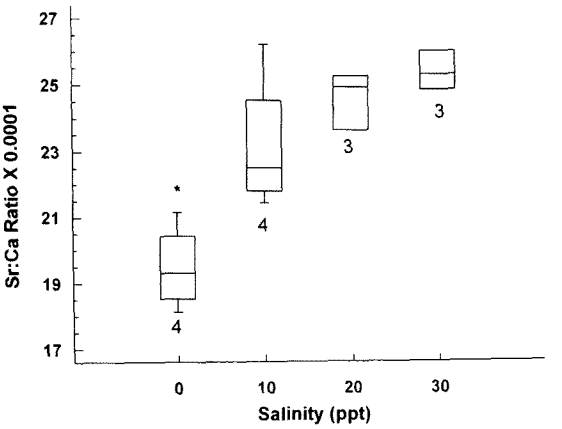

Fig. 1. Relationship between otolith Sr:Ca ratio and salinity in juvenile Japanese sea bass.

Box and Whisker plots of Sr:Ca ratio versus experimental salinity level for Experiment 1. Number of juveniles examined is indicated below each box plot. * represents Sr:Ca ratio at salinity 0 ppt is significantly lower ($P<0.01$) than those at 10, 20, and 30 ppt.

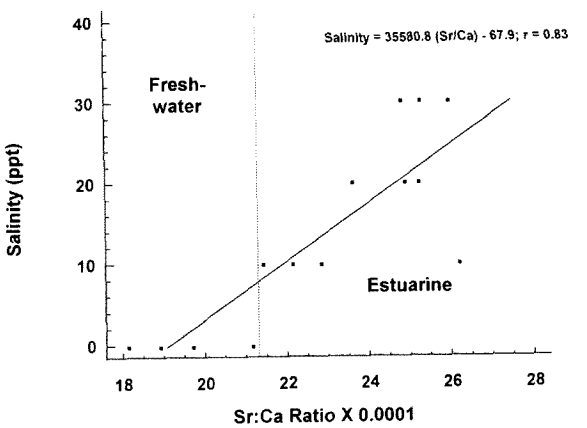

Fig. 2. Predictive regression of salinity based upon otolith Sr:Ca ratio in juvenile Japanese sea bass.

Regression and correlation coefficient are shown. The dashed line separating freshwater and estuarine Sr:Ca ratios was fitted by eye.

periphery of the otolith. Because daily increments became narrower as the experiment progressed (D. Secor, unpublished), the displacement of the nadir indicates that the constructed Sr:Ca chronologies provided better temporal resolution of the earlier portion of the experiment (i.e. decreasing salinity). The chronologies differed from each other in mean Sr:Ca levels, but the nadirs for both individuals were within the range expected for exposure to freshwater (Figs. 1 and 2).

The Sr:Ca chronology from an Ariake sea bass yearling showed a pattern in Sr:Ca ratio which indicated early freshwater ingress during the first year of life followed by a protracted period of freshwater residency (Fig. 4). The adult's Sr:Ca chronology (Fig. 5) indicated that freshwater residency extended past the time of first annulus formation, believed to occur during winter months (Ohta *et al.*, unpublished), and that this individual spent most of its lifetime in estuarine or marine habitats. However, the annual

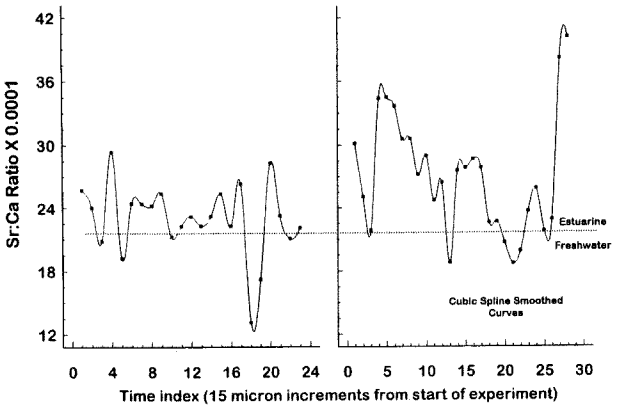

Fig. 3. Transect probe of Sr:Ca ratios for two juvenile Japanese sea bass exposed to a cycle of decreasing and then increasing salinity (Experiment 2).

Individual points for each transect probe were joined by a cubic spline. Time Index refers to 15 μm increments from an ALC mark which indicated the start of the experiment in the otolith's microstructure. Because daily increments became narrower as the experiment progressed, the Time Index did not linearly represent the experiment's time course. The estimated benchmark (see Fig. 2) for freshwater inhabitation is indicated as a dashed line.

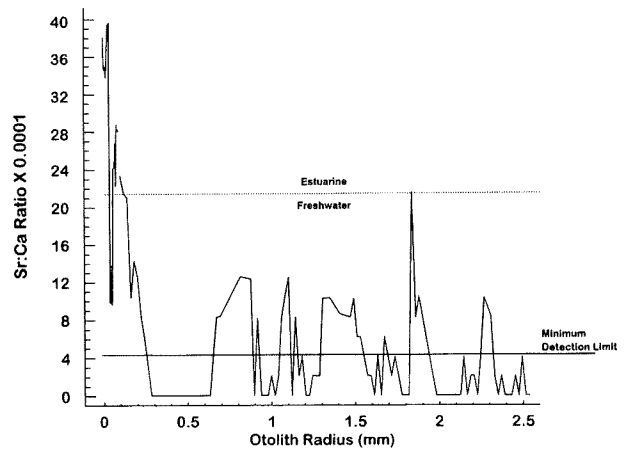

Fig. 4. Sr:Ca chronology of a yearling Ariake sea bass.

Benchmark for freshwater inhabitation (see Fig. 2) and minimum detection limit are shown.

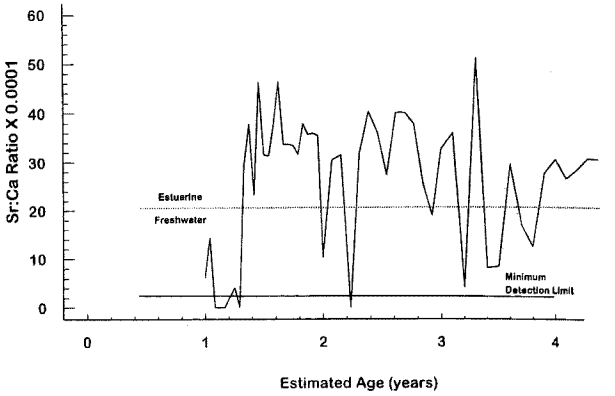

Fig. 5. Sr:Ca chronology of an adult Ariake sea bass.

Benchmark for freshwater inhabitation (see Fig. 2) and minimum detection limit are shown. Age was estimated by enumerating annuli in the otolith's microstructure.

nadirs during the adult's second and third year of life indicated that the fish may have seasonally and/or occasionally visited freshwater habitats.

Discussion

Japanese sea bass juvenile otoliths showed high levels of otolith Sr in comparison to other marine fish species, but were similar to those observed for striped bass.[10] The similarity in otolith Sr levels between the two species may be a consequence of their close taxonomic (and presumably phylogenetic) relationship. Also, both species exhibit life cycles which span fieshwater, estuarine and marine habitats. Salinity had a significant and positive influence on otolith Sr:Ca ratio for juvenile Japanese sea bass. Multiple range tests and regression analysis showed that freshwater inhabitation could be differentiated from estuarine or marine inhabitation. Otolith Sr:Ca ratio could not be used to confidently distinguish occurrences at 10, 20, or 30 ppt. This contrasted slightly with results for striped bass juveniles which showed rapid change in otolith Sr:Ca ratio with increasing salinity at salinity levels less than 20 ppt.

Tokyo Bay populations of Japanese sea bass were used in the current study. Recent mitochondrial DNA analysis on sea bass populations distributed in the east Asian coastal waters has revealed that a distinct population is distributed in the Ariake Sea, genetically different from both Chinese-Korean and Japanese (excluding Ariake Sea) populations.* Based on ecophysiological evidence, Tanaka *et al.*[16] proposed that Ariake sea bass has adapted more to freshwater habitat and evolved an amphidromous life cycle. Physiological characteristics may differ between the two populations of Japanese sea bass, this presumably influencing Sr:Ca ratios. Using a particle induced X-ray emission (PIXE), Ohta *et al.*[17] has preliminarily examined Sr:Ca ratio on otoliths of Ariake sea bass juveniles collected at various ranges of salinity including freshwater in the Chikugo River estuary, revealing nearly the same trend as obtained for laboratory-raised sea bass juveniles.

Sr:Ca chronologies performed on a yearling *L. japonicus* captured in the Chikugo River estuary, showed patterns of migration and habitat use consistent with those reported in the literature.[3,4,6] High levels of Sr:Ca in the core region of the otolith, corresponding to the larval period, indicated marine occurrence. The Sr:Ca ratio then dropped precipitously to levels indicative of freshwater occurrence. We believe that this pattern indicates rapid larval ingress from brackish water to freshwater habitats. For the first year of life (to the time of first annulus formation), otolith Sr:Ca levels were low, suggesting continued freshwater residency. Radtke *et al.*[18] observed a similar

* K. Nakayama, T. Nakabo, M. Tanaka, and M. Nishida: Advance Abstracts for the 29th Annual Meeting of the Ichthyol. Soc. Japan, Hakodate, 1996, p. 30.

drop in otolith Sr:Ca associated with freshwater ingress of Hawaiian gobies during their larval stage, followed by an extended period of residency.

Results supported the use of otolith microanalysis to reconstruct seasonal and ontogenetic patterns of dispersal in estuarine-dependent fishes.[9-11,18] Because salinity is an ecological scalar in estuaries, the otolith's chemical record could provide a chronometer of ontogenetic niche shifts.[19] In particular, we believe that otolith microanalysis will provide unique insight into the ecology of larval and juvenile dispersal into freshwater by Ariake sea bass in the Chikugo River. Research is underway to develop otolith-fish size relationships and ageing procedures[14] which will allow us to back-calculate size and ages of the sea bass larvae and juveniles as they ingress from Ariake Sea into the Chikugo River.

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