



Migration diversity of the freshwater goby *Rhinogobius* sp. BI, as revealed by otolith Sr:Ca ratios

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ABSTRACT: The migratory histories of the endangered freshwater goby *Rhinogobius* sp. BI (Bonin Island type) collected from 6 stations on Chichijima Island, part of the Bonin Islands chain in Japan, were studied by examining the environmental signatures of strontium and calcium concentrations in otoliths using electron probe microanalyses. The Sr:Ca ratios in the otoliths showed when the gobies had experienced various salinity environments such as freshwater, brackish water, and seawater. The otolith Sr:Ca ratios of almost all *Rhinogobius* sp. BI from rivers connected to the sea reflected the typical amphidromous life history. In contrast, fish collected upriver from a dam, disconnected from the downstream area, utilized freshwater habitats only, reflecting an artificially landlocked life history. Further flexible migration patterns were found in fish from areas downstream of the aforementioned dam (in a river connected to the sea), indicating that they utilized freshwater, brackish water, and seawater in their larval and adult life period. The present study clearly suggests that the migratory histories of *Rhinogobius* sp. BI are highly variable within habitats and that they have flexible migratory patterns, allowing them to utilize the full range of salinity during their life history.

KEY WORDS: *Rhinogobius* · Endangered species · Bonin Islands · Otolith Sr:Ca ratios · Flexible migration

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INTRODUCTION

Freshwater gobies, genus *Rhinogobius* (Pisces: Gobiidae), are widely distributed in East Asian countries, as far north as the Russian Amur River system and as far south as Mindanao Island, Philippines (Aonuma et al. 1998, Senou et al. 2004). In Japan, these fishes are also distributed widely from Hokkaido Island to the Ryukyu Archipelago, and more than 13 species have been reported; however, most of them remain taxonomically uncertain, although reproductive isolation among Japanese *Rhinogobius* species has been confirmed by both partner selection experiments and isozyme analyses (Mizuno et al. 2001, Senou et al. 2004). Therefore, their scientific name has not yet been decided and only common names have been given on

the basis of the colour of macules and morphological features (Mizuno et al. 2001).

The Bonin (Ogasawara) Islands consist of the small oceanic islands, Chichijima, Anijima, Hahajima, and the Mukojima Islands, located in the northwestern Pacific about 1000 km south of metropolitan Tokyo, Japan. These islands have never been connected by land to the continent since the time of their origin. *Rhinogobius* sp. BI (Bonin Island type) is an endemic freshwater goby distributed in the Chichijima, Anijima, and Hahajima Islands (Suzuki 1992, 2003, Akihito et al. 2000, Mizuno et al. 2001, Yokoi & Hosoya 2003, 2006). This species is designated an endangered species (Ministry of the Environment of Japan 2003) because of habitat disappearance caused by artificial river improvement, deforestation, and topsoil erosion,

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which continues even now (Suzuki 2003). Therefore, it is essential to take practical measures to protect the natural populations of this goby (Iwata 1997, Yokoi & Hosoya 2003, 2006). Ecological information about this goby is vital to implementing effective protective measures. In general, the life history patterns of the *Rhinogobius* species have been divided into 3 categories: amphidromous, fluvial and lacustrine (Mizuno et al. 2001). However, the migration history of the *Rhinogobius* sp. BI is still largely unknown.

Recent chemical analytical techniques have enabled the identification of life history events in individual fishes by detecting trace elements in the microstructure of their otoliths (Campana 1999, Arai 2002). Strontium (Sr) incorporation in fish otoliths is of special interest because of its potential utility as an indicator of past environmental (temperature and salinity) and physiological (ontogenetic change) conditions (Campana 1999). The deposition of Sr and calcium (Ca) in fish otoliths during their growth varies between freshwater and marine habitats (Campana 1999). Sr concentrations are 1 to 2 orders of magnitude higher in seawater than in freshwater (Campana 1999). Factors other than salinity have been shown to affect Sr deposition rates, including temperature, growth, developmental or reproductive stage, genetics, and diet (Campana 1999). These factors, unlike the salin-

ity effect, have not been systematically linked to the fluctuation of otolith Sr:Ca ratios. Although the values of the Sr:Ca ratios in otoliths are highly variable among species, ambient salinity is positively related to the Sr:Ca ratios of almost all fish species studied (Secor & Rooker 2000). Recently, Tsunagawa & Arai (2008) have found that otolith Sr:Ca ratios were positively related to ambient water salinity in *Rhinogobius* species. Therefore, this technique can be applied to reveal the migratory patterns of *Rhinogobius* sp. BI.

The objectives of the present study were to provide life history information by examining Sr:Ca ratios in the otoliths of the endangered freshwater goby *Rhinogobius* sp. BI. The results provide a basis for a discussion of the life history of this endangered species as well as information which should prove useful for attempts to protect them.

MATERIALS AND METHODS

Fish. The samples of *Rhinogobius* sp. BI used in the present study were collected from 6 stations in the Yatsuse River and the Sakaiura River on Chichijima Island, one of the Bonin Islands, Japan, from June 6 to 9, 2008, using dip nets (Fig. 1, Table 1). Sampling

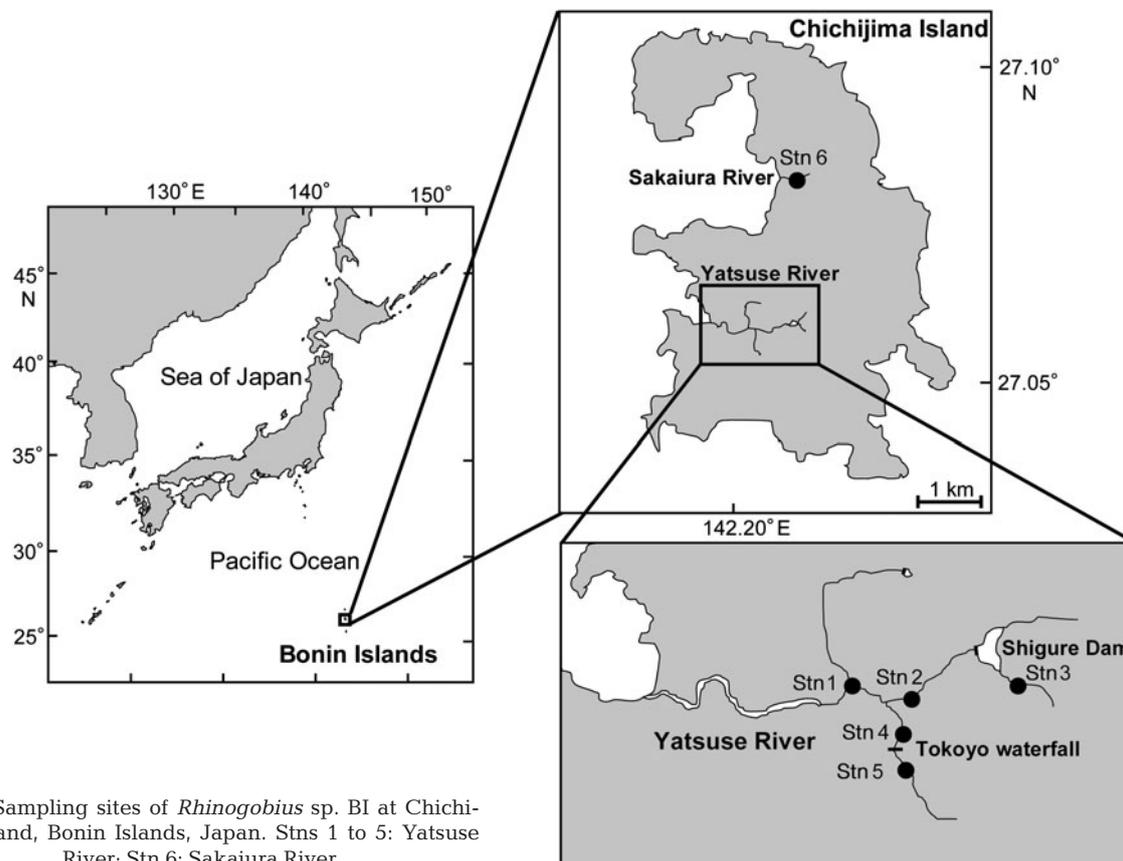


Fig. 1. Sampling sites of *Rhinogobius* sp. BI at Chichijima Island, Bonin Islands, Japan. Stns 1 to 5: Yatsuse River; Stn 6: Sakaiura River

Table 1. *Rhinogobius* sp. BI. Specimens used for otolith microchemistry analyses

Stn	Sampling location	Sampling date	No. of fish examined	Standard length (mm)	Migration pattern estimated from Sr:Ca ratios
1	Yatsuse River	7 June 2008	10	27.98–64.43	Typical amphidromous Long marine phase amphidromous Multiple inter-habitat amphidromous Short brackish water phase amphidromous
2	Yatsuse River	6 June 2008	10	27.59–44.57	Typical amphidromous Long marine phase amphidromous Artificially or naturally landlocked
3	Yatsuse River (upriver of Shigure Dam)	6 June 2008	5	29.21–42.08	Artificially landlocked
4	Yatsuse River (basin of Tokoyo waterfall)	7 June 2008	10	30.60–42.35	Typical amphidromous
5	Yatsuse River (upstream of Tokoyo waterfall)	7 June 2008	5	36.24–44.71	Typical amphidromous Short brackish water phase amphidromous
6	Sakaiura River	9 June 2008	5	17.53–46.58	Typical amphidromous

Stns 1 and 2 are in the main river in the Yatsuse River. There are no obstructions preventing fish from migrating either downstream to the sea or upstream from the sea via the river. Stn 3 is upriver from the Shigure Dam in the Yatsuse River. The dam wall is about 24 m tall and completely divides the river, thus preventing upstream migration, although it is thought that downstream migration is possible. Stns 4 and 5 are a basin and a section of river above the Tokoyo waterfall (respectively) in a tributary of the Yatsuse River. The height of the waterfall is about 20 m; therefore, upstream migration to Stn 5 is thought to be impossible, although migration from both stations to the sea is possible. Stn 6 is located in the Sakaiura River, which is connected to the sea without obstructions to upstream or downstream migration. All samples were collected from freshwater areas, with no influence from the rising tide and showed 0‰ salinity. After collection, all specimens were anesthetized, and thereafter fixed immediately by freezing. A total of 45 specimens were used for otolith microchemical analyses (Table 1).

Otolith preparation and otolith X-ray microprobe analyses. Sagittal otoliths were extracted from each fish, embedded in epoxy resin (Struers, Epofix), and mounted on glass slides after measuring the standard length of each fish. The otoliths were then ground to expose the core, using a grinding machine equipped with a diamond cup wheel (Struers, Discoplan-TS), and polished further with oxide polishing suspension on an automated polishing wheel (Struers, PdM-Force-20). 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 (Pt-Pd) coated by a high vacuum evaporator. Otoliths from all specimens were used for

life history transect analyses 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), as described in Arai et al. (1997). Wollastonite (CaSiO₃) and Tausonite (SrTiO₃) 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 5 μm in diameter, with measurements spaced at 5 μm intervals.

X-ray intensity maps of Sr and Ca were made using JEOL JXA-8900R as described by Arai et al. (2004) and Goto & Arai (2003). The beam current was 0.5 μA, counting time was 0.1 s and pixel size was 2×2 μm. The electron beam was focused on a point 1 μm in diameter, and the other analytical conditions followed those for the life history transect analyses.

Statistical analyses. Statistical analyses were performed using Systat (Systat Software). Differences among data of mean Sr:Ca ratios in otolith were tested by repeated measures ANOVA and *t*-test, and overall differences between groups were assessed using its methods.

RESULTS

Otolith Sr distribution

X-ray intensity maps showed that the otolith Sr concentrations were remarkably variable among the *Rhinogobius* sp. BI examined (Fig. 2). Thirty-two samples from Stns 1 (n = 6), 2 (n = 7), 4 (n = 10), and 5 (n = 4) in the Yatsuse River and Stn 6 (n = 5) in the Sakaiura River showed up as wide spaces of greenish

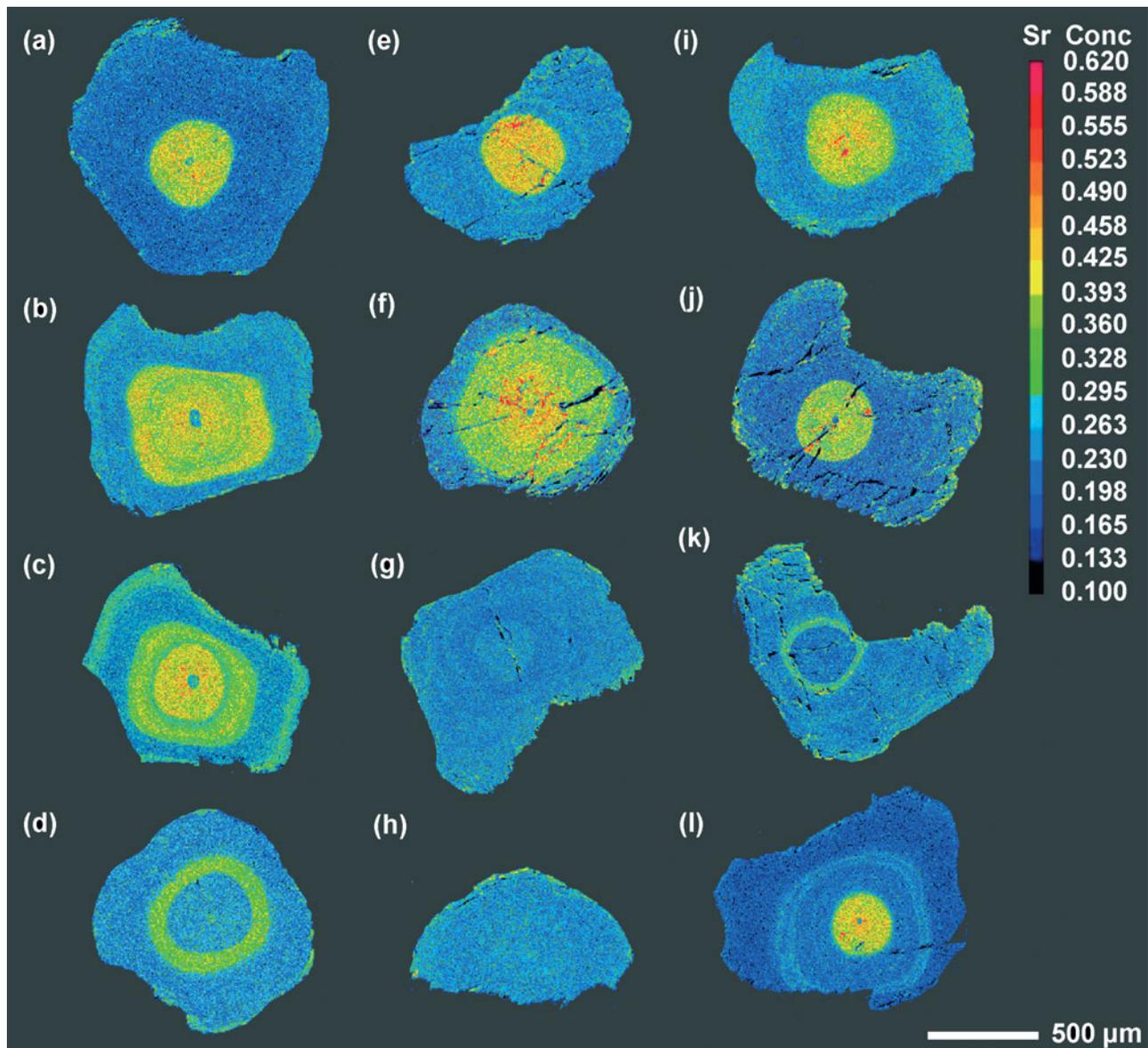


Fig. 2. *Rhinogobius* sp. BL. Two-dimensional imaging using X-ray electron microprobe analyses of the Sr concentration in the sagittal plane of the sagittal otolith samples from (a–d) Stn 1, (e–g) Stn 2, (h) Stn 3, (i) Stn 4, (j, k) Stn 5, and (l) Stn 6. See Fig. 1 & Table 1 for station locations. Values corresponding to Sr concentration are represented by 16 colours from red (highest) to yellow to green to black (lowest)

yellow, yellow and orange (high Sr) surrounded by blue and light blue (low Sr), except for the lowest otolith centre (Fig. 2a,e,i,j,l). A similar pattern of Sr concentration was found in 3 samples from Stns 1 ($n = 2$) and 2 ($n = 1$), indicating high Sr values around the core of the otoliths. However, the high-Sr regions were larger than those of the samples described above (Fig. 2b,f). One sample from Stn 1 featured concentric rings of yellow or orange (high Sr) and yellowish-green or green (medium Sr) surrounding the blue (low Sr) area in the otolith core. These high, medium and low Sr regions were surrounded by a wide space of blue

and light blue (low Sr) and a narrow space of green (medium Sr) (Fig. 2c). Five samples collected from Stn 3 showed a uniformly low concentration all over the otolith (blue and light blue; Fig. 2h). A similar consistently low concentration throughout the otolith was also found in 2 samples from Stn 2 (Fig. 2g). Two samples from Stns 1 ($n = 1$) and 5 ($n = 1$) featured a wide band of blue and light blue (low Sr) in the otolith core region surrounded by a narrow band of yellowish-green and green (medium Sr). Moreover, these regions were surrounded by blue and light blue areas (Fig. 2d,k).

Life history transects

The Sr:Ca ratios measured along a transect from the centre to the otolith edge of *Rhinogobius* sp. BI of all samples ($n = 5$) collected at Stn 3 showed consistently low ratios of $2.18 \times 10^{-3} \pm 0.95 \times 10^{-3}$ (mean \pm SD) (Fig. 3h). The same Sr:Ca ratio fluctuations were found in the 2 samples from Stn 2, which were uniformly low throughout the otolith ($2.11 \times 10^{-3} \pm 0.99 \times 10^{-3}$) (Fig. 3g).

In contrast, the otolith Sr:Ca ratios of 32 samples from Stns 1 ($n = 6$), 2 ($n = 7$), 4 ($n = 10$) and 5 ($n = 4$) in the Yatsuse River (Fig. 3a,e,i,j, respectively) and Stn 6 ($n = 5$) (Fig. 3l) in the Sakaiura River had low Sr:Ca ratios in 2 regions, one from the core to between 5 and 30 μm (with mean values ranging from 2.38×10^{-3} to 3.27×10^{-3}) and the other from between 150 and 245 μm to the edge (with mean values ranging from 1.17×10^{-3} to 2.55×10^{-3}). The Sr:Ca ratios from these samples were significantly higher in the wide area between the other regions (with mean values ranging from 5.92×10^{-3} to 6.70×10^{-3}) (repeated measures ANOVA, $p < 0.0001$ for both regions).

A similar fluctuation of Sr:Ca ratios was found in 3 samples collected from Stn 1 ($n = 2$) (Fig. 3b) and Stn 2 ($n = 1$) (Fig. 3f). The samples had low Sr:Ca ratios in 2 regions, from the core to between 15 and 30 μm (with mean values ranging from 2.16×10^{-3} to 3.50×10^{-3}) and from between 310 and 350 μm to the edge (with mean values ranging from 1.91×10^{-3} to 2.35×10^{-3}). Sr:Ca ratios in these samples were significantly higher in the wide area between the other regions (with mean values ranging from 5.79×10^{-3} to 6.25×10^{-3}) (repeated measures ANOVA, $p < 0.0005$ for the former region, $p < 0.0001$ for the latter region). The high Sr:Ca region near the otolith core of these samples was wider than that of the samples described in the previous paragraph.

One sample from Stn 1 (Fig. 3d) showed low Sr:Ca ratios in 2 regions, from the core to 175 μm ($2.35 \times 10^{-3} \pm 0.90 \times 10^{-3}$) and from 280 μm to the edge ($1.86 \times 10^{-3} \pm 0.87 \times 10^{-3}$), with significantly higher Sr:Ca ratios in the narrow area between the other regions ($4.65 \times 10^{-3} \pm 1.45 \times 10^{-3}$) (repeated measures ANOVA, $p < 0.0001$ for both regions). The same patterns were found in 1 sample from Stn 5 (Fig. 3k), with low Sr:Ca ratios from the core to 165 μm ($2.24 \times 10^{-3} \pm 0.94 \times 10^{-3}$) and from 265 μm to the edge ($2.77 \times 10^{-3} \pm 1.01 \times 10^{-3}$), and significantly higher ratios in the narrow area between the other regions ($3.98 \times 10^{-3} \pm 1.30 \times 10^{-3}$) (repeated measures ANOVA, $p < 0.0001$ for both regions).

One sample collected from Stn 1 (Fig. 3c) showed low Sr:Ca ratios in 2 regions, from the core to 20 μm ($2.74 \times 10^{-3} \pm 0.78 \times 10^{-3}$) and from 340 to 480 μm ($2.21 \times 10^{-3} \pm 0.84 \times 10^{-3}$). High Sr:Ca ratios were found

in the wide area around the otolith core region and the otolith edge. The former high ratio area was divided into 2 different values, from 25 to 170 μm ($7.31 \times 10^{-3} \pm 1.78 \times 10^{-3}$) and from 175 to 335 μm ($5.05 \times 10^{-3} \pm 1.28 \times 10^{-3}$). The latter high Sr:Ca ratio area in the otolith edge showed $3.61 \times 10^{-3} \pm 0.95 \times 10^{-3}$ from 485 μm to the edge. The Sr:Ca ratios of these 3 high value regions and the 2 low value regions were compared with all contrasts to estimate the environmental habitat use. The 3 high value regions are different from each combination (t -test, $p < 0.0001$). Moreover, these phases are remarkably higher than the 2 low ratio phases (t -test, $p < 0.0001$ to 0.05). Based on difference of the Sr:Ca ratios in these results, habitat environment were distinguished as follows: freshwater (Sr:Ca ratios $< 3.5 \times 10^{-3}$), brackish water (Sr:Ca ratios 3.5 to 5.5×10^{-3}) and seawater (Sr:Ca ratios $> 5.5 \times 10^{-3}$).

DISCUSSION

In the present study, the Sr contents or Sr:Ca ratios from the life history transects taken from otoliths were remarkably different among *Rhinogobius* sp. BI from each site. The Sr contents in the otoliths of teleost fishes were positively correlated with ambient salinity and the deposition of Sr and Ca in the fish otoliths during growth varied among freshwater, brackish water, and seawater environments (Secor et al. 1995, Arai et al. 2004). The Sr:Ca ratios of all fish collected from above the dam were consistently low, while nearly all fish from rivers connected to the sea had areas with high Sr:Ca ratios around their otolith cores, indicating that these fish moved downstream (toward the sea) over the course of their lives. Thus, the difference of Sr content in the *Rhinogobius* sp. BI otoliths was probably due to salinity effects. Besides salinity, temperature may also affect Sr:Ca ratios in fish otoliths (Kalish 1989, Secor et al. 1995, Arai 2002). Although the effect of temperature cannot be ruled out in the present study, there is little evidence that temperature was a major factor, as all specimens from each site had been subjected to similar temperature changes. In the Yatsuse River, the mean water temperature is about 26°C in the summer and about 18°C in the winter. However, no seasonal temperature effects were found in otoliths, probably because the fluctuations of the Sr:Ca ratios in fishes collected upriver from the dam showed constantly low values throughout their whole life. Therefore, the most important factor influencing the Sr content or Sr:Ca ratios in the otoliths of *Rhinogobius* sp. BI is probably ambient salinity changes.

Most specimens collected from below the dam in the Yatsuse River, which was connected to the sea, showed a typical amphidromous life history. This

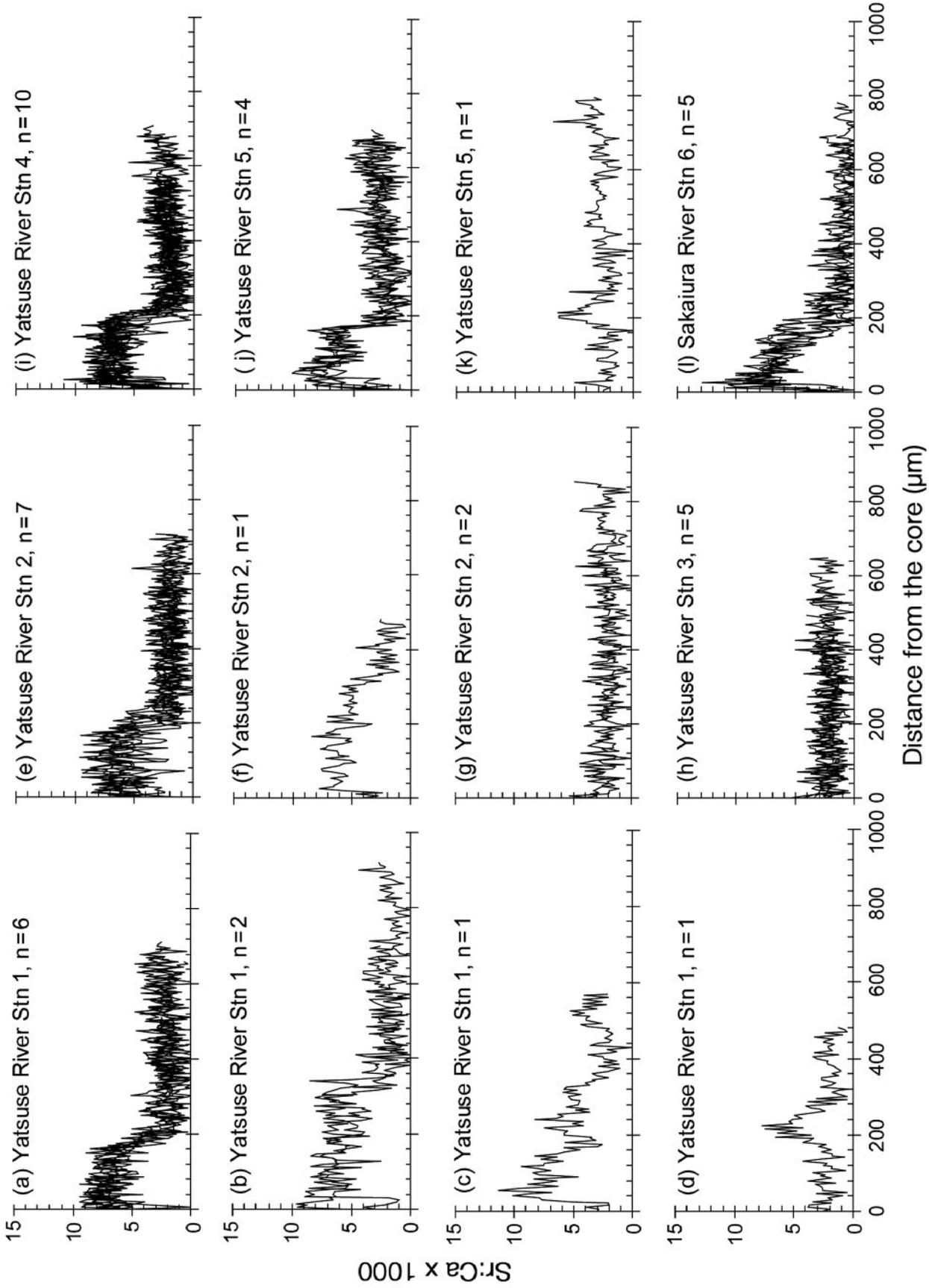


Fig. 3. *Rhinogobius* sp. Bl. Changes in otolith Sr:Ca ratio along line transects from the core to the edge of the sagittal otolith samples from (a–d) Stn 1, (e–g) Stn 2, (h) Stn 3, (i) Stn 4, (j,k) Stn 5, and (l) Stn 6. See Fig. 1 & Table 1 for station locations

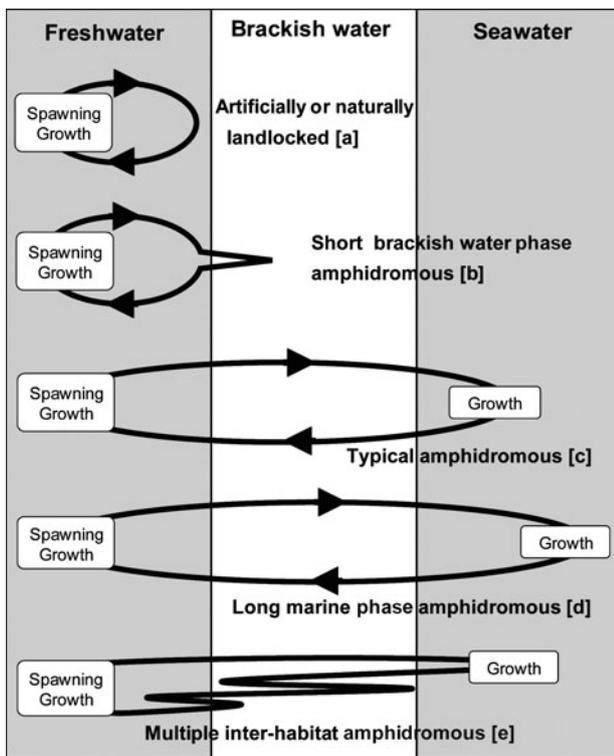


Fig. 4. *Rhinogobius* sp. BI. Life cycle, with special reference to migratory types as revealed by the otolith Sr:Ca ratio analyses along the life history transect. (a) Artificially or naturally landlocked type, (b) short brackish water phase amphidromous type, (c) typical amphidromous type, (d) long marine phase amphidromous type, (e) multiple inter-habitat amphidromous type

pattern was also found in all specimens from the Sakaiura River. As is common in freshwater goby with amphidromous life cycles (McDowall 1992, 1997), these fish drift to the sea just after hatching in a freshwater environment and spend their early lives in the sea for growth, thereafter ascending to and remaining in a freshwater environment (Figs. 2a,e,i,j,l, 3a,e,i,j,l & 4c). This migration pattern is thought to be the major pattern in *Rhinogobius* sp. BI and is similar to other amphidromous *Rhinogobius* species, such as *Rhinogobius* sp. CB (the cross-band type) and *Rhinogobius* sp. LD (the large dark type) inhabiting mainland Japan (Tsunagawa & Arai 2008).

Further, some specimens collected downstream of the dam in the Yatsuse River showed different amphidromous migration patterns and could be divided into 3 categories: (1) long marine phase amphidromous (Figs. 2b,f, 3b,f & 4d); (2) short brackish water phase amphidromous (Figs. 2d,k, 3d,k & 4b); (3) multiple inter-habitat amphidromous (movement among freshwater, brackish water, and seawater) (Figs. 2c, 3c & 4e). These migration patterns may be caused by specific

environmental conditions in the small oceanic islands, such as flooding after heavy rain, intermittent connection between rivers and sea, and habitat capacity.

In contrast, all samples collected from upriver of the Shigure Dam, an area disconnected both from the downstream area and the sea, indicated that these fish live in freshwater throughout their lives (Figs. 2h & 3h). Therefore, they are thought to have an artificially landlocked life history (Fig. 4a). This result suggests that an artificial obstacle, such as a dam wall, separating the upstream and downstream areas in rivers makes it more difficult for fish to ascend upstream than if only natural structures, such as waterfalls, were present because all fishes from upstream area of the Tokoyo waterfall exhibited an amphidromous life cycle. There have been similar findings for other amphidromous *Rhinogobius* species inhabiting Honshu and Shikoku Island in Japan (Tsunagawa & Arai 2008). Furthermore, similar migration patterns were also found in some specimens from below the dam (Figs. 2g & 3g). These fish appear to live in a freshwater environment during their whole lives after hatching either upstream or downstream from the dam, although it would be possible for them to move to a downstream area such as marine, brackish environment. Consequently, they are thought to be either an artificially or a naturally landlocked type (Fig. 4a). These results led us to conclude that *Rhinogobius* sp. BI has a polymorphic migration strategy, allowing it to adapt well to a given artificial or natural environment.

In the present study, the migration histories of the endangered freshwater goby *Rhinogobius* sp. BI are composed of 5 types: typical amphidromous, long marine phase amphidromous, short brackish water phase amphidromous, artificially or naturally landlocked, and multiple inter-habitat amphidromous types. Although the mechanism of this diverse migratory pattern is still unclear, it may be attributed to genetics or environmental adaptation as in other diadromous fishes (Nordeng 1983, Gross 1985). The Chichijima Island is an oceanic island, and its rivers are extremely small or short and surrounded by a mountainous landmass, so the number and quality of freshwater habitats is limited. Further, *Rhinogobius* sp. BI habitats are disappearing due to deforestation, topsoil erosion, and artificial river improvements (Suzuki 2003). In addition, deforestation may cause droughts during the dry season and freshets during the rainy season in the river habitat of this species. Thus, as a consequence of artificial environmental changes, *Rhinogobius* sp. BI might be forced to live in various environments such as freshwater, brackish water, and marine water areas. Further studies are needed to examine the factors affecting the apparently flexible pattern of migration of the species.

In this context, the detailed migratory histories of the *Rhinogobius* sp. BI in their entire range should be clarified using otolith microchemistry. *Rhinogobius* sp. BI is basically amphidromous but may become landlocked when seaward migration is obstructed. These results suggest that this species has a flexible life history strategy which enables them to utilize the full range of salinity in their given environments.

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