Variation in migration patterns of pond smelt, *Hypomesus nipponensis*, in Japan determined by otolith microchemical analysis

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In order to investigate the migration pattern of the pond smelt, *Hypomesus nipponensis*, the life history trajectories of populations from various localities in Japan were estimated using the otolith microanalysis technique. The strontium:calcium (Sr:Ca) ratio of otoliths was determined for pond smelt reared in fresh, brackish and sea water and was found to show a linear increase with increasing salinity. Salinity trajectories were constructed for populations based on a regression of Sr:Ca ratio to salinity. Some individuals in Lakes Abashiri and Ogawara, and all fish in Lakes Takahoko and Shinji showed values which were consistently low, indicating residence in these lakes throughout the life history. High values were also absent in individuals from the Ishikari River, so it was concluded that the populations migrated between fresh and brackish waters. However, pond smelt with high Sr:Ca ratio profiles indicating seawater residency were found in the Hei River, off Hachinohe, off Misawa, Lake Obuchi, Oppa Bay, and also some individuals from Lakes Abashiri and Ogawara. In these cases, residence in the sea appeared to have been short, except for Hei River and Lake Ogawara. These results indicate a wide flexibility in habitat selection by pond smelt, with brackish environments possibly playing an important role in the life history of the species.

Key words: Migration pattern, strontium concentration, Sr:Ca ratio, otolith microchemistry

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structure of otoliths makes them favorable to strontium-calcium (Sr:Ca) concentration ratio analyses. The strontium content of hard tissue of anadromous fish differs in the freshwater and seawater life stages and can be used confidently to distinguish freshwater and marine life history phases of individual fish (Reviewed by Arai, 2002; Pontual and Geffen, 2002).

The aim of the study reported here was to use otolith microanalysis of Sr:Ca ratio by EPMA (Electron Probe Microanalyzer) to determine the life history trajectory of pond smelt populations in various localities in Japan. The otolith Sr:Ca ratios in fresh, brackish and sea water were validated through a rearing experiment, making it possible to reconstruct the ontogenetic migration of pond smelt between their freshwater hatching area and estuarine-marine habitats based on the Sr:Ca ratio profile.

Materials and Methods
Rearing experiment
Parent pond smelt were captured in Lake Hinuma, Ibaraki Prefecture, Northern Central Japan, in February 1998. Eggs were artificially fertilized and the eggs and hatched larvae were reared at the Ocean Research Institute, University of Tokyo. Both the eggs and larvae were reared under the following conditions: 1/2 sea water (19.2 psu), around 20°C, constant light during the light period of the 12L : 12D cycle. Newly hatched larvae were initially fed 1/2-seawater-reared rotifers and then subsequently Artemia nauplii. In April 1998, they were exposed to one of three salinity conditions for about three months: freshwater (0.0 psu), 1/2 seawater (19.2 psu), seawater (32.8 psu). In July 1998, at the end of the experiment, the pond smelt were sacrificed and their sagittal otoliths removed, and the rearing waters of each regime were sampled for chemical analysis. Strontium concentrations in the water sampled at the end of the experiment were analyzed by an inductively coupled plasma mass spectrometry (ELEMENT, FinniganMAT, Bremen, Germany) using In (1 ppb) as an internal standard.

The environmental salinity can be documented well through otolith microchemistry. Although temperature and growth rate influence the Sr:Ca ratio, they have relatively minor effect compared to salinity (Secor et al., 1995; Secor and Rooker, 2000). Based on the result of the rearing experiment, regression analysis was conducted between the Sr:Ca ratio of otolith and ambient salinity, which provided the basis for the reconstruction of the salinity trajectory experienced by individual fish taken from the wild. Fresh, brackish and sea environments were defined as under 0.5 psu, 0.5–30 psu and over 30 psu, respectively (Mclusky, 1999).

Geography and aquatic characteristics of sampling areas
Natural populations of pond smelt were collected from various habitats in Japan (Table 1, Fig.1). Lake Abashiri is a brackish lake located on the Sea of Okhotsk side of Hokkaido. It is a eutrophic lake formed in the lower reaches of the Abashiri River, and the northernmost part of the lake is connected to the sea by a river mouth known as the Abashiri Bay. Freshwater is supplied to the lake from inflowing rivers which provide the spawning grounds for the pond smelt. Water in the lake is separated into two layers by a halocline formed at around 6–7 m depth. In the layer above the halocline, salinity changes considerably between 2.0 psu and 3.5 psu geographically and seasonally. The bottom layer remains highly saline (over 20 psu) and with very low oxygen levels all the year round (Imada et al., 1995). The Ishikari River, also in Hokkaido, flows into the Sea of

<table>
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<tr>
<th>Locality</th>
<th>Aquatic characteristics</th>
<th>Abbreviation</th>
<th>Sampling date</th>
<th>Sample size</th>
<th>Standard length (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>L. Abashiri</td>
<td>Brackish (2.0–3.5 psu)</td>
<td>A-L</td>
<td>Apr., 2001</td>
<td>3</td>
<td>84.3–90.0</td>
</tr>
<tr>
<td>Abashiri R.</td>
<td>Fresh water</td>
<td>A-R</td>
<td>Apr., 1999</td>
<td>4</td>
<td>75.1–105.1</td>
</tr>
<tr>
<td>Abashiri Bay</td>
<td>Brackish water</td>
<td>A-B</td>
<td>Dec., 1998</td>
<td>2</td>
<td>83.3–92.3</td>
</tr>
<tr>
<td>off Abashiri</td>
<td>Sea water</td>
<td>A-S</td>
<td>Oct., 2001</td>
<td>3</td>
<td>49.7–53.3</td>
</tr>
<tr>
<td>Ishikari R.</td>
<td>Fresh water</td>
<td>IS</td>
<td>Sep., 2001</td>
<td>3</td>
<td>46.9–55.6</td>
</tr>
<tr>
<td>L. Obuchi</td>
<td>Brackish (9.0–9.3 psu)</td>
<td>OB</td>
<td>Mar., 1998</td>
<td>5</td>
<td>76.2–91.0</td>
</tr>
<tr>
<td>L. Takahoko</td>
<td>Fresh water (Closed)</td>
<td>TH</td>
<td>Apr., 1995</td>
<td>4</td>
<td>51.8–72.2</td>
</tr>
<tr>
<td>L. Ogawara (Anadromus)</td>
<td>Brackish (0.5–0.6 psu)</td>
<td>OG-A</td>
<td>Apr., 1991</td>
<td>3</td>
<td>80.4–101.1</td>
</tr>
<tr>
<td>L. Ogawara (Resident)</td>
<td>Brackish (0.5–0.6 psu)</td>
<td>OG-R</td>
<td>Apr., 1991</td>
<td>4</td>
<td>48.2–54.5</td>
</tr>
<tr>
<td>off Misawa</td>
<td>Sea water</td>
<td>MS</td>
<td>Apr., 1995</td>
<td>2</td>
<td>67.5–88.0</td>
</tr>
<tr>
<td>off Hachinohe</td>
<td>Sea water</td>
<td>HC</td>
<td>Oct., 1994</td>
<td>3</td>
<td>57.7–91.1</td>
</tr>
<tr>
<td>Hei R.</td>
<td>Fresh water</td>
<td>HE</td>
<td>May, 2001</td>
<td>5</td>
<td>65.9–81.7</td>
</tr>
<tr>
<td>Oppa Bay</td>
<td>Sea water</td>
<td>OP</td>
<td>Mar., 2002</td>
<td>2</td>
<td>103.2–107.5</td>
</tr>
<tr>
<td>L. Shinji</td>
<td>Brackish (1–2 psu)</td>
<td>SN</td>
<td>Oct., 1991</td>
<td>3</td>
<td>89.9–99.0</td>
</tr>
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</table>
Japan. Lakes Obuchi, Takahoko and Ogawara are located on the Pacific side of the northernmost part of Honshu in Aomori Prefecture. Lake Takahoko is a freshwater lake separated from the sea by a water gate constructed in 1965. Lake Obuchi is a brackish lake (9.0–9.3 psu) (Katayama et al., 2000). Lake Ogawara is also brackish, but the water is far less saline at approximately 0.6 psu throughout the year (Kawasaki and Ito, 1995; Sato, 1953). Lake Ogawara is connected to the sea by the 6-km long River Takase. Part of the population was known to perform anadromous migration through the river. The Hei River is open to Miyako Bay, and the Kitakami River is open to Oppa Bay, located on the Pacific side of the Tohoku region. Lake Shinji is an oligohaline lake (1–2 psu), located in the western part of the side towards the Sea of Japan (Nakamura et al., 1988).

Otolith treatment and microchemical analysis

Specimens were frozen immediately after capture. Standard body length (SL) was measured to the nearest 0.1 mm and the age in years was determined by observing the otolith sagitta phase followed by otolithometry of Katayama and Kawasaki (1994). Measurement and analysis were made for 0+ aged fish only.

Sagittae were removed and prepared for transect probes to evaluate ontogenetic trends in migration and habitat utilization patterns. Otoliths were cleaned with 30% sodium hypochlorite solution and rinsed with distilled water. They were embedded in epoxy resin, sectioned along the transverse plane, and polished using 600–2000 grit carborundum and 0.5 and 0.3 μm alumina paste to reveal an incremental series from the core to the dorsal otolith edge. Samples were washed with distilled water and coated with carbon.

Strontium and calcium contents in the otolith were measured quantitatively with an X-ray wavelength dispersive electron microprobe (JEOL, JXA-8900). The electron beam was focused to 1 μm diameter, using an accelerating voltage of 15 kV and a beam current of about 3×10⁻⁷ A. Line analysis was conducted at 5 μm intervals from the otolith core to its margin along the otolith transverse line. CaSiO₃ and SrTiO₃ were used as standards for calibration of strontium and calcium measurements, respectively. Final elemental concentrations are presented as ratios of weight percentage. The Sr:Ca ratios are presented as the amount of strontium divided by the amount of calcium times 10³.

Sr:Ca ratio profiles were constructed in relation to back-calculated standard body length. The back-calculated body length was estimated by regression methods utilizing the regression formula of Katayama et al. (2000): SL (mm)=3.23X²+33.59X+3.66 (p<0.0001, r²=0.98), where x is otolith width.

Results

Rearing experiment

The strontium concentration and salinity of each rearing water (freshwater, 1/2 seawater, seawater) used to examine the relationship between salinity and Sr:Ca ratio in otoliths are shown in Table 2. Strontium concentration showed a positive relationship with salinity (r²=0.999, p<0.001, Fig. 2A). The Sr:Ca ratio of the otolith increased with salinity, and a linear regression was well fitted between salinity (X) and Sr:Ca ratio in the otolith (Y) as Y=0.0754X+4.14 (Fig. 2B, r²=0.847, p<0.001). This linear model was used to convert the Sr:Ca ratio of the otolith to the salinity of the habitat. Values less than 4.18, between 4.18 and 6.40, and over 6.40 of otolith Sr:Ca ratio corresponded to salinities under 0.5 psu (freshwater), 0.5–30 psu (brackish water) and over 30 psu (seawater), respectively.

Sr:Ca ratio profile (Fig. 3)

Of the 12 otoliths, only three samples showed a high Sr : Ca ratio (over 6.40) indicating clear traces of seawater residency (A-L-2, A-S-3, A-B-1). The Sr : Ca ratio of the other specimens fluctuated within the brackish and freshwater ranges. Among these specimens, four otoliths showed temporary rises to near 6.62 at about 20 mm body length (A-R-1, A-R-4, A-S-3 and A-B-1).

**Ishikari River (IS):** Three otoliths showed a similar pattern, where the Sr : Ca ratio stayed below 4.18 until approximately 20–30 mm SL, and then increased gradually. This pattern depicts a change in the habitat from river to brackish area with growth.

**Lake Takahoko (TH):** All four otoliths examined showed consistently low Sr : Ca ratios (below and around 4.18), suggesting continuous residence in freshwater.

**Lake Obuchi (OB):** Five otoliths showed a similar pattern, where the ratio fluctuated around 4–7, but rose temporarily above 6.62 in individuals larger than about 50 mm SL. All five fish examined lived basically in a brackish area, followed by a short-term residence in sea water.

**Lake Ogawara (OG):** Profiles of Sr : Ca ratio of resident fish (OG-R) were characterized by a low Sr : Ca ratio for the entire time series. The Sr : Ca ratio was about 4–5 after hatching and decreased slightly, showing permanent residence in the lake. The ratio of anadromous fish (OG-A) was about 4–5 around the time of hatching, as noted in the resident fish. At 20–50 mm body length, the Sr : Ca ratio increased markedly to over 6.40, and subsequently remained at this value, increased, or declined. These profiles show that after the seaward migration, the fish freely alternate their habitats between the sea and brackish areas.

**Off Misawa (MS):** The two sample otoliths displayed a wide range for the ratio from 2 to 8. MS-1 showed a gradual migration from freshwater to the sea with growth. MS-2 is interpreted to migrate irregularly numerous times between fresh, brackish and sea areas.

**Off Hachinohe (HC):** Two otoliths (HC-2, 3) showed a higher Sr : Ca ratio and marked fluctuations. One otolith showed a uniformly low ratio (under 4.18) with a slight increase at the otolith margin. It was estimated that two sample fish migrated many times between brackish and sea areas, and one fish lived almost entirely in freshwater.

**Hei River (HE):** Fish here were characterized by consistently high Sr : Ca ratios. After hatching, the ratio remained constant at around 5. Values then increased and rose over 6.40 at 30–50 mm SL. These profiles suggest that after living in a brackish area, all the fish migrated and resided in the sea.

**Oppa Bay (OP):** The Sr : Ca of OP-1 and OP-2 rose steeply above 4.18 and 6.40 after reaching 90 mm SL, respectively. The two individuals seem to have inhabited a freshwater area, and then migrated to brackish area or the sea at the end of their lives.

**Lake Shinji (SN):** All three otoliths examined kept a moderate value under 6.40, indicating residency in brackish water throughout their lives.

### Discussion

The relationship between the Sr : Ca ratio of fish otoliths and salinity has previously been reported for only a few species, Japanese sea bass (Secor et al., 1998), tropical goby (Radtke et al., 1988) and bay anchovy (Kimura et al., 2000). Secor et al. (1995) found a logistic relationship between salinity and the otolith Sr : Ca ratio. The Sr : Ca ratio of laboratory-reared pond smelt maintained at three salinity regimes showed a proportional relationship with salinity.
Figure 3. Sr : Ca ratio profiles from otoliths of pond smelt caught in various areas in Japan. For the explanation of abbreviation in each profile see Table 1. Dotted lines indicate 4.18 and 6.40 of Sr:Ca ratio.
The means and SDs of otolith Sr : Ca ratios for fish reared in freshwater and seawater were 4.21 ± 0.31 and 6.94 ± 0.72, respectively; however, this difference is small compared with the values of 0.018 mg/l and 7.51 mg/l for the Sr concentrations in the fresh and sea waters used for rearing. The same phenomenon was also found by Brown and Harris (1995), and is due mainly to physiological discrimination, the higher the concentration of Sr in the ambient water, the lower the efficiency of Sr incorporation (Mugiya, 1994). Elemental discrimination occurs at water-gill, blood-endolymph, and endolymph-crystal boundaries, resulting in the ratio of Sr to Ca in the otolith becoming far lower than that in the blood plasma or ambient water. The degree of discrimination varies among fish species (Campana, 1999). This suggests that the salinity encountered by a fish cannot be estimated solely from the absolute value of the Sr : Ca ratio. In addition, numerous other factors are known to relate to the Sr : Ca ratio (reviewed by Radtke and Shafer, 1992; Campana, 1999). A reliable reconstruction of the environmental history to provide biological information on the life history of an individual based on otolith microchemistry requires a rearing experiment to evaluate the effect of ambient salinity on the Sr : Ca ratio of the otolith.

The Sr : Ca ratio of otoliths of pond smelt from the Hei River indicate residency in seawater. However, the Sr : Ca ratio was much higher than that for pond smelt reared in the seawater. Factors such as temperature and growth rate can effect the Sr : Ca ratio, but, as mentioned above, are relatively minor, so the high Sr : Ca ratio observed in Hei River pond smelt had probably been enhanced by their food.

Pond smelt from different localities showed different migration patterns. It should be noted that the samples from off Abashiri, Abashiri Bay, off Hachinohe, and Lake Shiniji were all collected in autumn-winter, and were not mature adults that had completed a whole year of life. Therefore, the Sr : Ca profiles from these localities represent only part of the life history. As expected, the ratio for pond smelt inhabiting a freshwater area such as Lake Takahoko indicated a freshwater habitat. The Sr : Ca ratios of parts of the populations from Lakes Abashiri and Ogawara and all the population from Lake Shiniji were consistently low, indicating residence in the lakes throughout their lives. Pond smelt in Ishikari River did not show a high Sr : Ca ratio in the otolith, which would indicate residence in the sea, and it was concluded that the fish migrated between fresh and brackish waters. Conversely, pond smelt with ratios providing a clear indication of a seawater habitat were found in Lake Abashiri, Lake Obuchi, off Misawa, off Hachinohe, Hei River, Oppa Bay, and Lake Ogawara (anadromous fish). However, pond smelt with clear traces of seawater habitation showed relatively temporal residence in seawater, except for Hei River and Lake Ogawara (anadromous fish). Furthermore, the low Sr : Ca ratios at otolith margins of two individuals from off Abashiri and one from off Hachinohe indicated the short time residence in seawater environment, although these fish were captured in seawater area.

In Lake Abashiri and its adjacent areas, only a few specimens were concluded to have experienced seawater. In addition to these, temporal migration to seawater was considered to have occurred at about 20 mm body length for four specimens. Some of the Lake Abashiri population has been reported to descend from the lake in the juvenile period over 20 mm TL (Torisawa, 1998; Torao, 2001). Therefore, pond smelt in Lake Abashiri would mainly inhabit brackish areas after downstream migration toward the sea, and live only occasionally in seawater.

These results suggest that pond smelt show a wide flexibility in their habitat selection, and that the brackish area plays a significant role in the life history, even for a riverine population which agreed with previous research (Arai et al., 2006). Pond smelt of Lake Hinuma, a brackish lake in Ibaraki Prefecture, complete their life history inside the lake (Saruwatari, unpublished), similar to Japanese icefish, Salangichthys microdon (Saruwatari and Okiyama, 1992). The relative importance of the brackish habitat in the life history of pond smelt is probably determined by the geographic and climatic conditions of the brackish water system. The function of brackish water systems as highly productive nursery areas for teleost larvae is still not well understood and documented (e.g. Saruwatari, 1995). The application of otolith microchemical analysis, such as the Sr : Ca ratio, to determine the migratory history of individuals should aid in the understanding of the ways in which the fragile and valuable brackish water environment is utilized by teleosts.

In this study, pond smelt displayed various migration patterns, but habitat preference and the mechanism of habitat selection were not revealed. Resource-holding power, territory, pollution, drought, day length in the growth season, temperature, nutrient condition, discharge rate and population density are all suggested to be biological factors determining salmonid migration (see reviews by Baker, 1978; Randall et al., 1987). The three-spine stickleback, Gasterosteus aculeatus, and Japanese icefish are known for their wide plasticities in environmental adaptation and alternative life history similar to pond smelt (Wootton, 1984; Saruwatari, 1994; Saruwatari et al., 2002; Yamaguchi et al., 2004). However, their migration patterns have not yet been well documented. The mechanisms of fish migration and habitat selection would be disclosed through investigation and comparison of the migration patterns of these fishes, including pond smelt, among allopatric populations within a species and among sympatric species.
Migration pattern of pond smelt

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Reference


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耳石微量成分分析によって推定されたワカサギの多様な回遊パターン

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ワカサギの回遊パターンを明らかにするために、まず飼育実験によって飼育水の塩分と耳石Sr:Ca比の直線回帰式を求めた。次いで日本各地のワカサギ耳石の中心核から細胞に至るSr:Caプロファイルを基に、各水域における個体の塩分履歴を推定した。網走湖、小川原湖の一部の個体、および鷹巣沼、矢道湖の全個体のSr:Ca比は常に低い値を示し、一生を湖内で滞留していたことが示唆された。これに対して、海水域で生活したと判断される高いSr:Ca比を有していたのは、八戸沖、三沢沖、閉伊川、追波湾のワカサギと網走湖および小川原湖の一部の個体であった。しかし、閉伊川と小川原湖以外では、海水域の利用は一部的であり、主に汎域に生息しているものと考えられた。これらの結果から、ワカサギの回遊パターンは個体群によって様々であるが、汎水域における生活期間の生活史に占める割合が大きいことが示された。

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