Facultative catadromy of the eel *Anguilla japonica* between freshwater and seawater habitats

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ABSTRACT: To confirm the occurrence of marine residents of the Japanese eel, *Anguilla japonica*, which have never entered freshwater ('sea eels'), we measured Sr and Ca concentrations by X-ray electron microprobe analysis of the otoliths of 69 yellow and silver eels, collected from 10 localities in seawater and freshwater habitats around Japan, and classified their migratory histories. Two-dimensional images of the Sr concentration in the otoliths showed that all specimens generally had a high Sr core at the center of their otolith, which corresponded to a period of their leptocephalus and early glass eel stages in the ocean, but there were a variety of different patterns of Sr concentration and concentric rings outside the central core. Line analysis of Sr/Ca ratios along the radius of each otolith showed peaks (ca 15×10^{-3}) between the core and out to about 150 µm (elver mark). The pattern change of the Sr/Ca ratio outside of 150 µm indicated 3 general categories of migratory history: 'river eels', 'estuarine eels' and 'sea eels'. These 3 categories corresponded to mean values of Sr/Ca ratios of $\geq 6.0 \times 10^{-3}$ for sea eels, which spent most of their life in the sea and did not enter freshwater, of 2.5 to 6.0×10^{-3} for estuarine eels, which inhabited estuaries or switched between different habitats, and of $< 2.5 \times 10^{-3}$ for river eels, which entered and remained in freshwater river habitats after arrival in the estuary. The occurrence of sea eels was 20% of all specimens examined and that of river eels, 23%, while estuarine eels were the most prevalent (57%). The occurrence of sea eels was confirmed at 4 localities in Japanese coastal waters, including offshore islands, a small bay and an estuary. The finding of estuarine eels as an intermediate type, which appear to frequently move between different habitats, and their presence at almost all localities, suggested that *A. japonica* has a flexible pattern of migration, with an ability to adapt to various habitats and salinities. Thus, anguillid eel migrations into freshwater are clearly not an obligatory migratory pathway, and this form of diadromy should be defined as facultative catadromy, with the sea eel as one of several ecophenotypes. Furthermore, this study indicates that eels which utilize the marine environment to various degrees during their juvenile growth phase may make a substantial contribution to the spawning stock each year.

KEY WORDS: *Anguilla japonica* · Japanese eel · Catadromy · Migration · Migratory history · Otolith · Sr/Ca · Marine resident · Life history transect · Habitat transition

INTRODUCTION

Diadromy, the migration of fish between freshwater and ocean habitats, has long attracted the interest of biologists. Of special interest have been the catadromous migrations of freshwater eels of the genus *Anguilla*, which spawn in the mid-oceans but grow in freshwater streams and are arguably the most studied catadromous species (McDowall 1988).

The Japanese eel *Anguilla japonica* spawns in waters west of the Mariana Islands (presumably at some seamounts in the West Mariana Ridge: Tsukamoto 1996, Fricke & Tsukamoto 1998), and its transparent leaf-like larvae, or leptocephali, move within the North Equatorial and Kuroshio currents to the east Asian coasts of Taiwan, China, Korea and Japan (Tsukamoto 1992). They leave these currents

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after metamorphosing into glass eels, and all are generally thought to migrate up freshwater streams where they grow to the pre-adult silver eel stage. During the silver eel stage, their gonads begin maturing and they start their downstream migration into the ocean and back out to the spawning area, where they spawn and die. A similar life cycle is believed to occur in the Atlantic species, the American eel *A. rostrata* and the European eel *A. anguilla*. Both species spawn in the Sargasso Sea, and their larvae move westward within the southern Sargasso Sea. They enter the Gulf Stream and the North Atlantic Drift and are distributed throughout the coasts of North America and Europe, respectively, before their glass eels enter the rivers.

These spectacular life histories are the subject of a large literature on biology, and are so well accepted that the life cycle of freshwater eels goes without question. However, Tsukamoto et al. (1998), using Synchrotron Radiation Induced X-ray Fluorescence analysis for quantifying strontium and calcium deposits in their otoliths, have recently found populations of yellow and silver eels of both Japanese and European eels in marine areas adjacent to their typical freshwater habitats which have never migrated into freshwater and have spent their entire life history in the ocean. This discovery of marine residents ('sea eels') of both Japanese and European eels suggests that anguillid eels do not all have to be catadromous, and questions the generalized classification of diadromous fishes.

Both the Pacific and Atlantic eels are important commercial species and are harvested in rivers, estuaries and coastal waters. In addition, the glass eels are collected for intensive aquaculture throughout Asia and Europe. During the past 20 yr, the harvest of eels has declined by over 90% due to freshwater habitat destruction and over harvesting (Tsukamoto & Otake 1995). Because of the commercial importance of eels, many studies have been conducted on their physiology, ecology, morphology, phylogeny and population dynamics. In spite of the large amount of research that has been conducted, major components of their biology remain unknown. In particular, surprisingly little is known about the migratory and reproductive behavior of silver eels, the habitat choice of yellow eels, and the factors influencing the entry of glass eels into freshwater.

Recent chemical analytical techniques have enabled identification of life history events in individual fish by detecting trace elements in the microstructure of their otoliths (Kalish 1989, Radtke 1989, Radtke et al. 1990). Oxygen isotope ratios (Nelson et al. 1989, Tsukamoto et al. 1989) and strontium (Sr) incorporation (Kalish 1989, Radtke 1989, Radtke et al. 1990, Secor 1992, Townsend et al. 1992, Otake et al. 1994,

Tzeng and Tsai 1994, Secor et al. 1995, Arai et al. 1997) in fish otoliths are of special interest because of 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 their growth varies between freshwater and marine habitats (Casselman 1982, Kalish 1989, 1990, Radtke 1989, Secor 1992, Secor et al. 1995, Tzeng et al. 1997, Arai & Tsukamoto 1998). Freshwater contains low levels of Sr, while seawater contains high levels (Angino et al. 1966).

Analyses of Sr/Ca ratios in the otoliths of anguillid leptocephali and glass eels has recently been found to be very informative about the timing of life history events such as metamorphosis and recruitment to freshwater. Otake et al. (1994) found an ontogenetic change in the Sr/Ca ratios in the otoliths of *Anguilla japonica* glass eels at the onset of metamorphosis that was indicated by an abrupt drop in the high Sr level maintained during the leptocephalus stage. Similar results were confirmed in tropical eels (*A. celebesensis*, *A. marmorata*, *A. bicolor bicolor* and *A. bicolor pacifica*: Arai et al. 1999a,b) and other temperate eels in the southern hemisphere (*A. australis*: Arai et al. 1999c; *A. dieffenbachii*: Marui et al. 2001), suggesting that this ontogenic change in otolith Sr/Ca ratios is a common phenomenon during the leptocephalus stage of all anguillid eels. After a 7 mo rearing experiment using *A. japonica* elvers (young juveniles) in tanks of different salinities, Tzeng (1996) showed that otolith Sr/Ca ratios at the elver stage are directly related to the ambient salinity and concluded that the ratio could be used for determining the individual movements of elvers between habitats with differing salinities. Tzeng et al. (1997, 2000) examined otolith Sr/Ca ratios in *A. anguilla* yellow (older juveniles) and silver eels collected in brackish waters and freshwater lakes in Sweden. They reported several relatively higher Sr bands in eels from the brackish waters of the Baltic Sea but none in those from freshwater, suggesting that otolith Sr/Ca ratios may also be used as an environmental indicator to detect the migratory history of yellow eels between seawater and freshwater.

In this study we measured Sr/Ca ratios in the otoliths of both yellow and silver eels collected in marine and freshwater habitats around Japan to determine their migratory history. Thus, the objectives of this study were: (1) to confirm the occurrence of the sea eels reported by Tsukamoto et al. (1998), (2) to determine their distribution and relative abundance in Japanese coastal waters, estuaries and freshwater habitats, and (3) to consider their potential ecological significance and contribution to the spawning stock.

MATERIALS AND METHODS

Fish. Yellow and sliver eels of *Anguilla japonica* were collected from environments of various salinities (freshwater: 0 psu; brackish water: ca 5 to 30 psu; sea water: >30 psu) such as rivers, estuaries, port, coastal and offshore waters around Japan (Table 1, Fig. 1). The eels were collected by a variety of methods including dip-netting, eel pots, eel gaff (unagi-kama), and hook-and-line. Yellow eels from freshwater habitats were sampled in the Fukui River, Tokushima Prefecture, in April 2000, and silver eels from freshwater habitats were collected in the Tone River, Chiba Prefecture, in October 1992. Yellow eels from brackish water habitats were collected at the mouth of Tone River in June and July 1999, and at the mouth of Fukui River between February and March 1999. Yellow eels from the seawater habitat were sampled at the Yoshida Port, Shizuoka Prefecture, in July and August 1997, and at the Mishou Bay, Ehime Prefecture, in September 1999. Silver eels from the seawater habitat were sampled in the Hamana Lake (seawater lake), Shizuoka Prefecture, between October 1989 and February 1990, in the Mikawa Bay, Aichi Prefecture, in October and November 1998, at Hime Island, Nagasaki Prefecture, in November 1995, and at the Danjyo Islands, Nagasaki Prefecture, in January 1995. A total of 69 specimens were used in the present study; 2 yellow eels and 6 silver eels from freshwater habitats, 17 yellow eels from brackish water habitats, and 9 yellow eels and 35 silver eels from seawater habitats (Table 1). Total length (TL), body weight (BW) and gonad-somatic index (GSI) were measured.

Otolith preparation. Sagittal otoliths were extracted from each fish, embedded in epoxy resin (Struers, Epofix) after measuring their diameter, and mounted on glass slides. The otoliths were then ground to expose the core along an anterior-posterior direction in the frontal plane, using a grinding machine equipped with a diamond cup-wheel (Struers, Discoplan-TS), and polished further with 6 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 examination. The ground surfaces of the otoliths were examined at 200× with a light microscope (LM), and LM photographs were taken to measure the 'radius' of the elver mark (the distance from the otolith core to the elver check: Tzeng 1996).

Otolith X-ray microprobe analysis. For electron-microprobe analyses, all otoliths (69 specimens) were carbon or Pt-Pd coated by high-vacuum evaporator. Otoliths from 61 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 or JXA-733), as described by Arai et al. (1997, 1999a,b,c) and Arai & Tsukamoto (1998). Strontianite ($SrCO_3$) and calcite ($CaCO_3$) were used as standards. The accelerating voltage and beam current were 15 kV and 7 nA, respectively. The electron beam was focused on a point 5 or 10 µm in diameter, with measurements spaced at 5 or 10 µm intervals.

'X-ray intensity maps' of both elements were made of the otoliths of 26 specimens using a JEOL JXA-8900R. The beam current was 0.5 µA, counting time was 0.1 s, pixel size was 2×2 to 4×4 µm, the electron beam was focused on a point 1 µm in diameter, and other analytical conditions corresponded to those for the life-history transect analyses. Otoliths from 18 specimens were used for both life-history transects and X-ray intensity-map analyses.
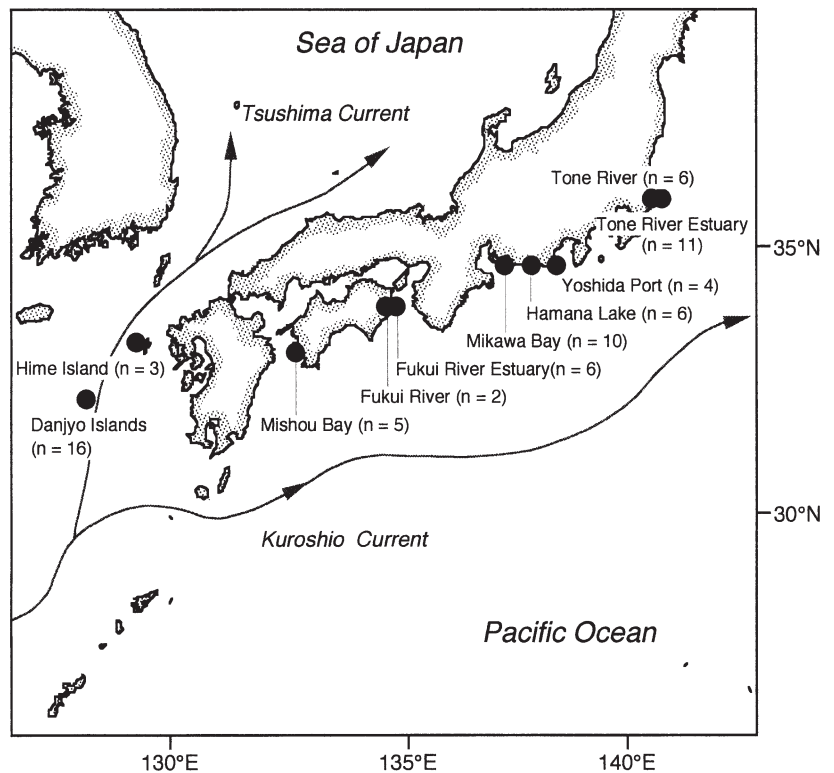

Fig. 1. Sampling sites for the Japanese eel *Anguilla japonica* along the coast of Japan, adjacent to the Kuroshio Current and at offshore islands in the East China Sea. Sample sizes of specimens examined at each location are given in parentheses

Table 1. *Anguilla japonica* used for otolith microchemistry analyses. F: female; M: male; GSI: gonad-somatic index; –: no data

Sampling location	Salinity	(psu)	Sampling date	Sampling gear	Develop-. mental stage	No. fish examined	Sex ratio F(M+F)$^{-1}$
Tone River	Freshwater	(0)	12–26 Oct 1992	Eel gaff	Silver	6	100
Tone River Estuary	Brackish water	(5–29)	Jun–Jul 1999	Eel gaff	Yellow	11	100
Yoshida Port	Seawater	(30–33)	20 Jul–16 Aug 1997	Hook-and-line	Yellow	4	–
Hamana Lake	Seawater	(30–33)	1 Oct 1989–2 Feb 1990	Set net	Silver	6	100
Mikawa Bay	Seawater	(30–33)	Oct–Nov 1998	Set net	Silver	10	–
Fukui River	Freshwater	(0)	21 Apr 2000	Eel pot	Yellow	2	–
Fukui River Estuary	Brackish water	(7–29)	Feb–Mar 1999	Eel pot	Yellow	6	–
Mishou Bay	Seawater	(30–34)	26 Sep 1999	Eel pot	Yellow	5	–
Hime Island	Seawater	(30–34)	30 Nov 1995	Hand net	Silver	3	100
Danjyo Islands	Seawater	(30–34)	8–13 Jan 1995	Hand net	Silver	16	81

RESULTS

Otolith strontium distribution

Two-dimensional images of the Sr concentration in the otoliths of *Anguilla japonica* revealed a remarkable variation among the specimens examined (Fig. 2). However, all specimens had a high Sr area at the center of the otolith (red oval spot about 200 to 250 µm in long axis) surrounded by an elver mark that could be observed with a light microscope. This 'high Sr core' was considered to correspond to the oceanic lepto-cephalus stage up to the early glass eel stage just after metamorphosis, which would be that part of the life history from the spawning grounds to coastal waters (Otake et al. 1994). The typical pattern of the Sr con-centration in the river samples from freshwater habi-tats was a uniformly low Sr concentration over the whole otolith (blue color) except for the high Sr core (Fig. 2: 2 [Tone River-2] and 8 [Fukui River-2]). Other samples from brackish and seawater habitats (except 15 [Danjyo Island-1]) displayed concentric annulus-like rings of both low and high Sr bands surrounding the high Sr core. Most seawater samples were charac-terized by a yellowish color (higher Sr concentration) from the otolith core to the edge (Fig. 2: 11 [Mishou Bay-3], 13 [Hime Island-7] and 16 [Danjyo Islands-4]), while the estuarine samples from the brackish water habitats (and some seawater samples) displayed either concentric rings with a continuous pattern of relatively less yellowish color (lower Sr) outside the high Sr core (Fig. 2: 3 [Tone River estuary-39], 7 [Mikawa Bay-11] and 10 [Fukui River estuary-8]), or a wide space of bluish color (low Sr) just outside the high Sr core that was surrounded by concentric rings with higher Sr concentrations (Fig. 2: 5 [Hamana Lake-24], 6 [Mikawa Bay-3] and 9 [Fukui River Estuary-3]). However, sam-ples from the Danjyo Islands, the most oceanic locality

in the study, included a uniformly low Sr pattern (15: Danjyo Islands-1) and an intermediate pattern (17: Danjyo Islands-6), as well as the relatively higher Sr concentration (16: Danjyo Islands-4) that was charac-teristic of other seawater samples.

Life-history transects

In all specimens examined, the otoliths had a high Sr central region that corresponded to the leptocephalus stage and showed lower Sr levels after metamorphosis into the glass eel stage (Fig. 3). Each otolith had a peak in the Sr/Ca ratio (ca 15×10^{-3}) between the otolith core and out to about 150 µm, which corresponded to the high Sr core visible in the 2-dimensional images of Fig. 2. The high Sr content in the central core region during the leptocephalus stage may derive from the large amounts of gelatinous extracellular matrix that fills the body until metamorphosis. This material is composed of sulfated glycosaminoglycans (GAG), which are converted into other compounds during metamorphosis (Pfeiler 1984). The drastic decrease in Sr at the outer otolith region in both river and seawater samples after metamorphosis to glass eels, may occur because these sulfated polysaccharides have an affin-ity to alkali earth elements, and are particularly high in Sr, suggesting that a high Sr content in the body has a significant influence on otolith Sr content through sac-cular epithelium in the inner ear, and the sudden loss of Sr-rich GAG during metamorphosis probably results in the lower Sr concentration in otoliths after metamor-phosis (Otake et al. 1994).

Outside the high Sr core, there was a wide range of Sr/Ca values in the otoliths of eels from the different habitats. Most of the river samples (6 of 7 specimens examined) had consistently low Sr/Ca values of less than 5×10^{-3} (e.g. Tone River-3 and Fukui River-2:

Total length (mm)		Body weight (g)		GSI (%)		No. fish analyzed		
Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range	Line analyses	Map analyis	Line & map analyis
716 ± 5.8	629–784	634 ± 136	384–822	–	–	2	1	3
670 ± 14.3	424–865	507 ± 299	75–1008	1.0 ± 0.8	0.2–2.7	7	1	3
580 ± 10.0	530–730	–	–	–	–	4	0	0
715 ± 23.6	675–737	511 ± 78.9	412–639	2.0 ± 0.6	1.0–2.70	4	1	1
507 ± 30.7	461–563	–	–	–	–	7	0	3
–	351–441	–	72–109	–	0	0	0	2
466 ± 37.1	410–507	125 ± 42.3	55–167	0.2 ± 0.1	0.1–0.4	4	1	1
390 ± 49.9	344–473	82 ± 50.2	52–166	0	–	3	0	2
745 ± 130	626–883	729 ± 492	346–1284	1.7 ± 0.4	1.3–2.2	0	0	3
F 700 ± 95	543–849	551 ± 273	270–1155	2.8 ± 0.3	2.1–3.3	10	3	0
M 518 ± 63	450–574	216 ± 90	143–316	0.5 ± 0.1	0.4–0.6	2	1	0

Fig. 3), suggesting a long-term residence in a freshwater habitat after upstream migration during the elver stage. This type of Sr/Ca change was observed in a total of 14 specimens collected not only from river but also from estuarine and seawater habitats. We regarded such specimens with consistently low Sr/Ca values of less than 5×10^{-3} as apparent river migrants or 'river eels' with a standard catadromous migration, which had entered the freshwater habitat after arrival in the estuary and had continued growing in the freshwater habitat until collection.

In contrast, some samples from seawater had relatively high values of around 5 to 10×10^{-3} outside the high Sr core out to the otolith edge, but showed large fluctuations, with some sharp decreases, or inverted spikes to almost zero (e.g. Mishou Bay-2 and, -4, Hime Island-7 and -8, and Danjyo Islands-6 and -10: Fig. 3). These sudden spikes were probably measurement errors caused by tiny flaws on the surface of the samples or an unevenness in the carbon or Pt-Pd coating that greatly reduced the reflection of the X-ray from the sample. This would have no biological significance in relation to migration or habitat transition, especially since each spike only represented 1 or 2 data points of 5 to 10 μm beam diameter, equivalent to a period of about 1 wk. Therefore, disregarding these spikes and focusing on the general trend, these specimens were characterized by a constantly high Sr level that suggested a long-term residence in a seawater habitat (salinity of >30 psu), although a few specimens (e.g. Hime Island-8: Fig. 3) showed an initial drop for a short period just outside the high Sr core that suggested they had experienced lower salinities before moving into the seawater habitat. This pattern of relatively high Sr/Ca ratio outside the otolith core was observed in a total of 12 specimens out of 61 fish examined, 11 from the seawater habitat and 1 from an estuary, Fukui River Estuary-8 (Fig. 3) that had presumably moved

from the sea to the estuary shortly before collection. Thus, we conventionally regarded the specimens with consistently high Sr values of around 5 to 10×10^{-3} as marine residents or 'sea eels' that had spent their entire life in the sea, and showed no evidence of ever having migrated into freshwater habitats.

Besides these 2 distinctive types of eels with consistently high or low Sr/Ca ratios, there were others with intermediate or variable Sr/Ca ratio patterns, and for convenience we generally refer to these specimens as 'estuarine eels'. Furthermore, these estuarine eels were roughly divided into the following 3 patterns.

Specimens belonging to the first pattern showed changes in their Sr/Ca ratios that indicated a single movement from 1 salinity habitat to the next ('secondary migrants'). Most of these showed a temporary decrease in their Sr/Ca ratio ($<5 \times 10^{-3}$) between 300 and 800 μm along the radius, returning to higher values ($\geq 5 \times 10^{-3}$) out to the otolith edge (Yoshida Port-3, Hamana Lake-24, and Mikawa Bay-3: Fig. 3). This pattern was consistent with the 2-dimensional images of 5 (Hamana Lake-24) and 6 (Mikawa Bay-3) in Fig. 2, in which peaks of Sr/Ca values roughly correspond to the annulus-like yellow rings in Fig. 2. These specimens presumably went upstream and settled in the freshwater habitat before returning to the ocean, where they were collected. A few specimens classified as sea eels above (e.g. Hime Island-8: Fig. 3) showed an initial drop in the Sr/Ca ratio just outside the high Sr core similar to those of secondary migrants of the intermediate type (e.g. Hamana Lake-24: Fig. 3). However, we could distinguish between secondary migrants and sea eels by their mean Sr/Ca values during the initial drop between 300 and 800 μm: secondary migrants had lower values, equivalent to freshwater specimens, whereas sea eel values never dropped to the freshwater level. It was noteworthy that there were many secondary migrants from the freshwater to the seawater
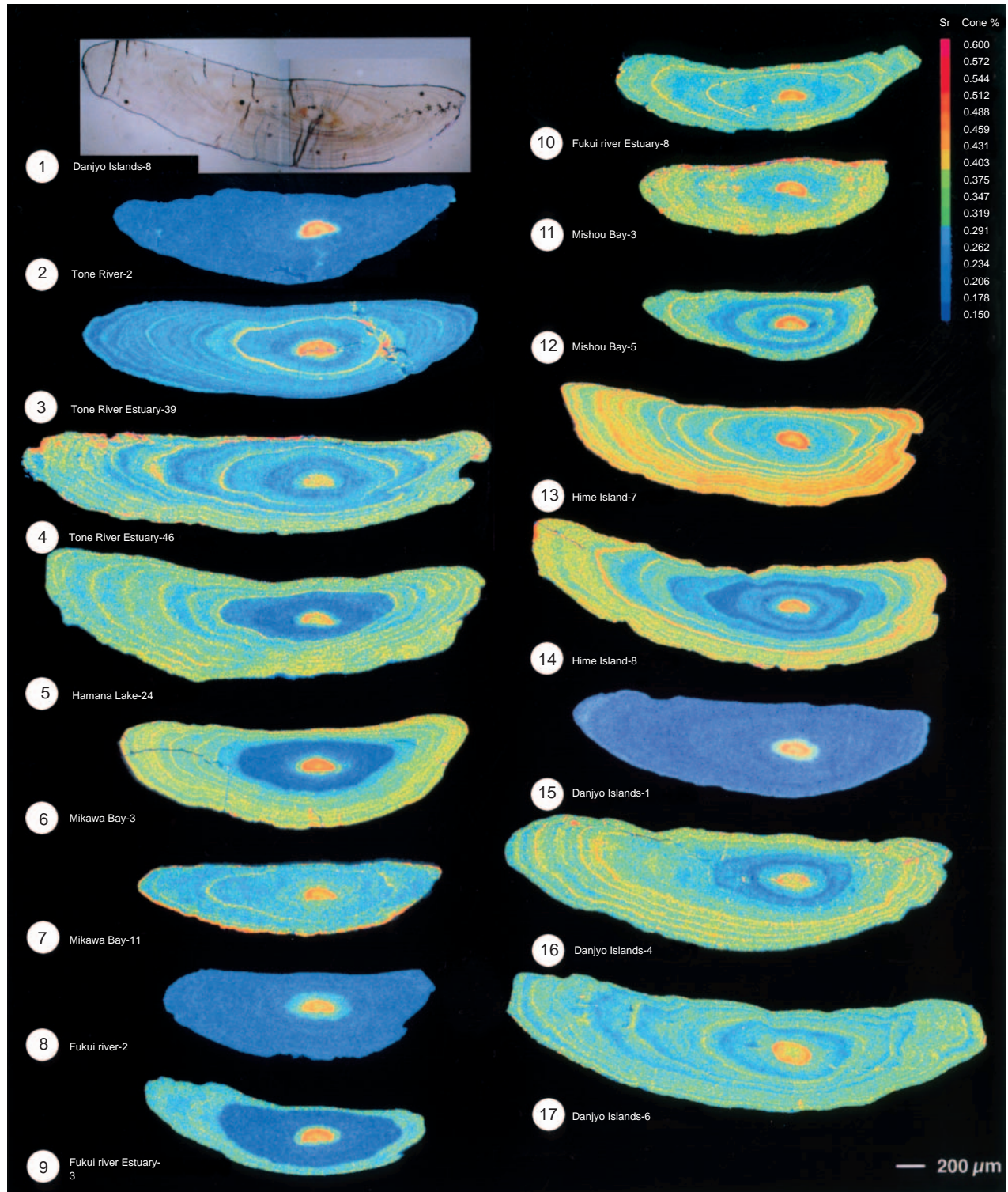
Fig. 2. *Anguilla japonica*. Two-dimensional imaging using X-ray electron-microprobe analysis of the Sr concentration in the frontal plane of sagittal otoliths of the Japanese eel collected from 10 localities in Japanese coastal waters and rivers. Values preceding locality names correspond to Sr concentrations, represented by 17 colors from red (highest), through yellow, green, to blue (lowest), numbers following locality names are specimen identification numbers. On top left is a photograph of an otolith taken with a light microscope (1: Danjyo Islands-8)

habitat among the intermediate types, but only a few specimens among the estuarine samples from the Tone River showed a reverse shift from the marine to the freshwater habitat. These specimens may have spent their glass eel and early yellow eel stages in brackish water, and then undertaken a delayed secondary migration into freshwater.

Specimens displaying the second pattern were characterized by a frequent transition between freshwater, estuarine, or seawater habitats ('transients'). They had several peak values higher than 5 to 10×10^{-3} outside the high Sr core, with lower values between (Tone River Estuary-39 and 46: Fig. 3). This pattern was also consistent with the 2-dimensional images in 3 (Tone River Estuary-39) and 4 (Tone River Estuary-46): Fig. 2, in which peaks of Sr/Ca values roughly correspond to the annulus-like yellow rings in Fig. 2.

Specimens exhibiting the third pattern was characterized by consistent intermediate values (around 3 to 5×10^{-3}) from the high Sr core out to the otolith edge (Tone River Estuary-53 and Mikawa Bay-14: Fig. 3), suggesting that they had stayed in the brackish water habitat from their arrival in the estuary until our collection ('estuarine residents').

The classifications of these 3 patterns for the intermediate types were not rigid, since some specimens showed a mixed pattern or intermediate changes and thus were difficult to classify.

Index of seawater residence

In order to quantitatively estimate the general habitat use of each specimen based on its mean Sr/Ca ratio values, we calculated an index for the degree of seawater residence as follows. Since all specimens had experienced the same common marine life as a preleptocephalus, leptocephalus, metamorphosing larva and early glass eel during their long trip from the spawning area to coastal waters, the values of Sr/Ca inside the
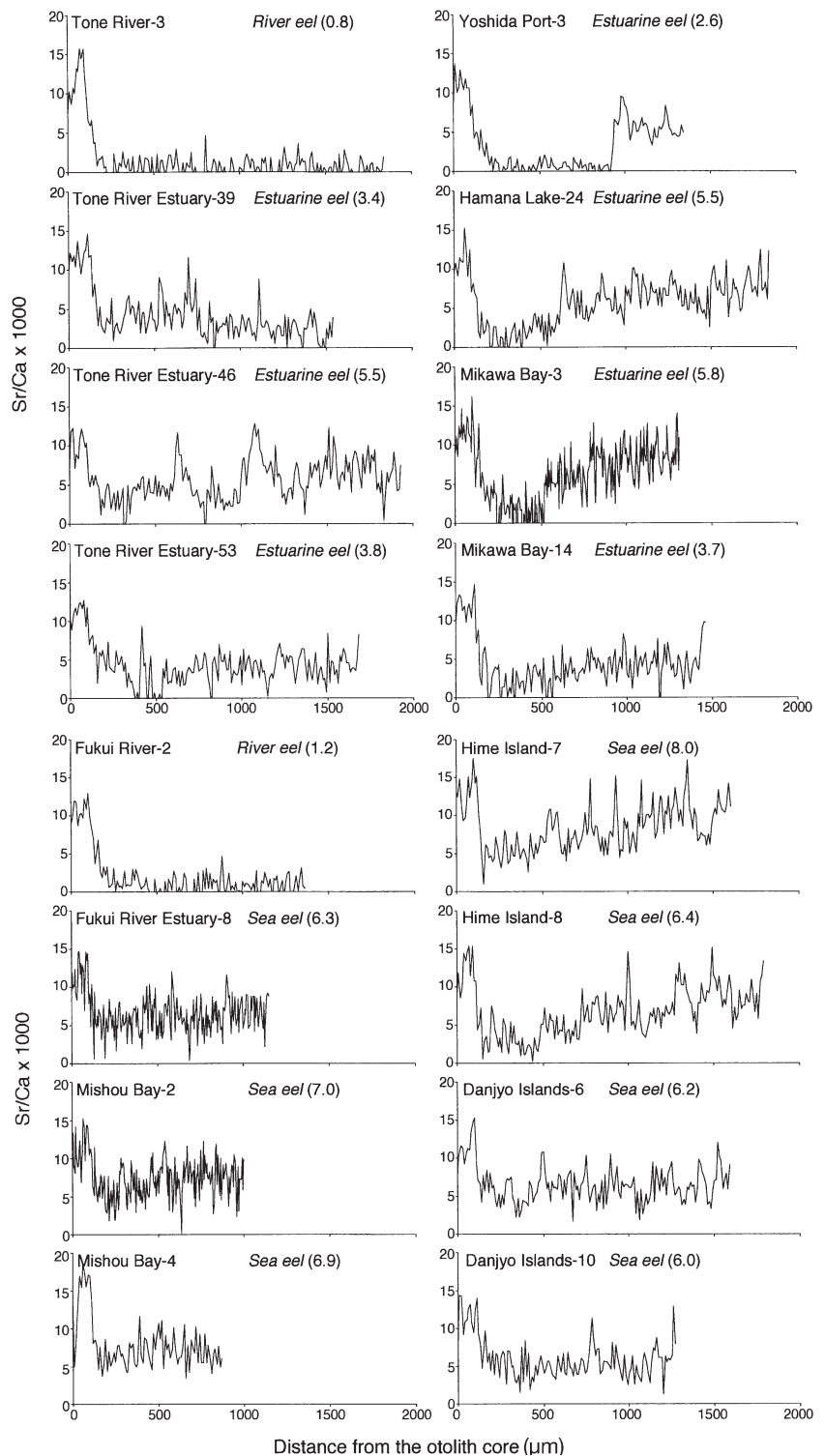

Fig. 3. *Anguilla japonica*. Typical changes in otolith Sr/Ca ratio along line transects from the core (0 µm) to the edge in the frontal plane of sagittal otoliths of specimens collected from various localities. Specimen identification numbers are given after locality names, and the type of migratory history of each specimen, classified based on Sr/Ca pattern of change outside the 150 µm elver mark is indicated in italics in each panel: mean Sr/Ca ratio values outside elver mark (×1000) are in parentheses
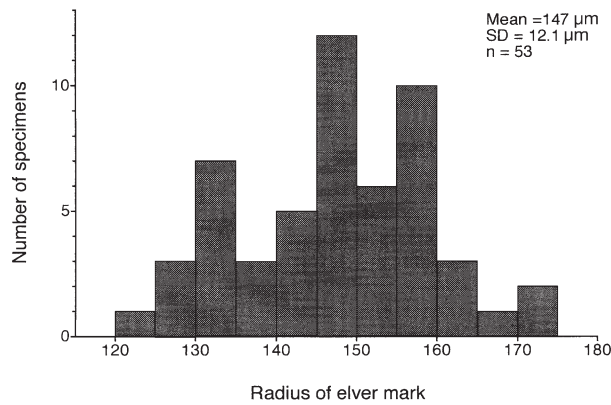
Fig. 4. *Anguilla japonica*. Frequency distribution of mean radius of elver mark (distance from the otolith core to the elver mark) in otoliths

elver mark could be excluded from each life-history transect (see Fig. 3) when estimating the degree of sea-water residence for the juvenile stages of each individual after arrival in coastal waters at the early glass eel stage. The radius of the elver mark ranged from 121 to 173 µm and the mean ± SD was 147 ± 12.1 µm (Fig. 4). Based on this, the mean value of Sr/Ca outside 150 µm along the radius to the otolith edge was calculated for all samples. The mean Sr/Ca ratio values outside 150 µm ranged from 0.8 to 8.0×10^{-3}, with some peaks at 1.0 to 1.5, 2.5 to 3.0, 5.0 to 5.5 and 6.0 to 6.5×10^{-3}, respectively (Fig. 5). Specimens with mean values less than 2.5×10^{-3} were all river eels that had consistently low Sr/Ca ratio values along their otolith transects, with no shifting to higher values, while those classified as sea eels had mean values larger than 6.0×10^{-3}, and specimens with values between 2.5 and 6.0×10^{-3} were those grouped as estuarine eels (but these included
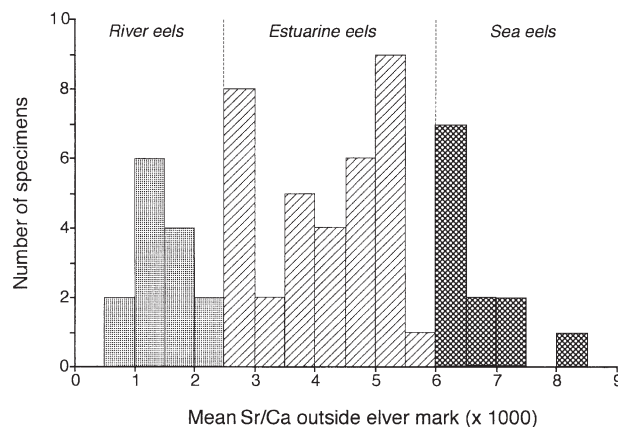

Fig. 5. *Anguilla japonica*. Frequency distribution of the mean values of Sr/Ca ratio outside the elver mark (150 µm in radius) in each otolith of specimens of the Japanese eel

individuals displaying a variety of habitat-use patterns). Thus, the index of seawater residence showed non-overlapping values of mean Sr/Ca ratios and supported the visual classification of the 3 general types of eels based on the line-transect analyses.

Distribution of the 3 eel types

Of the specimens examined, sea eels comprised 20% of all specimens, river eels 23% and estuarine eels were the most abundant (57%) of the 3 types (Fig. 6). When separated into developmental stages, sea eels split into similar percentages of the total number of yellow eels (19%) and silver eels (20%), while about twice as many river eels were silver eels (29%) than yellow eels (15%). Among the 3 habitats, the highest percentage of sea eels was found in the samples from the seawater habitat (28%). In contrast, no sea eels occurred in the samples from freshwater habitat, where river eels were most abundant (86%). Estuarine eels were dominant in the samples from the brackish water habitat (80%).

Sea eels were dominant in some of the localities with seawater habitats, but estuarine eels were dominant in some of the other seawater habitats and were found in all seawater localities (Fig. 7). The percentage of sea eels in the samples were 80% at Mishou Bay, 67% at Hime Island, and 42% at Danjyo Island. However, at the most offshore seawater habitat, the Danjyo Islands, 3 river eels (25%) and 4 estuarine eels (33%) were also found. Estuarine eels were exclusively found at the other seawater habitats at Yoshida Port (100%), Hamana Lake (100%) and Mikawa Bay (70%), and no sea eels were among the samples. At Mikawa Bay, 3 silver-phase river eels (30%) were found. River eels were dominant in the Tone River (80%) and Fukui River (100%), but in their estuaries, estuarine eels were dominant, with percentages in the Tone River Estuary and the Fukui River Estuary of 90 and 60%, respectively. One sea eel was found in Fukui River Estuary. The 3 males from the Danjyo Islands were categorized as 2 river eels and 1 estuarine eel, and the others were all female.

DISCUSSION

Definition of *Anguilla japonica* migration

The most significant finding of this study was that the occurrence of resident sea eels that had never migrated into freshwater habitat was confirmed at many localities in Japanese coastal waters including the Danjyo Islands, where they were first reported by
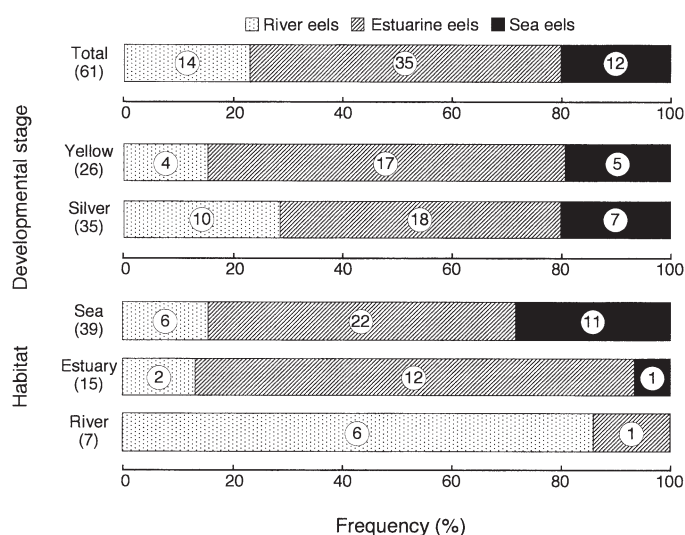
Fig. 6. *Anguilla japonica*. Frequency composition of the 3 designated types of eels with different migratory histories (river eel, estuarine eel and sea eel) based on mean Sr/Ca ratio outside the elver mark in the otolith of all specimens examined (Total), for specimens of the developmental stages of yellow- and silver-phase eels, and for the specimens collected in the 3 different habitats (Sea, Estuary, River). Numbers in parentheses on left are the total number of specimens analyzed, and numbers within each bar indicate the number of eels belonging to each type

Tsukamoto et al. (1998). Another significant result was the finding of intermediate types of eel migration (estuarine eels), within which 3 different patterns were recognized: secondary migrants, transients, and estuarine residents (Fig. 3). These findings strongly suggested that *Anguilla japonica* has a flexible migration strategy with a high degree of behavioral plasticity and an ability to utilize the full range of salinity as juveniles. In detail, however, some specimens were difficult to classify into 1 of these patterns because of a complex life-history transect or an intermediate change in values. Mark-recapture studies using microdata loggers will be needed to determine the precise correspondence between fish movement and Sr/Ca changes in their otoliths, and to evaluate whether the annulus-like rings seen in the otolith Sr concentration maps are due to habitat transition or simply a result of annulus formation or seasonal temperature changes within the same habitat.

Tzeng et al. (1997) also found 3 specimens of the intermediate type among *Anguilla anguilla* yellow eels collected from brackish water along the Swedish coastline (which would be classified as 'estuarine residents' in this study) with stable intermediate values of Sr/Ca ratio (about 5×10^{-3}). Four specimens collected from freshwater lakes (Groups 4 and 5 in their paper) showed a unique pattern of otolith Sr/Ca ratios, with

both a series of intermediate values (ca 5×10^{-3}) and subsequent low values (ca 0×10^{-3}) from the inside to the edge of otolith. This shift in the ratio appeared to correspond to the artificial transplantation of the eels (collected in a brackish water habitat) into the freshwater lakes. Among silver eels collected in offshore brackish waters of the Baltic Sea, Tzeng et al. (2000) also observed 'estuarine eels' with stable intermediate Sr/Ca ratios.

Considering that there were estuarine eels at all the localities of this study except the Fukui River, it appears that it is not unusual for *Anguilla japonica* to live in estuarine or seawater habitats for long periods of time, and this appears to be a geographically widespread phenomenon that is not limited to *A. japonica* (Tsukamoto et al. 1998). It is well known among fishermen that large numbers of immature yellow eels occur in estuarine waters in Japan. In addition, male yellow eels of the American eel *A. rostrata* are common in estuarine habitats in the southeastern USA (Helfman et al. 1987), and over 80 % of European eels in the commercial ocean catch in the North Sea are yellow; i.e. not yet in the silver phase (F. W. Tesch pers. comm.). It has been generally assumed that the yellow eels found
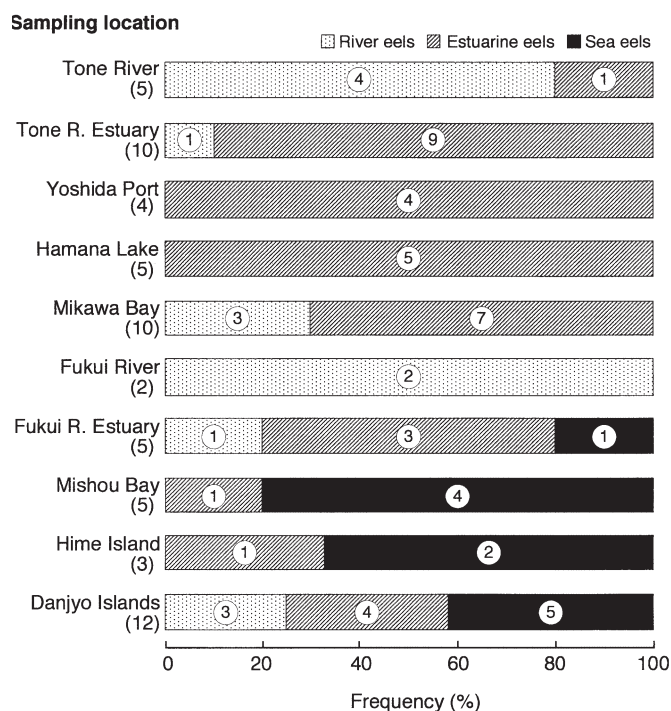

Fig. 7. *Anguilla japonica*. Frequency composition of 3 types of eels (river eel, estuarine eel and sea eel) collected from the 10 localities. Numbers in parentheses on left are total number of specimens analyzed at each locality, and numbers within each bar indicate the number of eels belonging to each type.

in coastal waters have been washed out of rivers by floods, but this has not been tested. Tagging studies have shown that some sea eels in the North Sea migrated into brackish or freshwater areas (Lowenberg 1980), suggesting a secondary movement after first settlement ('reverse' secondary migrants in this study). Our results have demonstrated that the eels found in coastal and estuarine habitats can be resident in these areas and also may move back and forth between freshwater and saltwater (Fig. 8). Thus, the classification of anguillid eels in all major ichthyology texts as being catadromous and having a freshwater growth stage clearly needs revision, because it is now evident that their movement into freshwater is not an obligate migratory pathway, and should be defined as a facultative catadromy, with ocean and estuarine residents as ecophenotypes.

Cause of occurrence of sea residents

In anadromous salmon, freshwater residents or landlocked populations that do not migrate to the ocean often occur, especially near the southern limit of their geographical distribution (McDowall 1988). The ancestors of salmon, which originated in freshwater, expanded their growth habitat into the ocean while their breeding place remained in freshwater. Since reproduction is physiologically costly, it was hypothesized that the migratory behaviour remained conservative through its evolutionary process (Tcharnavin 1939). Freshwater eels of the genus *Anguilla* are considered to have originated from a marine ancestor (Tsukamoto & Aoyama 1998), and all anguilliform fishes except *Anguilla* are marine species; thus, the marine breeding habits of *Anguilla* are probably a conservative trait. This suggests the hypothesis that at least some species of catadromous eels have never lost the ability to be resident in marine habitats during the juvenile growth phase, but it is unknown whether this is due to a remnant genetic trait that determines if an individual will enter freshwater or not, or if it is simply due to behavioral plasticity that enables each species to use the maximum range of habits.

Fish migration is generally explained by a difference in food abundance between marine and freshwater habitats (Gross 1987). Juvenile anadromous salmon utilize low-productivity freshwater habitats at high latitudes, and they migrate to higher-productivity habitats in the ocean for growth before returning to freshwater for breeding. In contrast, catadromous freshwater eels that recruit at low latitudes, migrate upstream into freshwater habitats of higher productivity for growth before returning to the ocean for breeding. Therefore, a latitudinal cline might be predicted in which marine resident freshwater eels would occur more frequently at higher latitudes where the productivity of the freshwater habitat is lower compared to the ocean. An analysis of otolith Sr/Ca ratios needs to be made on silver-phase tropical eels during their spawning migration, and an index of seawater residence should be compared between tropical and temperate eels.

Another hypothesis for the occurrence of marine resident anguillid eels would be ecological competition with other species (Moriarty 1978). In the case of European eels, they may have strong competition with the conger eel *Conger conger*, especially in regard to predation at lower latitudes, and thus there are few reports of the occurrence of European eels in the ocean from the Central and South European and Mediterranean coasts where conger eels are plentiful. In contrast, the North Sea and Baltic Sea have no conger eels, but European eels are abundant and commercially exploited (at least 3.1 kg ha^{-1}; Lowenberg 1980). Sea eels do occur in the East China Sea, how-
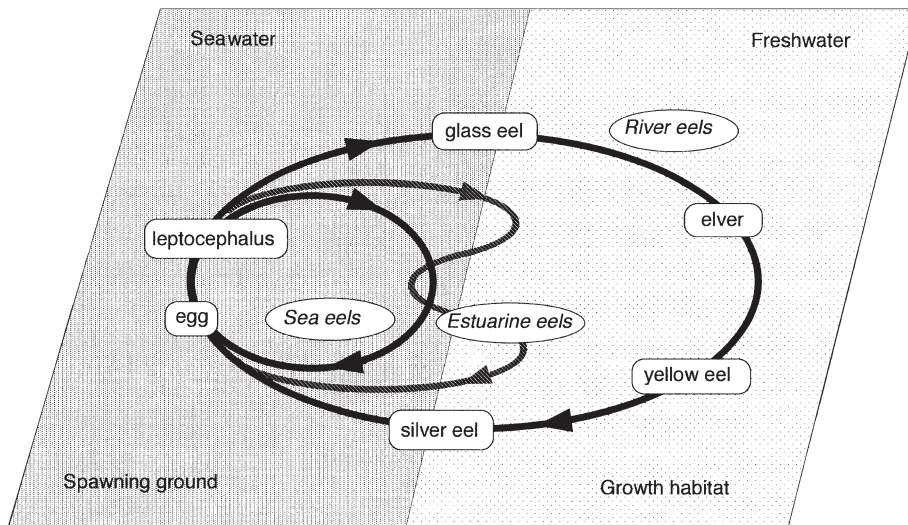

Fig. 8. *Anguilla* spp. Diagrammatic presentation of the life cycle of freshwater eels with special reference to their migratory histories. River eels migrate upstream after arriving at the estuary during the glass eel stage and stay in the freshwater habitat until their downstream spawning migration as silver-phase eels, while sea eels never migrate into the freshwater habitat and spend their entire life in the ocean. An intermediate type of estuarine eels occurs mainly in the brackish water habitat in estuaries which may move back and forth between freshwater, brackish water and/or seawater

ever, in spite of a huge stock of *C. myriaster* (more than 1000 tons harvested yr^{-1}). Although no freshwater eels have been found in the stomach contents of the conger eel in the East China Sea (U. Yamada unpubl. data), there may be other species-specific factors in each geographic area that determine the distribution and abundance of sea eels.

Contribution of sea eels to the next generation

The findings of this study indicate that *Anguilla japonica* may use seawater habitats on a widespread basis, that it appears to mature in the habitats in the same way as in freshwater, and that the silver phase estuarine and sea eels presumably migrate to the spawning areas to reproduce just as do typical freshwater eels. All the sea eels at the Danjyo and Hime Islands and about half of the 35 estuarine eels of this study were in the silver phase, indicating that they were close to migrating or were in the early stages of migration. Tsukamoto et al. (1998) also reported that all 12 sea eels collected at the Danjyo Islands in November 1994 were silver-phase eels. However, the eels in our Danjyo Islands samples collected in January 1995 (also within the same migratory season) included 25% river eels, 42% sea eels and 33% of the intermediate type. In addition, some of the silver eels regarded as sea eels by Tsukamoto et al. (1998) would have been considered as estuarine eels in this study, indicating that a variety of silver eels with different migratory histories were collected from the Danjyo Islands during both sampling periods. This collection of sea eels, estuarine eels and river eels in the same area around the Danjyo Islands, relatively far from any substantial freshwater or estuarine habitat, suggests that eels from several regions were collected as they passed through the area during their reproductive migrations, and that estuarine and sea eels probably migrate to the spawning area in a fashion similar to that of silver eels from freshwater.

To make a rough estimation of the contribution of sea eels to spawning stock, we collected as many specimens as possible in this study from various habitats around Japan. As a result we obtained a frequency distribution for the degree of seawater residence (Fig. 5), which shows the relative proportion of the 3 types based on these samples. However, this distribution may be somewhat biased because of the different number of specimens available in the various habitats. If we examined more specimens under an ideal sampling regime, a different distribution of the 3 types might be obtained. Additional research on the relative abundance of eels in habitats of different salinities habitats would be required to estimate the proportion

of sea eels, estuarine eels and river eels that contribute to the spawning population of *Anguilla japonica* each year. However, this study does indicate that eels which utilize the marine environment to various degrees during their juvenile growth phase may make a substantial contribution to the spawning stock each year, especially if escape from freshwater habitats is relatively low due to commercial harvesting.

Applied significance of sea eels

The results of this study, which indicate that most of the eels examined in ocean and estuarine habitats were either resident there or had moved between freshwater and marine habitats, have several important commercial implications. Firstly, it has demonstrated that eel aquaculture probably can be carried out entirely in seawater without the need for a freshwater stage. Growth rates, and rates of disease infection need to be tested in freshwater and seawater to determine which of these is best suited for aquaculture. Secondly, if eel resources are declining due to the destruction of freshwater habitats and intensive harvesting by the commercial fishery in freshwater and estuaries of East Asia, the sea eel as an ecophenotype of the Japanese eel, may be very important in maintaining stocks of this species. Detailed studies, focusing in estimation of population sizes for the 3 types of eels, or a time-series sampling of coastal migrants of silver eel using set nets, are needed to understand the population dynamics of *Anguilla japonica* and the relative importance of the different ecophenotypes of this species.

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