J. Mar. Biol. Ass. U.K. (2003), **83**, 223–230
Printed in the United Kingdom

Use of otolith microchemistry to estimate the migratory history of the threespine stickleback, *Gasterosteus aculeatus*

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Ontogenic change patterns in otolith Sr:Ca ratios in the threespine stickleback, *Gasterosteus aculeatus*, collected from Japanese brackish waters and freshwater, were examined by wavelength dispersive X-ray spectrometry on an electron microprobe. Two-dimensional images of the Sr concentration in the otoliths showed a variety of patterns of Sr concentration relative to salinity of habitat in all specimens collected in Hokkaido Island. Otolith Sr:Ca ratios in line analysis of all specimens collected in Hokkaido Island fluctuated strongly during the life history transect in accordance with the migration (habitat) pattern from sea to freshwater, via brackish water. In contrast, the Sr concentration or the Sr:Ca ratios of the Otsuchi River stickleback remained at consistently low levels throughout the otolith (1.5×10^{-3} in the Sr:Ca ratios). The higher ratios ($3.2–6.3\times10^{-3}$) in sticklebacks collected in Hokkaido Island probably reflect the ambient salinity or the seawater−freshwater gradient in Sr concentration. The findings clearly indicated that otolith Sr:Ca ratios reflected individual life histories, and that the stickleback had a flexible migration strategy in the ambient water.

INTRODUCTION

The threespine stickleback, *Gasterosteus aculeatus*, is widely distributed in coastal regions throughout the Northern Hemisphere. It occurs in marine, brackish water, and freshwater in a bewildering array of morphological, physiological, and ecological forms. The species includes anadromous and freshwater forms (Wootton, 1984). Anadromous sticklebacks migrate into freshwater areas from the sea to breed in the spring and early summer, whereas freshwater sticklebacks permanently reside in freshwater areas. Freshwater sticklebacks are believed to have been derived from anadromous sticklebacks (Bell & Foster, 1994). These different forms are usually allopatric, but are occasionally parapatric or sympatric (Heuts, 1947; Hagen, 1967; Moodie, 1972; Larson, 1976; McPhail, 1984, 1993; Reimchen et al., 1985; Zuiganov et al., 1987). Recently, the threespine sticklebacks around Japan were shown to have two genetically divergent forms (Nei's D=0.482), the Pacific Ocean and the Japan Sea forms, which are reproductively isolated from each other sympatrically (Higuchi & Goto, 1996). The Pacific Ocean form are composed of both anadromous and freshwater sticklebacks, whereas the Japan Sea form is monomorphic, consisting of only anadromous fish (Higuchi & Goto, 1996; Higuchi et al., 1996). It has been assumed that although the initial divergence started in allopatry, competitive interactions in sympatry played a major role in the evolution of reproductive isolation and resource partitioning (McPhail, 1993). Thus, the migratory pattern in the life cycle of this species is variable and complicated. Information on individual migratory histories would provide basic knowledge about migration and ecological behaviour in the sticklebacks.

Recent chemical analytic techniques have enabled identification of life history events in individual fish by detecting trace elements in the microstructure of their otoliths. Strontium (Sr) incorporation (Kalish, 1989; Secor et al., 1995; Arai et al., 1997; Arai, 2002) in fish otoliths are of special interest for their potential utility as indicators of past environmental (temperature, salinity) and physiological conditions (ontogeny, migration). The deposition of strontium (Sr) and calcium (Ca) in fish otoliths during growth varies between freshwater, brackish water, and marine habitats (Secor et al., 1995; Tzeng et al., 1997; Arai & Tsukamoto 1998; Arai & Miyazaki, 2001; Tsukamoto & Arai, 2001).

Compared with other diadromous fish, little is known about the migratory history of the stickleback. Discrimination between anadromous and freshwater types of sticklebacks has previously been determined on the basis of external morphological characteristics such as number of plates and size distribution. Anadromous sticklebacks are a completely plated morph, whereas those in freshwater have a comparatively small number of plates (low plate morph) (Ikeda, 1933; Hagen, 1967; Zuiganov et al., 1987). Bimodal size distribution of the large- and small-sized fish has been suggested to allow for the coexistence of both anadromous and freshwater fish in one habitat (Mori, 1990; Higuchi et al., 1996). However, findings of evidence useful for discrimination between anadromous and freshwater sticklebacks have rarely been reported, and such findings are applicable only to a limited area due to the local variation in characteristics (Munzing, 1963; Wootton, 1976; Hagen & Moodie, 1982).

In this study we measured Sr:Ca ratios in the otoliths of sticklebacks collected in marine, brackish, and freshwater habitats around Japan to determine the individual

https://doi.org/10.1017/S0025315403007008h Published online by Cambridge University Press

Table 1. Gasterosteus aculeatus. *Specimens used for otolith microchemistry analyses.*

Sampling location	Life history style estimated from morphology	Salinity (psu)	Sampling date	No. fish examined	Total length (mm)		Sr:Ca ratios		Migration pattern estimated from Sr:Ca ratios
					Mean ±SD	Range	Mean ±SD	Range	
Otsuchi River	anadromous	0	28–29 May 2001	5	81.9 ±11.9	71.7–96.6	1.5 ±0.14	1.3–1.6	freshwater resident
Otsuchi River	freshwater resident	0	28–29 May 2001	5	50.5 ±3.88	46.2–56.0	1.5 ±0.19	1.2–1.7	freshwater resident
Lake Akkeshi (Pacific Ocean form)	anadromous	18–31	7 May 2001	5	93.0 ±2.77	90.1–96.4	5.3 ±0.38	5.0–5.9	anadromous
Lake Akkeshi (Japan Sea form)	anadromous	18–31	7 May 2001	5	72.9 ±3.79	69.3–77.8	6.3 ±0.87	5.3–7.5	anadromous
Hyoutan Marsh	anadromous	16–31	5 May 2001	5	87.3 ±2.44	84.3–88.7	5.4 ±0.34	5.1–6.0	anadromous
Hyoutan Marsh	freshwater resident	16–31	5 May 2001	5	59.6 ±4.96	54.7–66.0	5.1 ±0.18	4.9–5.3	anadromous
Shiomi River	anadromous	1–28	8 May 2001	5	88.3 ±3.08	85.1–92.8	5.6 ±0.22	5.3–5.8	anadromous
Shiomi River	freshwater resident	1–28	8 May 2001	5	58.1 ±4.64	53.1–64.3	5.9 ±0.08	5.8–6.0	anadromous
Numajiri River	anadromous	3–4	6 May 2001	5	89.7 ±2.80	87.8–94.6	3.9 ±0.27	3.5–4.2	anadromous
Numajiri River	freshwater resident	3–4	6 May 2001	5	61.4 ±4.94	54.2–66.4	4.1 ±0.11	4.0–4.3	anadromous
Lake Takkobu	anadromous	0	7 May 2001	5	92.2 ±1.87	90.1–94.2	3.2 ±0.20	3.0–3.5	anadromous
Lake Takkobu	freshwater resident	0	7 May 2001	5	75.7 ±4.30	71.9–82.1	6.0 ±1.3	4.0–7.5	anadromous

migratory histories of fish. Thus, the objectives of this study were: (i) to describe the ontogenic changes in otolith Sr:Ca ratios of the threespine stickleback, *Gasterosteus aculeatus*; and (ii) to examine the usefulness of this technique for reconstructing the migratory history of the species.

MATERIALS AND METHODS

Fish

The threespine stickleback, *Gasterosteus aculeatus*, was collected by casting and seine nets in environments of various salinities (freshwater: 0–5 psu, and brackish water: 5–30 psu) such as rivers, lakes, marshes, estuaries, and coastal waters around northern Japan (Table 1; Figure 1). Sticklebacks in a freshwater habitat were sampled at the head of the Otsuchi River, Iwate Prefecture, between 28 and 29 May 2001. Sticklebacks of the Pacific Ocean form (Higuchi & Goto, 1996) in a brackish water habitat were collected at the Hyoutan Marsh of the Shiomi River basin, at the watercourse of the Shiomi River basin, and at Lake Akkeshi, Hokkaido Island, between 5 and 8 May 2001. Those in freshwater were collected at the Numajiri River of the Lake Harutori basin, and at Lake Takkobu, Hokkaido Island, between 6 and 7 May 2001. Sticklebacks of the Japan Sea form (Higuchi & Goto, 1996) in a brackish water habitat were collected at Lake Akkeshi, Hokkaido Island, on 7 May 2001. These sampling sites, with the exception of the Otsuchi River and Lake Takkobu, are influenced by the rising tide. Lake Takkobu, an inland sea-lake, is connected to the sea by an intermittent small stream, although a 0 psu salinity was indicated. The Otsuchi River, around the riverhead, is not influenced by fluctuations in tide because the downstream has a weir. A total of 60 specimens were used in the present study; 20 sticklebacks from a freshwater habitat and 40 from brackish habitat (Table 1). Total length (TL) was measured, then the specimens were differentiated by migration pattern (anadromous or freshwater resident types) based on morphological analyses and habitat environment, following the method of Higuchi & Goto (1996) and Higuchi et al. (1996). As a result, these specimens were divided into 30 anadromous and 25 freshwater resident sticklebacks from the Pacific Ocean form, and five anadromous fish from the Japan Sea form.

Otolith preparation

Sagittal otoliths were extracted from each fish, embedded in epoxy resin (Struers, Epofix) and mounted on glass slides. The otoliths were then ground in order to expose the core, using a grinding machine equipped with a

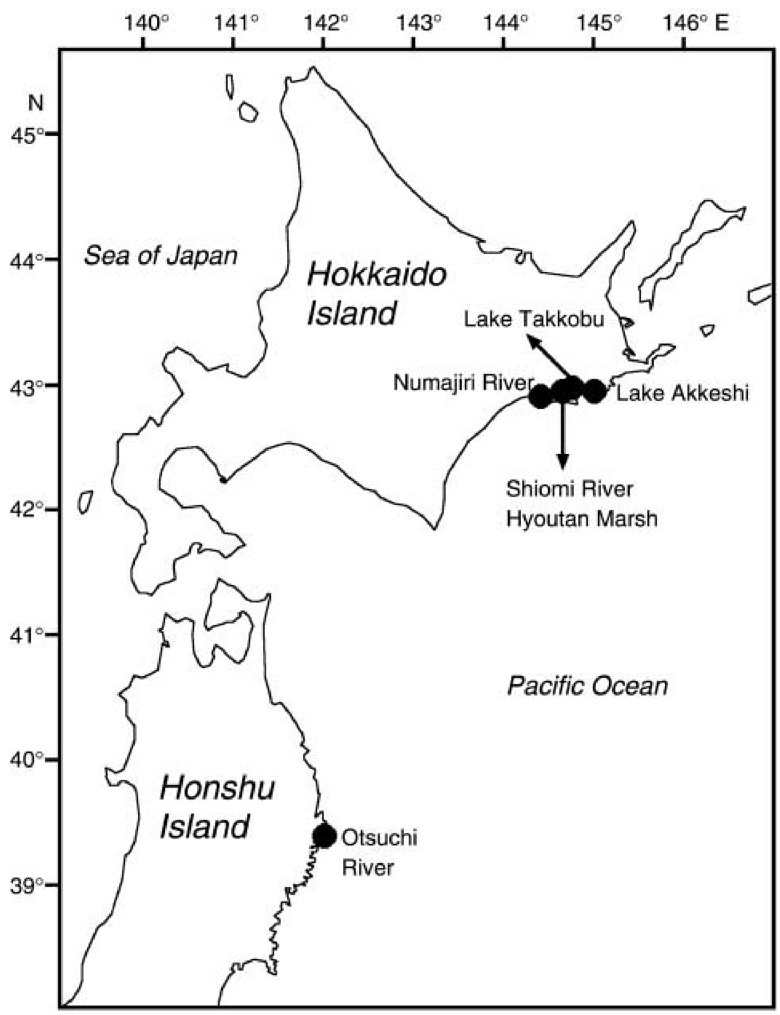

Figure 1. Sampling sites for the threespine stickleback, *Gasterosteus aculeatus*, along the coast of northern Japan.

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diamond cup-wheel (Struers, Discoplan-TS), and polished further with 6 μm and 1 μm diamond paste on an automated polishing wheel (Struers, Planopol-V). Finally, they were cleaned in an ultrasonic bath and rinsed with deionized water prior to examinations.

Otolith X-ray microprobe analysis

For electron microprobe analyses, all otoliths were Pt-Pd coated by a high vacuum evaporator. Otoliths from 52 specimens were used for 'life-history transect' analysis of 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), as described in Arai et al. (1997) and Arai & Tsukamoto (1998). Calcite ($CaCO_3$) and strontianite ($SrCO_3$) were used as standards. The accelerating voltage and beam current were 15 kV and 1.2×10^{-8} A, respectively. The electron beam was focused on a point 2 μm in diameter, with measurements spaced at 2 μm intervals.

'X-ray intensity maps' of both elements were made of the otoliths of six specimens using JEOL JXA-8900R as described by Tsukamoto & Arai (2001) and Arai et al. (2002). The beam current was 0.5 μA, counting time was 0.1 s, pixel size was 2×2 μm, the electron beam was focused on a point of 1 μm in diameter, and other analytical conditions followed those for the life-history transect analyses. Otoliths from two specimens were used for both life-history transects and X-ray intensity map analyses.

Statistical analyses

Differences between data were tested by Mann–Whitney U-test. Differences among data were tested by an analysis of variance (ANOVA) and afterwards with Scheffé's multiple range tests for pair-wise comparisons (Sokal & Rohlf, 1969).

RESULTS

Otolith strontium distribution

Two-dimensional images of the Sr concentration in otoliths showed remarkable variation among the specimens examined (Figure 2). However, all specimens had a low Sr area around the core of the otolith (bluish or less yellowish colour). The typical pattern of the Sr concentration in the Otsuchi River samples of freshwater resident type fish from a freshwater habitat was a uniformly low Sr concentration all over the otolith (bluish colour) (Figure 2A). Samples of fish from a brackish habitat showed both low and high Sr levels in the outer region, beyond a low Sr centre. The Numajiri River samples of both anadromous and freshwater resident types showed a wide space of bluish colour (low Sr) radiating from the centre, which was surrounded by concentric rings with higher Sr concentrations (Figure 2B,D). The Shiomi River sample of a freshwater resident type was characterized by yellowish and reddish colours (higher Sr concentration) from the otolith core to the edge (Figure 2E), whereas the Lake Akkeshi samples from brackish water showed either low Sr rings with a continuous pattern of relatively high yellowish and red colours (lower Sr) beyond the high Sr centre (Figure 2C). However, samples from Lake Takkobu, the locality of lowest salinity in the study, included a high Sr pattern in the outermost region of the otolith (Figure 2F).

Life history transects of freshwater type from freshwater habitat

The Sr:Ca ratios measured along a transect from the core to the otolith edge of the Otsuchi River of freshwater resident type samples showed consistently low Sr:Ca values of $1.5\times10^{-3}\pm0.19\times10^{-3}$ (mean $\pm$SD) (Figure 3), suggesting continuous residence in a freshwater habitat after hatching. Otolith Sr:Ca ratios from the Numajiri River samples of the freshwater resident type (mean $\pm$SD from the core to the edge: $4.1\times10^{-3}\pm0.11\times10^{-3}$) had a slightly high Sr:Ca phase (Phase-H) between the point 200 μm from the core and the edge. Significant differences occurred between the Sr:Ca ratios in the low Sr:Ca phase (Phase-L) from the core to the point 200 μm (mean $\pm$SD; $3.7\times10^{-3}\pm0.34\times10^{-3}$, range; 3.5×10^{-3}–3.9×10^{-3}), and the ratios in the latter phase from the point 200 μm to the edge (Phase-H) (mean $\pm$SD; $5.1\times10^{-3}\pm0.51\times10^{-3}$, range; 4.5×10^{-3}–6.3×10^{-3}) (Mann–Whitney U-test, $P<0.05$–0.0001) in all specimens. The type from Lake Takkobu showed relatively high Sr:Ca ratios around the core to the edge, (mean $\pm$SD; $6.0\times10^{-3}\pm1.3\times10^{-3}$), excepting one specimen that showed a fluctuation pattern similar to that of the Numajiri River samples. Significant differences were found between the Otsuchi River sample and the Numajiri River sample (ANOVA, $P<0.0001$), between the Otsuchi River sample and the Lake Takkobu sample (ANOVA, $P<0.0001$) and between the Numajiri River sample and the Lake Takkobu sample (ANOVA, $P<0.01$).

Life history transects of anadromous type from freshwater habitat

All samples from the Otsuchi River of the anadromous type had consistently low Sr:Ca values, averaging $1.5\times10^{-3}\pm0.14\times10^{-3}$ ($\pm$SD) from the centre to the edge, suggesting continuous residence in a freshwater habitat after hatching, as was the case for the freshwater types from the same location (Figure 3). Otolith Sr:Ca ratios in the Numajiri River samples of the anadromous type (mean $\pm$SD from the core to the edge: $3.9\times10^{-3}\pm0.27\times10^{-3}$) had a high Sr:Ca phase between the point 200 μm from the core to the edge. Significant differences were observed between the Sr:Ca ratios in the former phase from the core to the point 200 μm (Phase-L) (mean $\pm$SD; $3.1\times10^{-3}\pm0.08\times10^{-3}$, range; 2.6×10^{-3}–3.6×10^{-3}) and the ratios in the latter phase from the point 200 μm to the edge (Phase-H) (mean $\pm$SD; $5.1\times10^{-3}\pm0.19\times10^{-3}$, range; 4.1×10^{-3}–6.4×10^{-3}) (Mann–Whitney U-test, $P<0.005$–0.0001) in all specimens. Otolith Sr:Ca ratios in Lake Takkobu samples of the anadromous type (mean $\pm$SD from the core to the edge: $3.2\times10^{-3}\pm0.20\times10^{-3}$) had a high Sr:Ca phase between the point 250 μm from the core to the edge. Significant differences were observed between the Sr:Ca ratios in the former phase from the core to the point 250 μm (Phase-L) (mean $\pm$SD; $2.2\times10^{-3}\pm0.11\times10^{-3}$, range; $2.1\times10^{-3}-2.4\times10^{-3}$) and the ratios in the latter

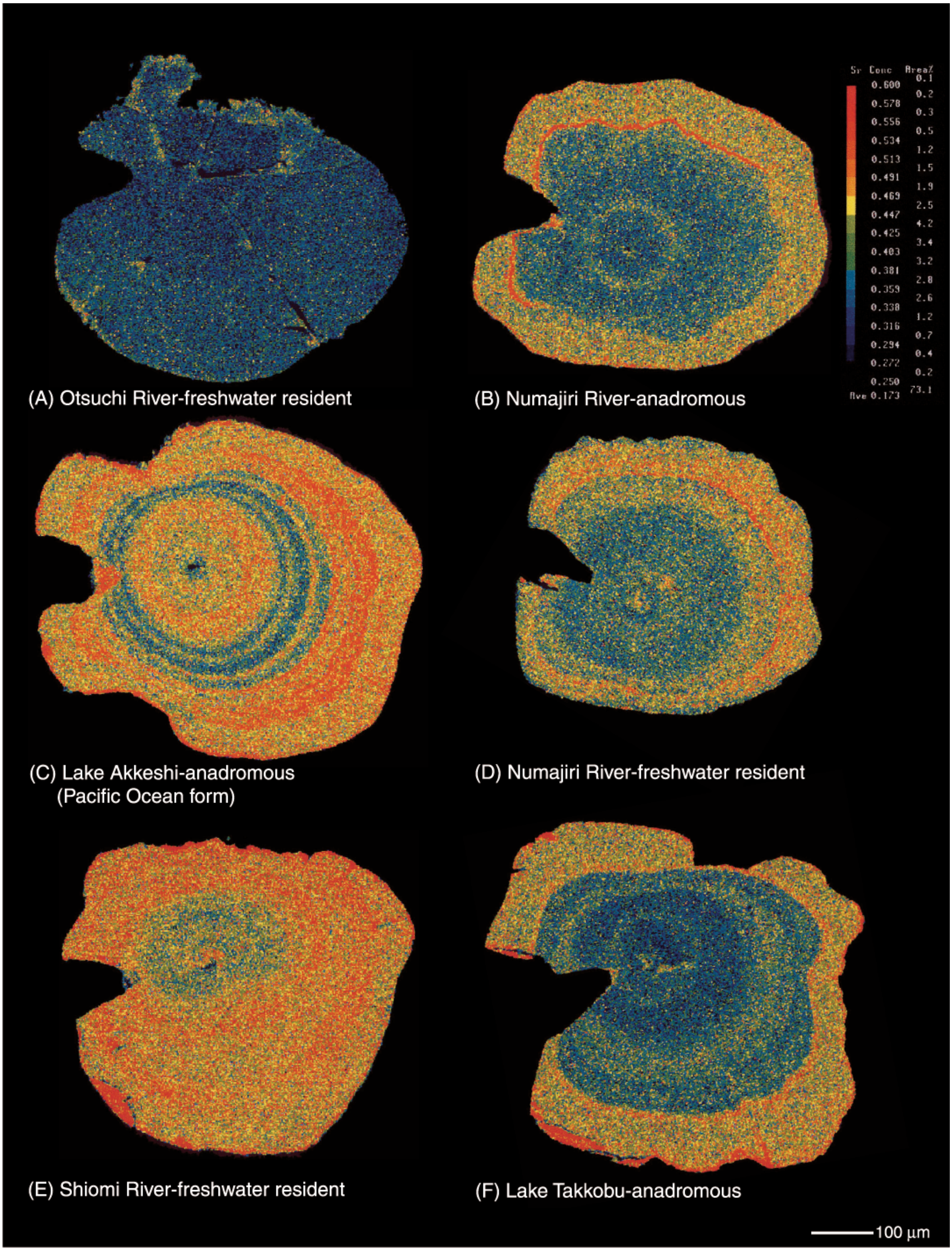

Figure 2. *Gasterosteus aculeatus*. (A–F) Two-dimensional imaging using X-ray electron microprobe analysis of the Sr concentration in the sagittal plane of sagittal otoliths of the threespine stickleback samples collected in Japanese coastal waters and rivers. The values corresponding to Sr concentration are represented by 17 colours from red (highest) to yellow to green to blue (lowest).

https://doi.org/10.1017/S0025315403007008h Published online by Cambridge University Press

phase from the point 250 μm to the edge (Phase-H) (mean ±SD; $4.9\times10^{-3}\pm0.49\times10^{-3}$, range; 4.1×10^{-3}–5.4×10^{-3}) (Mann–Whitney *U*-test, $P<0.0001$) in all specimens. Significant differences were observed between the Otsuchi River sample and the Numajiri River sample (ANOVA, $P<0.0001$), between the Otsuchi River sample and the Lake Takkobu sample (ANOVA, $P<0.005$), and no significant difference was observed between the Numajiri River sample and the Lake Takkobu sample (ANOVA, $P>0.5$). Furthermore, a significant difference was found between the freshwater resident type and the anadromous type from Lake Takkobu samples (ANOVA, $P<0.0001$), though differences between the migration types at other locations were not significant (ANOVA, $P>0.5$).

Life history transects of freshwater type from brackish water

Otolith Sr:Ca ratios in the Hyoutan Marsh samples of freshwater resident type (mean ±SD from the core to the edge: $5.1\times10^{-3}\pm0.18\times10^{-3}$) had a high Sr:Ca phase between the point 100 μm from the core to the otolith edge (Figure 3). Significant differences were found between the Sr:Ca ratios in the former phase from the core to the point 100 μm (Phase-L) (mean ±SD; $3.8\times10^{-3}\pm0.17\times10^{-3}$, range; 3.4×10^{-3}–4.2×10^{-3}) and the ratios in the latter phase from the point 100 μm to the edge (Phase-H) (mean ±SD; $5.8\times10^{-3}\pm0.25\times10^{-3}$, range; 5.4×10^{-3}–5.9×10^{-3}) (Mann–Whitney *U*-test, $P<0.0001$) in all specimens. Otolith Sr:Ca ratios in the Shiomi River of the freshwater resident type (mean ±SD from the core to the edge: $5.9\times10^{-3}\pm0.08\times10^{-3}$) had a high Sr:Ca phase between the point 100 μm from the core and the otolith edge. Significant differences were found between the Sr:Ca ratios in the former phase from the core to the point 100 μm (Phase-L) (mean ±SD; $4.2\times10^{-3}\pm0.12\times10^{-3}$, range; 3.2×10^{-3}–4.7×10^{-3}) and the ratios in the latter phase from the point 100 μm to the edge (Phase-H) (mean ±SD; $6.6\times10^{-3}\pm0.16\times10^{-3}$, range; 6.3×10^{-3}–6.9×10^{-3}) (Mann–Whitney *U*-test, $P<0.0001$) in all specimens.

Life history transects of anadromous type from brackish water

Otolith Sr:Ca ratios in both Pacific Ocean and Japan Sea forms from Lake Akkeshi showed relatively high Sr:Ca ratios from the core to the edge; $5.3\times10^{-3}\pm0.38\times10^{-3}$ (mean ±SD) and $6.3\times10^{-3}\pm0.87\times10^{-3}$, respectively (Figure 3). In the Hyoutan Marsh of the anadromous type, these samples were divided into the following two patterns. The characteristics of the first pattern showed frequent transition among three salinity habitats (two of five specimens). These specimens were significantly divided into three corresponding phases (ANOVA, $P<0.0001$), with higher values 5.9×10^{-3}–8.1×10^{-3} between the point 150 and 260 μm from the core, decreasing to the otolith edge. The characteristics of the second pattern showed changes in Sr:Ca ratios that indicated a single movement from one salinity habitat to another (the remaining three of the five specimens).

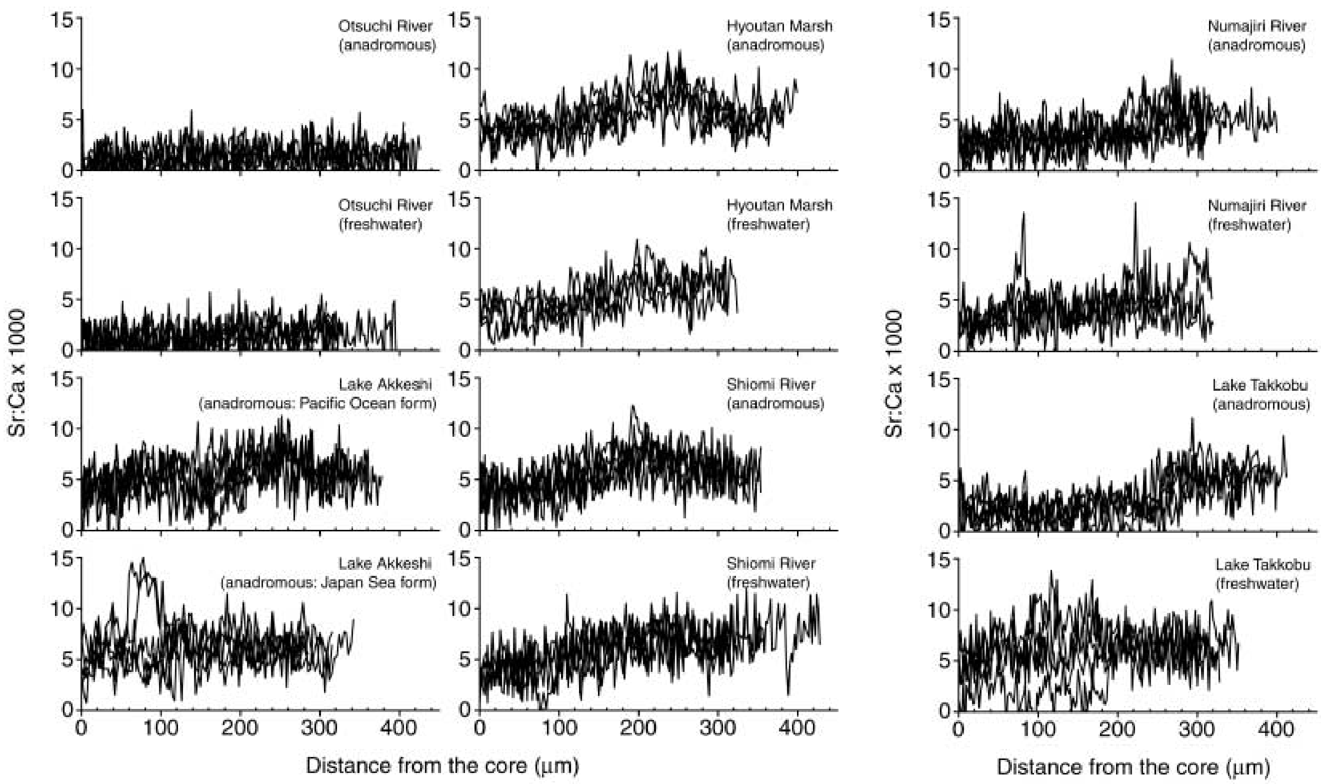

Figure 3. *Gasterosteus aculeatus*. Typical changes in otolith Sr:Ca ratio along line transects from the core (0 μm) to the edge in the saggital plane of sagittal otoliths of specimens collected at various localities. The sampling location is shown, as is the type of migratory history of each specimen, classified based on morphology. Life history analyses based on otolith Sr:Ca ratios for all specimens in the study are shown.

These specimens showed a temporary increase in Sr:Ca ratio between the point 150 μm from the core (Phase-L) (mean $\pm$SD; $3.8\times10^{-3} \pm 0.17\times10^{-3}$, range; 3.4×10^{-3} -4.2×10^{-3}) and from the point 150 μm to the edge (Phase H) (mean $\pm$SD; $3.8\times10^{-3} \pm 0.17\times10^{-3}$, range; 3.4×10^{-3}–4.2×10^{-3}). Otolith Sr:Ca ratios in the Shiomi River samples of the anadromous type (mean $\pm$SD from the core to the edge: $5.6\times10^{-3} \pm 0.22\times10^{-3}$) had a high Sr:Ca phase between the point 100 μm from the core and the otolith edge. A significant difference was found between the Sr:Ca ratios in the former phase from the core to the point 100 μm (Phase-L) (mean $\pm$SD; 4.1 $\pm$0.19, range; 3.6×10^{-3}–4.4×10^{-3}) and the ratios in the latter phase from the point 100 μm to the edge (Phase-H) (mean $\pm$SD; $6.2\times10^{-3} \pm 0.15\times10^{-3}$, range; 6.1×10^{-3} -6.6×10^{-3}) (Mann–Whitney U-test, $P<0.0001$) in all specimens.

Sr:Ca ratios in sympatric pairs of anadromous and freshwater sticklebacks

Otolith Sr:Ca ratios in sympatric pairs of anadromous and freshwater sticklebacks in each habitat showed a significant difference between those types in Lake Takkobu (Mann–Whitney U-test, $P<0.0001$), and no significant difference between those types in other locations (Mann–Whitney U-test, $P>0.5$).

DISCUSSION

This study confirmed that otolith Sr:Ca ratios in the threespine stickleback reflected changes in ambient environmental conditions and could indicate habitat transitions. Strontium contents or Sr:Ca in the otoliths were significantly different between fish sampled from brackish water and those sampled from freshwater environments, regardless of the migration patterns estimated from morphology. All fish collected from brackish water had a transition point (TP) from Phase-L to Phase-H in the Sr:Ca ratio and thereafter, maintaining constantly high Sr:Ca ratios toward the edge, and some of them had consistently high Sr:Ca values from the core to the otolith edge. In contrast, all fish collected from the Otsuchi River did not have a TP, and maintained constantly low Sr:Ca ratios from the core to the edge. The Otsuchi River sample seems to be a standard freshwater resident type, which may be explained by the fact that the riverhead is landlocked; the downstream has a weir, by which the upstream migration of anadromous sticklebacks is completely prevented. Accordingly, both anadromous and freshwater types might be unable to migrate downstream after hatching. Therefore, the difference in Sr:Ca ratio might be the result of an otolith from an individual stickleback that underwent brackish water or freshwater life history phases. Salinity was quite different between coastal brackish waters and the inland riverhead. Strontium contents in the otolith of teleost fish were positively correlated to ambient salinity (Secor et al., 1995; Tzeng, 1996). Therefore, the difference in Sr content in otoliths of the sticklebacks between brackish and freshwater environments was probably due to the effects of ambient salinity. A similar phenomenon has been observed in various diadromous fish: e.g. the salmonid, *Oncorhynchus masou* (Arai & Tsukamoto, 1998); the striped bass, *Morone saxatillis* (Secor et al., 1995); and freshwater eels, *Anguilla anguilla* (Tzeng et al., 1997, 2000) and *A. japonica* (Tsukamoto & Arai, 2001).

Anadromous sticklebacks migrate from the sea into freshwater areas in order to breed in the late spring and early summer, whereas freshwater sticklebacks permanently reside in freshwater areas. All fish collected from freshwater, except those from the Otsuchi River, also had a TP. Those freshwater resident type fish might have experienced brackish water or a seawater environment, because their sampling locations are influenced by the tides or may have contact with the estuarine or sea via a small intermittent stream (Figure 1). Therefore, in the present study, those specimens were discriminated as anadromous type based on otolith microchemistry fingerprinting (Table 1). For higher accuracy, however, mark-recapture studies will be needed to determine the precise correspondence between fish movement and Sr:Ca ratios in their otoliths.

Freshwater sticklebacks have been derived from anadromous sticklebacks (Bell & Foster 1994). These findings strongly suggest that the threespine stickleback has a flexible migration strategy with a high degree of behavioural plasticity and an ability to utilize the full range of salinity in its life history.

Most fish collected in freshwater did not show a clear Phase-L (low Sr:Ca ratio), though a slight decrease was detected in several specimens. This might be due to timing; the fish may have recently immigrated into the river and lake in order to breed. Accordingly, low freshwater Sr:Ca ratios would not be incorporated into the edge of otolith. Another factor possibly influencing relatively high otolith Sr:Ca ratios might be ontogenic variations in Sr:Ca ratios in stickleback related to gonad maturation and spawning. Booke (1964) and Elliot et al. (1979) suggested that during the period leading up to spawning, anadromous salmonids were characterized by a gradual increase in levels of both plasma Ca and plasma protein, both of which are involved in the synthesis of gonadal tissue. Kalish (1989) suggested that seasonal variations in Sr:Ca ratios in the 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 this period. However, further studies on the growth patterns of the otolith and on the Sr metabolism in otoliths are required to understand the minute ontogenic changes in otolith Sr:Ca ratios.

Fish migration is generally explained by a difference in food abundance between marine and freshwater habitats (Gross, 1987). Juvenile anadromous salmon utilize freshwater habitats at high latitudes with low productivity, and migrate to higher productivity habitats in the ocean for growth before returning to freshwater for breeding. Similarly, both anadromous and freshwater types of sticklebacks that inhabit high latitudes might frequently migrate downstream into brackish water and seawater habitats of higher productivity for growth before returning to the breeding grounds. A latitudinal cline in which marine-dependent sticklebacks would occur more frequently at higher latitudes, where the productivity of the freshwater habitat is lower compared to the ocean, might be predicted. Analysis of the otolith Sr:Ca ratio needs to be made on various latitudinal distributions, and mean otolith Sr:Ca ratios as an index of the environmental life history should be compared among habitats.

We are grateful to Messrs T. Kitamura, M. Kume, T. Noda, and S. Takeyama, and the staff of Otsuchi Town Office, for their assistance in sampling. This work was supported in part by Grant-in-Aid no. 13760138 from the Ministry of Education, Science, Sports and Culture, Japan.

REFERENCES

Arai, T., 2002. Migratory history of fishes: present status and perspectives of the analytical methods. *Japanese Journal of Ichthyology*, **49**, 1–23. [In Japanese with English abstract.]

Arai, T., Marui, M., Miller, M.J., Otake, T. & Tsukamoto, K., 2002. Growth history and inshore migration of the tropical eel, *Anguilla marmorata* in the Pacific. *Marine Biology*, **140**, 309–316.

Arai, T. & Miyazaki, N., 2001. Use of otolith microchemistry to estimate the migratory history of the Russian sturgeon, *Acipenser guldenstadti*. *Journal of the Marine Biological Association of the United Kingdom*, **81**, 709–710.

Arai, T., Otake, T. & Tsukamoto, K., 1997. Drastic changes in otolith microstructure and microchemistry accompanying the onset of metamorphosis in the Japanese eel, *Anguilla japonica*. *Marine Ecology Progress Series*, **161**, 17–22.

Arai, T. & Tsukamoto, K., 1998. Application of otolith Sr:Ca ratios to estimate the migratory history of masu salmon, *Oncorhynchus masou*. *Ichthyological Research*, **45**, 309–313.

Bell, M.A. & Foster, S.A., 1994. *The evolutionary biology of the threespine stickleback*. Oxford: Oxford University Press.

Booke, H.E., 1964. Blood serum protein and calcium levels in yearling brook trout. *The Progressive Fish-Culturist*, **26**, 107–110.

Elliot, J.A., Bromage, K.N. & Whitehead, C., 1979. Effects of oestradiol 17β on serum calcium and vitellogenin levels in the rainbow trout. *Journal of Endocrinology*, **83**, 54–55.

Gross, M.R., 1987. Evolution of diadromy in fishes. *American Fisheries Society Symposium*, **1**, 14–25.

Hagen, D.W., 1967. Isolating mechanisms in the threespine sticklebacks (*Gasterosteus*). *Journal of the Fisheries Research Board of Canada*, **24**, 1637–1692.

Hagen, D.W. & Moodie, G.E.E., 1982. Polymorphism for plate morphs in *Gasterosteus aculeatus* on the East coast of Canada and an hypothesis for their global distribution. *Canadian Journal of Zoology*, **60**, 1032–1042.

Heuts, M., 1947. Experimental studies on adaptive evolution in *Gasterosteus aculeatus* L. *Evolution*, **1**, 89–102.

Higuchi, M. & Goto, A., 1996. Genetic evidence supporting the existence of two distinct species in the genus *Gasterosteus* around Japan. *Environmental Biology of Fishes*, **47**, 1–16.

Higuchi, M., Goto, A. & Yamazaki, F., 1996. Genetic structure of threespine stickleback, *Gasterosteus aculeatus*, in Lake Harutori, Japan, with reference to coexisting anadromous and freshwater forms. *Ichthyological Research*, **43**, 349–358.

Ikeda, K., 1933. The distribution and the morphological variations of the sticklebacks in Japan. *Zoological Magazine*, **45**, 141–173. [In Japanese with English abstract.]

Kalish, J.M., 1989. Otolith microchemistry: validation of the effects of physiology, age and environment on otolith composition. *Journal of Experimental Marine Biology and Ecology*, **132**, 151–178.

Larson, G., 1976. Social behaviour and feeding ability of two phenotypes of *Gasterosteus aculeatus* in relation to their spatial and trophic segregation in a temperate lake. *Canadian Journal of Zoology*, **54**, 107–121.

Mcphail, J.D., 1984. Ecology and evolution of sympatric sticklebacks (*Gasterosteus*): morphological and genetic evidence for a species pair in Enos Lake, British Columbia. *Canadian Journal of Zoology*, **62**, 1402–1408.

McPhail, J.D., 1993. Speciation and the evolution of reproductive isolation in the sticklebacks (*Gasterosteus*) of southwestern British Columbia. In *The evolutionary biology of the threespine stickleback* (ed. M.A. Bell and S.A. Foster), pp. 400–437. Oxford: Oxford University Press.

Moodie, G.E.E., 1972. Morphology, life history, and ecology of an unusual stickleback *Gasterosteus* populations of the Queen Charlotte Islands. *Systematic Zoology*, **25**, 49–61.

Mori, S., 1990. Two morphological types in the reproductive stock of three-spined sticklebacks, *Gasterosteus aculeatus*, in Lake Harutori, Hokkaido Island. *Environmental Biology of Fishes*, **27**, 21–31.

Munzing, J., 1963. The evolution of variation and distributional patterns in European populations of the three-spined stickleback, *Gasterosteus aculeatus*. *Evolution*, **17**, 320–332.

Reimchen, T.E., Stinson, E.M. & Nelson, J.S., 1985. Multivariate differentiation of parapatric and allopatric populations of threespine stickleback in the Sangan River watershed, Queen Charlotte Islands. *Canadian Journal of Zoology*, **63**, 2944–2951.

Secor, D.H., Henderson-Arzapalo, A. & Piccoli, P.M., 1995. Can otolith microchemistry chart patterns of migration and habitat utilization in anadromous fishes? *Journal of Experimental Marine Biology and Ecology*, **192**, 15–33.

Sokal, R.R. & Rohlf, F.J., 1969. *Biometry*. San Francisco: W.H. Freeman.

Tsukamoto, K. & Arai, T., 2001. Facultative catadromy of the eel, *Anguilla japonica*, between freshwater and seawater habitats. *Marine Ecology Progress Series*, **220**, 265–276.

Tzeng, W.N., 1996. Effects of salinity and ontogenetic movements on strontium:calcium ratios in the otoliths of the Japanese eel, *Anguilla japonica* Temminck and Schlegel. *Journal of Experimental Marine Biology and Ecology*, **199**, 111–122.

Tzeng, W.N., Severin, K.P. & Wickström, H., 1997. Use of otolith microchemistry to investigate the environmental history of European eel *Anguilla anguilla*. *Marine Ecology Progress Series*, **149**, 73–81.

Tzeng, W.N., Wang, C.H., Wickström, H. & Reizenstein, M., 2000. Occurrence of the semi-catadromous European eel *Anguilla anguilla* in the Baltic Sea. *Marine Biology*, **137**, 93–98.

Wootton, R.J. 1976. *The biology of the sticklebacks*. London: Academic Press.

Wootton, R.J. 1984. *A functional biology of sticklebacks*. London: Croom Helm.

Zuiganov, V.V., Golovatjuk, G.J., Savvaitova, K.A. & Bugayev, V.F., 1987. Genetically isolated sympatric forms of threespine stickleback, *Gasterosteus aculeatus*, in Lake Azabachije (Kamchatka-peninsula, USSR). *Environmental Biology of Fishes*, **18**, 241–247.

Submitted 5 June 2002. Accepted 16 October 2002.