

# Migratory history of wild and released ayu (*Plecoglossus altivelis*) in the Kurobe River, Japan

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**Abstract**—In order to discuss the influence of dam discharge on the movement of ayu *Plecoglossus altivelis* in a river and the stocking effectiveness of released ayu, the migratory history of 83 juveniles of ayu (standard length=67.9–142.4 mm sL) collected in the Kurobe River, Japan during 25 June–23 August 2004 was determined by otolith microchemistry, and the origin of fish was identified as being either native amphidromous ayu or released amphidromous or landlocked fish. Except for otoliths with abnormal morphology (N=10), most specimens (96%, N=70) had inner layers (radius: >400  $\mu\text{m}$ ) of higher Sr:Ca ratios and outer ones of low values. These specimens were identified as native amphidromous fish that migrated upstream from the sea. Only 4% (N=3) were released ayu (reared fish), which were further divided into two origins, amphidromous fish (N=2) with smaller inner layers (radius: <400  $\mu\text{m}$ ) of high Sr:Ca ratios, and landlocked fish (N=1) with constantly low values over the whole otolith. There were no specimens with an increase in Sr:Ca ratios in the outer layers of the low Sr:Ca region of the otolith after their upstream migration in spring, suggesting that it is unlikely that any of these ayu had been washed-out from the river by cold and turbulent river water.

**Key words:** ayu, *Plecoglossus altivelis*, migratory history, otolith, Sr:Ca ratios, Kurobe River, stocking

## Introduction

The ayu, *Plecoglossus altivelis*, is an osmerid fish with an annual life cycle, which spawns in freshwater in autumn. Their newly hatched larvae drift downstream to over-winter in coastal waters, and then they migrate upstream as juveniles in spring (Tsukamoto et al. 1987). Besides the typical amphidromous populations, landlocked populations are also known from Lake Biwa (Azuma 1973) and several other lakes in Japan, which have never migrated into seawater and have spent their entire life history in freshwater.

Ayu is one of the most important fishes for freshwater fisheries and recreational fishing in Japan. Juvenile ayu have been extensively stocked in many rivers of Japan for more than several decades: Most stocked fish are juveniles from the landlocked population in Lake Biwa (70%), and the rest are comprised of amphidromous wild juveniles (10%) and hatchery-reared fish (20%) (Otake and Uchida 1998). However, the stocking has not necessarily resulted in an enhancement of the wild stock in most rivers, although it has been successful for establishing an artificial resource of one generation for game fishing and fisheries. One possible reason for this could be that progeny of the landlocked fish from Lake Biwa that were released and matured in a river were suggested to show a high mortality in the sea because of their

low tolerance to elevated salinity at a higher water temperature in the sea (Azuma 1973, Tabata and Azuma 1986, Pastene et al. 1991).

The Kurobe River originates in the center of the Northern Alps of Japan and flows northwards in the eastern part of Toyama Prefecture. It has a length of 85 km and discharges a huge amount of water into the Japan Sea. The Kurobe River is famous for the construction of many hydro-electric power plants since 1924 because of its rapid and steep waters. The dams on the river were multiple-purpose for preventing floods, decreasing the collapse of the coastline, providing a source of water, and generating electricity. However, these dams may also have some unfavorable influences on the environment including impacts on the riverine fishes from the coldwater discharge from the dams and from the dredging up of sand deposited in dams. However, knowledge is still insufficient about the influence of dams on the riverine environment of this river.

The Kurobe River is not an exception for the stocking of the ayu and has an extensive release with a biomass of more than 9,000 kg every year. In 2004, 10,400 kg of juvenile ayu were released, about three fourths of which were from Lake Biwa. Released ayu have the possibility to be washed out into the coastal area by the cold and turbid water released by the dam on the river. If this occurs, it is not clear if these ayu can survive in the sea and migrate back into the Kurobe River.

Recently, ratios of strontium (Sr) to calcium (Ca) in the otoliths of fishes have been shown to reflect the migration history of an individual fish (Otake et al. 1994; Secor et al. 1995, Tsukamoto et al. 1998). This method has also been confirmed to be applicable to the ayu to distinguish between stocked landlocked fish or native amphidromous ones (Otake and Uchida 1998, Otake et al. 2002).

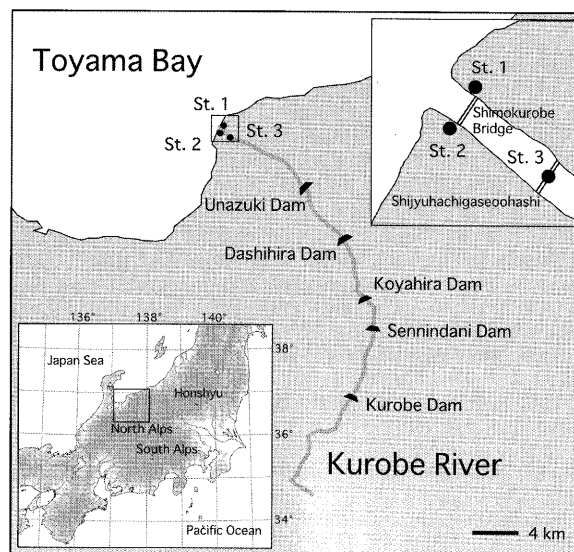
The objectives of the present study were to examine the migratory history of ayu collected in the Kurobe River and to evaluate the possibility of whether they were washed out into the sea by the dam discharge and if they had migrated back into the Kurobe River. In addition, we estimated the origin of ayu as being either released ayu or native fish in the study area, for a further evaluation of the stocking effectiveness of ayu in the river. This study is a first assessment of the use of otolith microchemistry to be applied to an evaluation of stocking effectiveness of a large-scale release program of the ayu.

## Materials and Methods

A total of 83 ayu *Plecoglossus altivelis* were collected by cast net at three sites of the lower reaches of the Kurobe River (Fig. 1) on 25 June, 5 and 20 July, and 2 and 23 August 2004. The standard length (SL) and fork length (FL) of these "sample fish" were measured to the nearest 0.1 mm, and their body weight without the internal organs (BW\*) were also measured to the nearest to 0.1 g (Table 1).

The sagittal otoliths were extracted from all sample fish. All otoliths were observed using a dissecting microscope (Nikon, SMZ-U) and photographed by a digital camera attached to the microscope. The maximum otolith diameter (OD), otolith radius (OR) and otolith weight (OW) were measured. After measurement, the otoliths were used for otolith microchemistry analysis. Each otolith was embedded in epoxy resin (Struers, Epofix), mounted on a glass slide, then ground to expose the core on the sagittal plane using a grinding machine equipped with a diamond cup-wheel (Struers, Discoplan-TS), and polished further with OP-S suspension on an automated polishing wheel (Struers, Planopol-V). The surface was cleaned with distilled water and ethanol, and coated with Pt-Pd using a high vacuum evaporator.

All otoliths with abnormal morphology were excluded from further otolith analyses. Normal otoliths were examined in "life-history transect" analysis of Sr and Ca concentrations along a line down the longest axis of each otolith from the core to the posterior edge using a wave-length dispersive X-ray electron microprobe (JEOL, JXA-8900R) as described in Tsukamoto and Arai (2001). Strontianite ( $\text{SrTiO}_3$ ) and calcite ( $\text{CaCO}_3$ ) were used as standards. The accelerating voltage and beam current were 15 kV and 12 nA, respectively. The electron beam was focused on a point of 5  $\mu\text{m}$  diameter, with



**Fig. 1.** Sampling sites for the ayu, *Plecoglossus altivelis*, in the Kurobe River, Japan.

**Table 1.** The sampling dates, number of fish examined (N), body length (SL, FL) and body weight (BW) of ayu collected from 25 June to 23 August 2004 in the Kurobe River. SL=standard length, FL=fork length, and BW\*=body weight without internal organs.

Date	N	SL(mm) mean $\pm$ SD	FL(mm) mean $\pm$ SD	BW*(g) mean $\pm$ SD
25 Jun 04	7	84.8 $\pm$ 20.7	93.2 $\pm$ 23.0	8.7 $\pm$ 8.8
05 Jul 04	14	86.6 $\pm$ 10.5	93.8 $\pm$ 12.1	8.0 $\pm$ 3.5
20 Jul 04	20	91.6 $\pm$ 10.7	100.1 $\pm$ 12.4	9.5 $\pm$ 4.1
02 Aug 04	20	92.0 $\pm$ 13.2	101.1 $\pm$ 14.2	9.5 $\pm$ 5.6
23 Aug 04	22	114.0 $\pm$ 14.7	124.0 $\pm$ 16.7	22.0 $\pm$ 10.5
Total	83	96.3 $\pm$ 17.1	105.1 $\pm$ 18.9	12.5 $\pm$ 9.0
Max		142.4	153.3	45.9
Min		67.9	75.4	3.5

measurements spaced at 5  $\mu\text{m}$  intervals (each counting time was 4.0 s).

"X-ray intensity maps" of both elements were made of the otoliths of 5 normal specimens. The beam current was 50 nA, counting time was 0.4 s, pixel size was 4.0 $\times$ 4.0  $\mu\text{m}$ , and the electron beam was focused on a point of 4  $\mu\text{m}$ .

In the Kurobe River, the releasing of landlocked ayu from Lake Biwa and amphidromous ayu collected in the coastal waters of Wakayama Prefecture in winter-early spring has been done every year. These fish of known origins were all reared in freshwater culture ponds for a couple of months before transportation to the Kurobe River for release. In order to compare with native ayu in the Kurobe River, ten fish each of the landlocked fish from Lake Biwa and amphidromous fish from Wakayama Prefecture were also sampled as "control fish" for otolith examination just before the release into the Kurobe River on 4 and 10 June 2004. The

**Table 2.** The sampling dates, number of fish examined (N), body length (SL, FL) and body weight (BW) of ontrol fish of ayu examined in the study.

Date	N	Origin	SL(mm) mean±SD	FL(mm) mean±SD	BW(g) mean±SD
4 Jun 04	10	Wakayama, amphidromous	117.5±10.2	127.7±11.0	27.2±7.4
10 Jun 04	10	Lake Biwa, landlocked	131.5±4.8	143.2±6.5	36.8±5.9
Total	20		124.4±10.9	136.1±12.2	31.0±8.0
		Max	138.8	150.3	45.3
		Min	104.0	112.7	19.4

SL, FL, and total body weight (BW) of the control fish were also measured (Table 2). Sagittal otoliths of three normal specimens of the control fish from both origins were also used for the otolith microchemistry analyses of Sr and Ca concentrations. X-ray intensity map analyses of these control fish were also made.

## Results

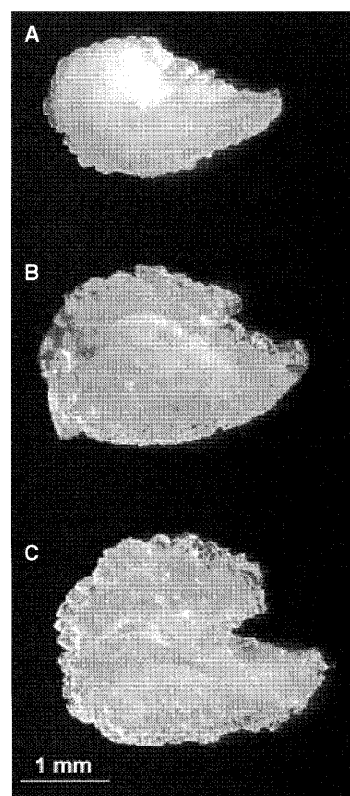
### Fish size

The SL of ayu collected in the Kurobe River ranged from 67.9 to 142.4 mm ( $96.3 \pm 17.1$ : mean±SD), the FL were 75.4 to 153.3 mm ( $105.1 \pm 18.9$ ), and BW\* were 3.5 to 45.9 g ( $12.5 \pm 9.0$ ) (Table 1). Both body lengths and body weights increased with the catching dates (ANOVA,  $p < 0.001$ ). There was no significant difference in these sizes among sampling stations (ANOVA,  $p > 0.05$ ).

The SL, FL and BW of control fish that would be released in the Kurobe River ranged from 104.0 to 138.8 mm ( $124.4 \pm 10.9$ : mean±SD), 112.7 to 150.3 mm ( $136.1 \pm 12.2$ ), and 19.4 to 45.3 g ( $31.0 \pm 8.0$ ), respectively (Table 2). Despite the earlier timing of sacrifice, the control fish had significantly larger mean SL, FL and BW than the sample fish collected in the Kurobe River in later months (ANOVA,  $p < 0.001$ ).

### Otolith morphology

The normal sagittal otoliths (N=73, 88%) of sample fish were oval with an elongated frontal side and had a white color, with a smooth surface (Fig. 2A). These otoliths had an approximately centric core and clear concentric daily increments from the core to edge. In contrast, the abnormal otoliths (N=10, 12%) were larger in length ( $t$ -test,  $p < 0.01$ ), 1.5 times heavier in weight ( $t$ -test,  $p < 0.01$ ), deeper in height, thinner, and more transparent with rough surface compared to the normal otoliths (Fig. 2B, C). One type (N=6, 7%) of the abnormal otolith was semi-abnormal with an inner normal part and outer half of a thin and yellowish translucent layer. Another type (N=4, 5%) was completely abnormal from core to edge with a several eccentric cores in different



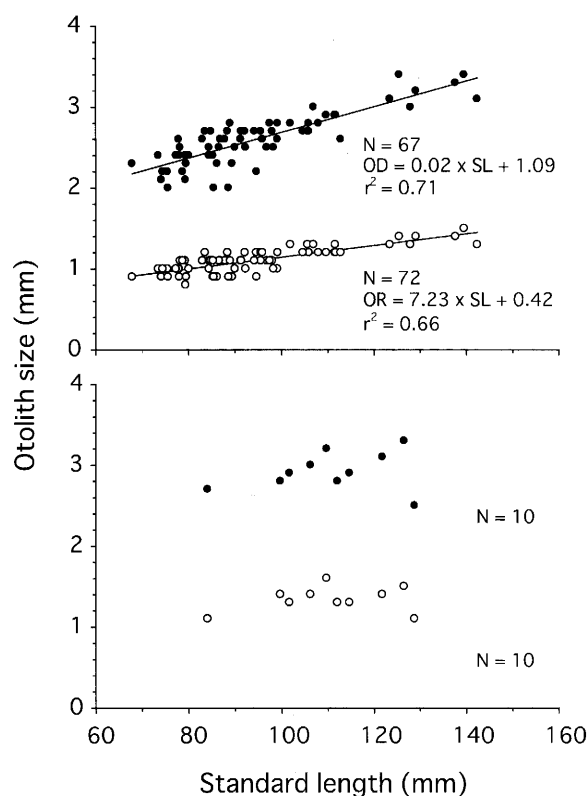
**Fig. 2.** Light microscope photos of sagittal otoliths (right side) of ayu. A: normal otolith (84.9 mm SL); B: semi-abnormal otolith with a normal part at inner layers (99.3 mm SL); C: fully abnormal otolith (105.8 mm SL).

growth planes where unclear daily increments spread towards the different directions around different cores. In control fish, the abnormal otolith occurred in 40% of the fish (landlocked fish: 60%, amphidromous fish: 20%), which was remarkably higher than those of sample fish collected in the river.

The mean OD of the normal otoliths were  $2.6 \pm 0.32$  mm, OR were  $1.1 \pm 0.15$  mm, and OW were  $0.8 \pm 0.25$  mg (Table 3), while those of abnormal otoliths were  $2.9 \pm 0.23$  mm,  $1.3 \pm 0.16$  mm, and  $1.21 \pm 0.24$  mg, respectively. The regressions of OD to SL, and OR to SL in normal otoliths showed linear relationships, while those for abnormal otoliths showed no relationship (Fig. 3). The regression of OW and SL in both normal and abnormal otoliths

**Table 3.** Otolith characters of the ayu collected from 25 June to 23 August 2004 in the Kurobe River. OD=otolith diameter, OR=otolith radius, and OW=otolith weight.

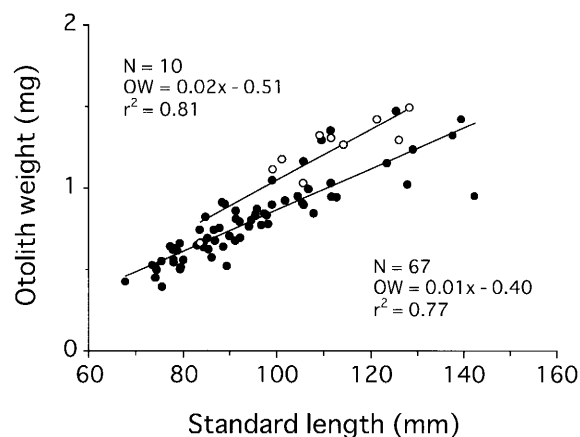
Abnormality	Sampling	N (%) Date	OD(mm) mean±SD	OR(mm) mean±SD	OW(mg) mean±SD
Normal	25 Jun 04	6 (7.2)	2.3±0.18	0.9±0.05	0.55±0.12
	05 Jul 04	13 (15.7)	2.3±0.22	0.9±0.10	0.61±0.19
	20 Jul 04	18 (21.7)	2.6±0.17	1.1±0.08	0.78±0.24
	02 Aug 04	20 (24.1)	2.7±0.26	1.1±0.11	0.84±0.20
	23 Aug 04	16 (19.3)	2.9±0.26	1.3±0.12	1.00±0.19
Total		73 (88.0)	2.6±0.32	1.1±0.15	0.80±0.25
Abnormal	25 Jun 04	1 (1.2)	2.5	1.1	1.50
	05 Jul 04	1 (1.2)	3.2	1.6	1.33
	20 Jul 04	2 (2.4)	2.8±0.10	1.3±0.17	0.89±0.32
	02 Aug 04	0	—	—	—
	23 Aug 04	6 (7.2)	2.9±0.51	1.3±0.39	1.41±0.12
Total		10 (12.0)	2.9±0.23	1.3±0.16	1.21±0.24

**Fig. 3.** Relationship between fish size (SL) and otolith diameter (closed circle), and SL and otolith radius (opened circle). Top: normal otolith. Bottom: abnormal otolith.

showed a linear relationship (Fig. 4).

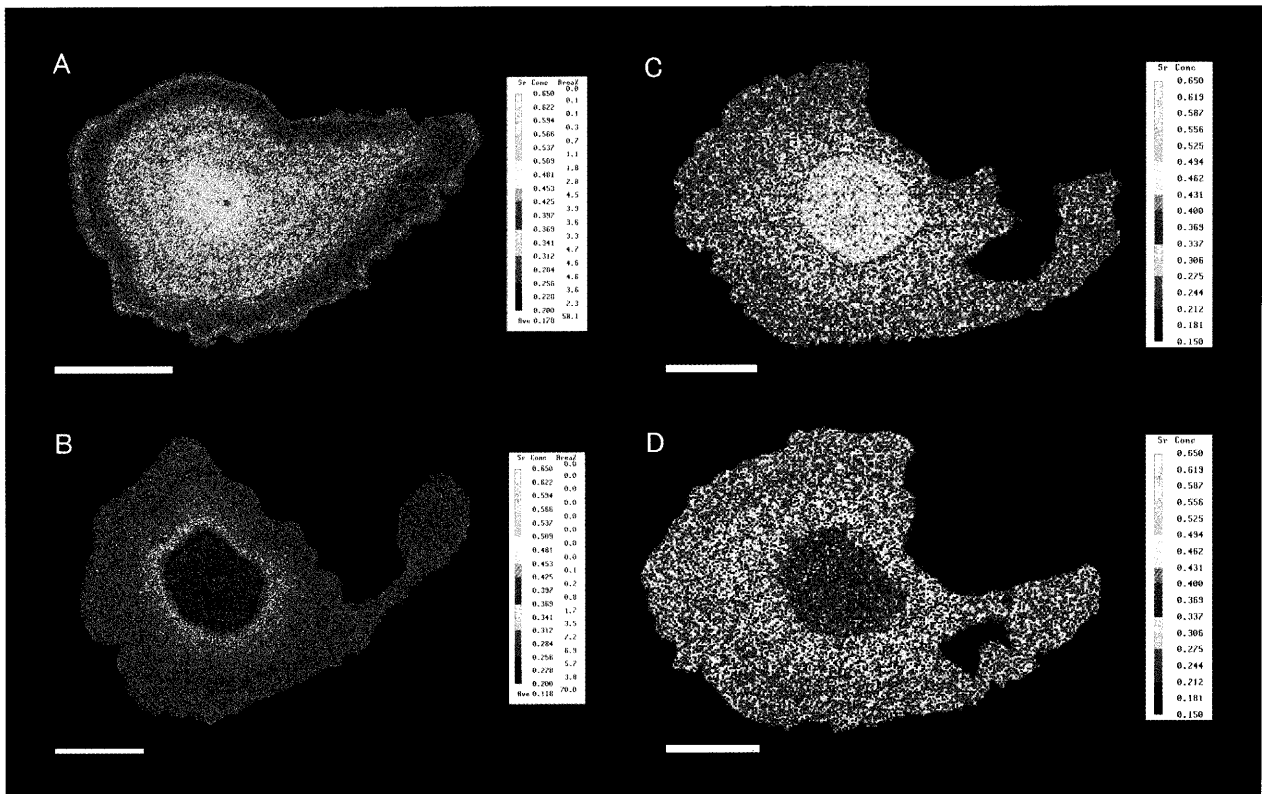
#### Otolith Sr : Ca ratio

The X-ray intensity maps showed various types of Sr distribution according to each origin (Fig. 5). Most of the sample fish collected in the river showed a higher Sr concentration (red) in the center followed by a gradual decrease (yellow-green) in the outer part, and then very low values

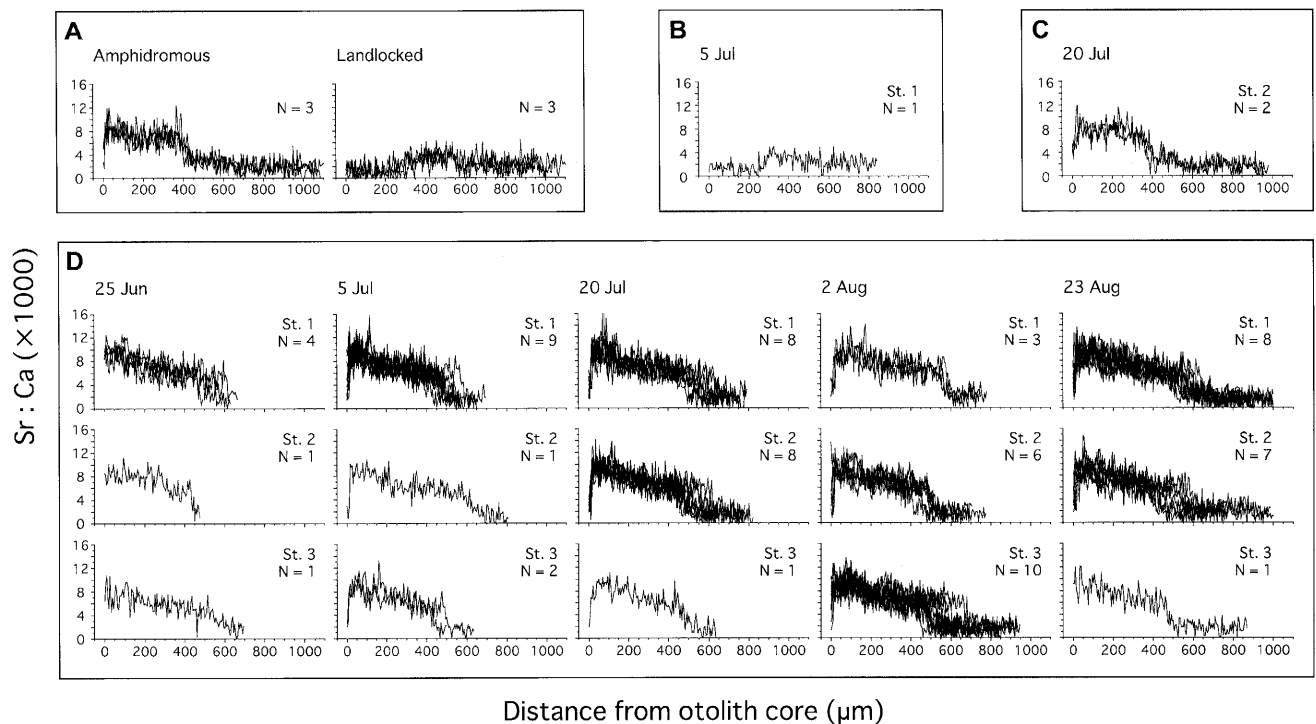
**Fig. 4.** Relationship between fish size (SL) and otolith weight. Closed circle: normal otolith. Opened circle: abnormal otolith.

(blue) to the outermost layer (Fig. 5A). Control fish of amphidromous ayu (Fig. 5C) showed similar color change patterns. These types of images appeared to correspond to the native amphidromous fish (see below). Contrary to this, one otolith showed a low value (blue) over the whole area with a relatively higher Sr content of a light yellowish circle between 300–550  $\mu\text{m}$  from the otolith core (Fig. 5B). This fish caught in the Kurobe River apparently showed a whole life history in freshwater, showing that this individual was a landlocked fish released in the river. Landlocked control fish showed fundamentally the same pattern with the otolith of the landlocked fish in Fig. 5B, with a little broader yellowish ring at 300–600  $\mu\text{m}$  from the otolith core (Fig. 5D).

The Sr:Ca ratios also showed various patterns corresponding to each origin and migratory history (Fig. 6). In control fish, an individual of amphidromous origin showed a low Sr:Ca ratio of  $2.24 \times 10^{-3}$  at the center of otolith, reflecting riverine freshwater at the spawning ground, and afterwards increased to high mean values of  $7.26 \times 10^{-3}$  until



**Fig. 5.** X-ray intensity maps of Strontium (Sr) contents in otoliths of ayu. Red color shows high concentration of Sr, while blue color shows low concentration. A: native amphidromous ayu (77.9 mm SL); B: released landlocked ayu (105.9 mm SL). C: control fish of amphidromous ayu reared in a fresh water pond in Wakayama; and D: control fish of landlocked ayu reared in a freshwater pond. Scale bars indicate each 500  $\mu\text{m}$ .



**Fig. 6.** Profiles of Sr:Ca ratios from the core to the edge of the sagittal otolith in the ayu. A: control fish of amphidromous ayu (left) and landlocked ayu (right); B–D: sample fish collected in the Kurobe River of different origins B: released landlocked ayu, C: released amphidromous ayu, and D: native amphidromous ayu.

about 400  $\mu\text{m}$  in radius (Fig. 6A). Subsequently Sr:Ca ratios drastically dropped down to  $2.29 \times 10^{-3}$  and this low level continued to the otolith edge. Control fish of landlocked origin had low mean Sr:Ca ratios of  $2.31 \times 10^{-3}$  from the otolith core to the edge, showing a history in freshwater throughout their life.

Based on the Sr:Ca pattern of control fish, sample fish collected in the Kurobe River were roughly divided into two types, amphidromous and landlocked types as shown in Fig. 6A. Only one specimen showed a landlocked type pattern (Fig. 6B). It had low Sr:Ca ratios (mean:  $2.14 \times 10^{-3}$ ) throughout the life history transect with a slightly higher values (mean:  $4.97 \times 10^{-3}$ ) corresponding to the light yellowish circle in Fig. 5B at 300  $\mu\text{m}$  radius. Others ( $N=72$ ) were all basically the amphidromous type that had a inner high Sr:Ca layers and outer low Sr:Ca layers. Among them, only two individuals with a large radius of 900–1000  $\mu\text{m}$  showed an abrupt drop of Sr:Ca ratios from  $7.25 \times 10^{-3}$  to  $2.31 \times 10^{-3}$  at less than 400  $\mu\text{m}$  in radius (Fig. 6C), while the others ( $N=70$ ) showed the abrupt drop at more outer layers from the core ( $>400 \mu\text{m}$ ) or the decrease occurred gradually without a sharp drop (Fig. 6D). Thus the former two were estimated as the released fish of amphidromous origin, while the latter were regarded as a native amphidromous fish.

The three individuals identified as released fish (two amphidromous in Fig. 6C and one landlocked fish in Fig. 6B) were larger (range: 105.9–115.0 mm SL, 108.8–115.0 mm FL) than the native ones ( $N=70$ , mean: 93.6 mm SL, 101.9 mm FL).

None of the fish had a rise of Sr:Ca ratio in the outer half of their otolith radius, suggesting no fish had experienced being washed-out by low temperature and turbid dam discharges to the sea, before migrating back to the river again.

## Discussion

### Otolith morphology

Otolith analyses have been used as a useful tool to learn about the age and growth of fishes by examining their daily or annual increments, and to determine their migration patterns using otolith chemistry (Campana 1999, Campana and Thorrold 2001). In the case of ayu, the microstructure of daily increments is extremely clear and otolith microchemistry analyses have contributed to a better understanding of the origins and migration history of this species (Tsukamoto and Kajihara 1987, Tsukamoto et al. 1989, Otake and Uchida 1998, Otake et al. 2002). However, we found many abnormal otoliths (12% of sample fish) in the present study. The differences between normal and abnormal otoliths were not only the appearance, but also the size, such as diameter and weight. In the reared ayu (control fish), the abnormality was

much higher (40%) than in the collected ones. This finding may give an interesting suggestion for further study on the mechanism of otolith formation.

### Identification of origin of ayu by Sr:Ca ratios

We could identify the origin of sample fish collected in the Kurobe River using otolith Sr:Ca analysis. It was easy to identify the landlocked or amphidromous origin by the Sr concentration in the inner layers of the otoliths (Otake and Uchida 1998). We could also distinguish the released amphidromous ayu from native amphidromous ones using the timing (radius:  $<400 \mu\text{m}$ ) of abrupt decrease in Sr:Ca ratios or the slope of the decrease. Since the released amphidromous fish were caught at the larval stage in coastal waters in late winter or early spring and then transported into a freshwater rearing pond, their otoliths would have incorporated evidence of this sudden change in salinity in their inner layer. Contrarily, the native fish grew to the juvenile stage in coastal waters or the estuary and then could adapt to freshwater gradually before starting their upstream migration into the river. In this case, the decrease in Sr:Ca would occur in the outer layer (radius:  $>400 \mu\text{m}$ ) at larger body sizes or its change would be moderate. This was a good key for the identification of reared (released) and native fish. However, a few specimens had a sharp drop of Sr:Ca ratio at around a 400  $\mu\text{m}$  radius or more and these individuals were a little difficult to identify as one type or the other. Since, all these fish had much smaller otoliths than the control fish (released fish) that had larger otoliths of about 9,000–1,000  $\mu\text{m}$  in radius, we identified those fish with smaller sized otolith as native fish that would have experienced an abrupt change in salinity at a relatively earlier time than other ordinary fish.

### Ayu stocking in the Kurobe River and the influence of dam discharge

Intensive release of ayu has been carried out by the Fisherman's Union in the Kurobe River, and the amount of released fish reached 10,400 kg (about 335,484 individuals) from June to August in 2004, of which 75% were landlocked fish from Lake Biwa. In sample fish collected in the Kurobe River, however, only 4% of the fish we analyzed were released ayu and the rest (96%) were all recognized as native amphidromous fish. If we adopt a simple Petersen's method to these data, population size of native amphidromous ayu would be as many as 7,827,960 individuals in the Kurobe River. However, this number is apparently an overestimate. Furthermore, the ratio of released landlocked and amphidromous ayu was about 2:1, while the ratio of recaptured fish was 1:2; although the number of fish was very small. Such discrepancies appeared to be caused mainly by the bias of the sampling sites of the study. Our sampling sites were all limited to the lower reaches of the river near estuary, while the releasing points were scattered over the middle and lower

reaches. Since native ayu migrate upstream from the sea through the estuary to middle reaches, the fish density would be larger in general in the lower reaches than the upper reaches, causing a bias of distributions between native and released fish. Furthermore, landlocked ayu, which were the majority of released fish, have shown a tendency to go upstream after release (Tsukamoto et al. 1990). These considerations can explain the above overestimate of native fish in the river. In addition, fishing mortality of landlocked fish would be higher by the decoy fishing technique of "tomo-zuri", which is a popular method for catching ayu using their behavioral characteristics of territoriality, than for the native fish since landlocked fish show a stronger tendency to exhibit territorial behavior. Another factor is that bacterial coldwater disease has recently appeared and caused mass mortalities of ayu. Although it is not clear if the released fish have been infected or were carriers, because of the risk of this, the proportions of properly released ayu should be low.

Dam discharge also may have an unfavorable effect on the population of ayu, because both native and released fish could possibly be washed out to sea by its cold temperature, turbidity and strong flow. However, there were no specimens that had an elevated Sr:Ca ratios (due to seawater) in the outer layers of their otoliths after their upstream migration in spring for either amphidromous or landlocked ayu, even if the sampling was over a month after a big discharge of the dam. It appeared to be a reasonable conclusion that the ayu in the Kurobe River could maintain themselves in their riverine habitat even during a strong flow of cold, turbid water to some degree and were not carried into the sea by the dam discharge. However, another interpretation is that the dam discharges would carry fish downstream into coastal waters, but these fish could come back immediately to the Kurobe River, and this experience in sea water was not recorded in their otoliths because of too short of a period in the sea. Furthermore, we would not be able to detect the fish that were lost through death or had migrated upstream to other rivers than the Kurobe River. To resolve this problem, further study is needed using a greater number of specimens analyzed together with other techniques such as biotelemetry or extensive mark-recapture studies.

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