

# Age and body size of Japanese eels, *Anguilla japonica*, at the silver-stage in the Hamana Lake system, Japan

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**Abstract**— Effects of salinity-different habitats on growth rate, age and body size of Japanese eels at the silver-stage were investigated. Fish were sampled in one drainage system, that includes the brackish Hamana Lake and its inlet freshwater rivers, and were evaluated using otolith strontium:calcium (Sr : Ca) analysis to determine whether the eels had been living in estuarine or freshwater habitats. The growth rate of estuarine eels was found to be significantly higher than that of river eels for both females and males. In male silver eels, there was no difference in age between estuarine and river eels, but the total length and body mass (BM) of estuarine eels were significantly larger than river eels. In female silver eels, the age of river eels was significantly older than those of estuarine eels and the BM of river eels was significantly greater than in estuarine eels. These results indicate that effects of habitat use and salinity differences on age and body size at the silver-stage were different between female and male eels in this system.

**Key words:** Silver eel, growth rates, body size, otolith microchemistry, salinity

## Introduction

The Japanese eel, *Anguilla japonica*, has a complex migratory life cycle because it is a catadromous anguillid eels that uses both freshwater and marine environments during its life history. This species spawns far offshore in the ocean in the seamount area of the southern part of West Mariana Ridge in the western North Pacific (Tsukamoto et al. 2011), and its larvae are transported to continental waters in East Asia (Tsukamoto 2009) where they spent most of their life as yellow eels (Aoyama 2009). After a long growth period, they metamorphose from yellow eels to silver eels and migrate back towards the ocean for spawning (Aoyama and Miller 2003).

Anguillid eels are traditionally known as freshwater fishes during their juvenile growth phase as yellow eels, but evidence from otolith microchemistry demonstrates that some eels in continental waters reside estuarine areas or nearby coastal areas (Tsukamoto and Arai 2001, Daverat et al. 2006). It has also been reported that the type of habitat used by several anguillid species can affect their growth rates, with higher growth rates in brackish or seawater than in freshwater within a particular river system (Tzeng et al. 2002, Morrison et al. 2003, Arai et al. 2004, Daverat and Tomas 2006, Lamson et al. 2009). Contrary to these reports, Kotake et al. (2005) found only minor effects of habitat salinity on age and body size of silver eels of the Japanese eel

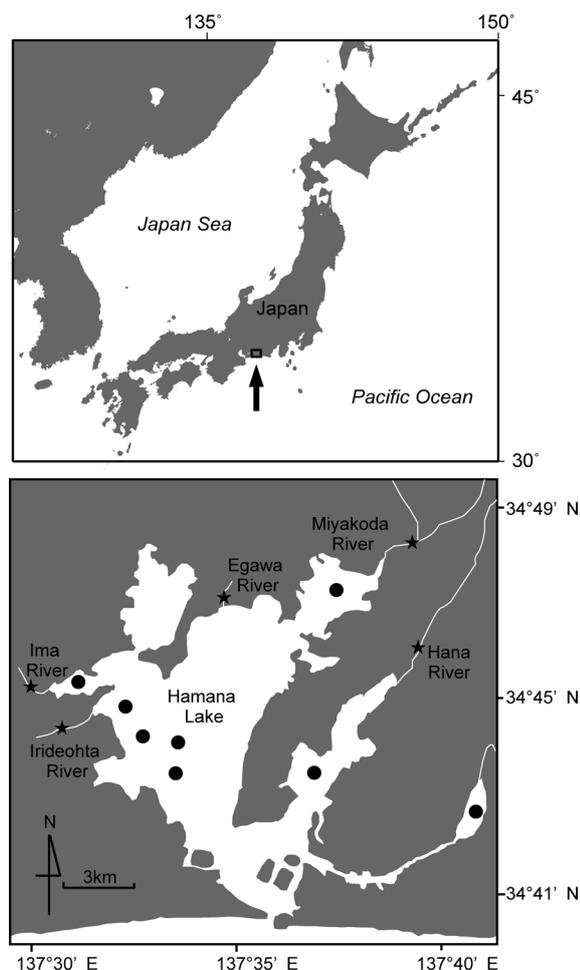
in Mikawa bay, Japan. Because silver eels leaving continental habitats are the only chance to evaluate the eels that may contribute to reproduction, it is important to accumulate more basic data about silver eels, such as age and body size, which can lead to understanding demographic aspects of eels that use different habitats and provide useful information for resource conservation.

In this study, we investigated the effects of habitat salinity-different habitats on growth rate, age and body size of Japanese eels at the silver-stage. To exclude the effect of latitude difference that can cause growth rate variability in anguillid eels (Cairns et al. 2009), we sampled fish in one freshwater and estuarine system that is the brackish Hamana Lake and its inlet freshwater rivers. However, because of inter-habitat movements or downstream migration, the locations in which the eels were collected does not necessary reflect their growth habitat. To solve this problem, we conducted otolith strontium:calcium (Sr:Ca) analysis to determine whether the eels had lived in the brackish lake or in freshwater rivers.

## Materials and methods

### Study area

The Hamana Lake system is located in central Japan



**Fig. 1.** Maps showing the location of the sampling areas in Hamana Lake and its inlet rivers in Shizuoka Prefecture of Japan. Lake sampling sites (●), and river sampling sites (★) are shown.

(34°45'N, 137°35'E), and is comprised of Hamana Lake and more than 17 inlet streams and rivers (Fig. 1). Hamana Lake is a brackish lake with surface salinity ranging from 22 to 33 psu depending on tide, with a connection to the Pacific Ocean only by the Imakireguchi channel (200-m width), and all its inlet tributaries are pure freshwater (salinity close to zero).

### Fish

In this study, we collected a total of 252 Japanese eels in Hamana Lake and its inlet rivers (Hana River, Miyakoda River, Egawa River, Ima River, and Irideohta River) from 2006 to 2008 (Table 1). In Hamana Lake, sampling was performed by commercial fishermen using fyke nets and eel pots. In rivers, eels were collected by electro-fishing. Immediately after collections, the live eels were quickly transported to the laboratory and divided into yellow eels and silver eels, according to a silvering index for the Japanese eel (Okamura et al. 2007). Then, they were anesthetized with 0.08% 2-phenoxyethanol. The total length (TL) of all eels

**Table 1.** Sampling year, month and the number of eels collected in Hamana Lake system.

Year	Month	Lake		River
		Female	Male	Female
2006	May	4	1	
	Jun	5		
	Jul	3	2	
	Aug	9		6
	Sep	10		
	Oct	10		5
2007	Jul	17	1	2
	Sep	9	2	8
	Oct	26	4	12
	Nov	14	2	5
2008	Dec	6	1	4
	Sep	6	18	
	Nov	16	35	
Total		144	66	42

was measured to the nearest 0.1 cm, and body mass (BM) to the nearest 0.1 g. Eels were sacrificed by decapitation while still anesthetized, and then their saggital otoliths were collected.

### Otolith microchemistry

The extracted otoliths were embedded in epoxy resin (Struers, Epofix) and ground to expose the core along the anterior-posterior direction in the frontal plane using a grinding machine equipped with a diamond cup-wheel (Struers, Discoplan-TS). They were further polished with OP-S liquid (Struers), cleaned using distilled water and ethanol, and dried in an evaporator prior to examination. For electron microscope analysis, otoliths were Pt-Pd coated by a high vacuum evaporator. Life history transect analysis for Sr and Ca concentrations in all specimens was carried out from the core to the edge using a wavelength-dispersive X-ray electron microprobe (JEOL JXA-8900R), as described previously (Tsukamoto and Arai 2001).  $\text{CaCO}_3$  and  $\text{SrTiO}_3$  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  $10\mu\text{m}$  in diameter, with measurements spaced at  $10\mu\text{m}$  intervals.

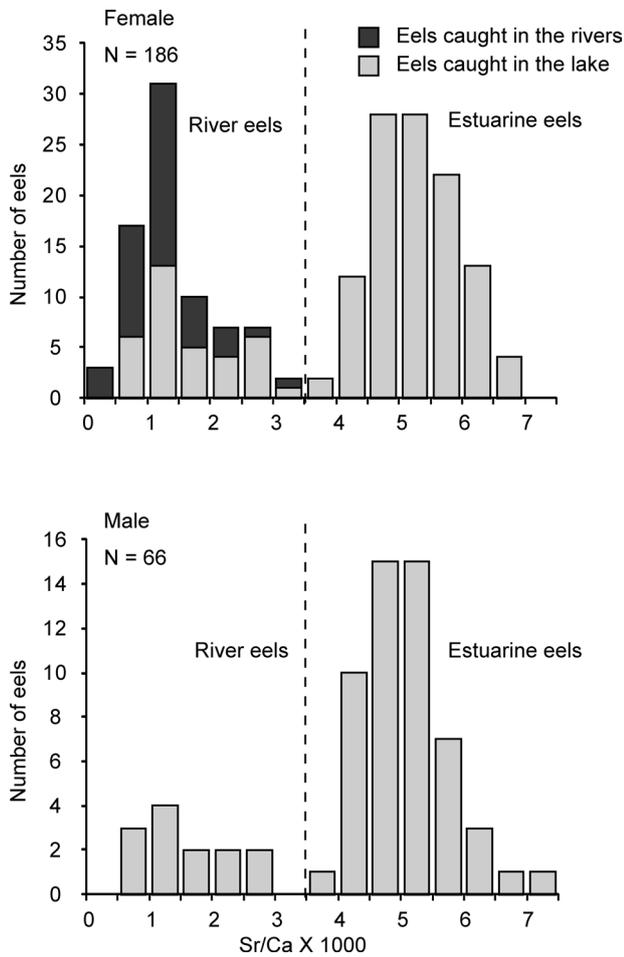
### Habitat determination

To determine the habitat use of the eels, we calculated the average Sr:Ca ratios for the values outside the elver mark. We grouped all specimens into the two categories: 'river eels' ( $\text{Sr:Ca} < 3.5 \times 10^{-3}$ ) that inhabited a freshwater river after upstream migration and 'estuarine eels' ( $\text{Sr:Ca} \geq 3.5 \times 10^{-3}$ ) that stayed in the brackish Hamana Lake, based on their mean otolith Sr:Ca ratios. The otolith Sr:Ca ratios cri-

teria of this study was based on the frequency distribution of the mean values of Sr:Ca ratio, which showed a bimodal distribution (Fig. 2).

**Age and growth**

Following the electron microscope analysis, the otoliths



**Fig. 2.** Frequency distribution of the mean values of Sr:Ca ratio outside the elver mark in the otolith of the Japanese eels examined in this study.

were polished to remove the coating, etched with 1% HCl, and thereafter stained with 1% toluidine blue. The age of specimens was determined by counting the number of blue-stained transparent zones outside the elver mark, following the method of Nagiec & Bahonsawy (1990). Annual growth rate was estimated by size and age as follows: growth rate = (TL-6.0)/Age, where 6.0 is the mean length (cm) of glass eels when they recruit to estuaries (Tsukamoto 1990, Tzeng et al. 2000).

**Data analysis**

Each parameter was presented as the mean±standard deviation (SD). Comparisons between groups were made by two-tailed Mann-Whitney U-test, using Excel Stat 2008 software (SSRI, Tokyo).

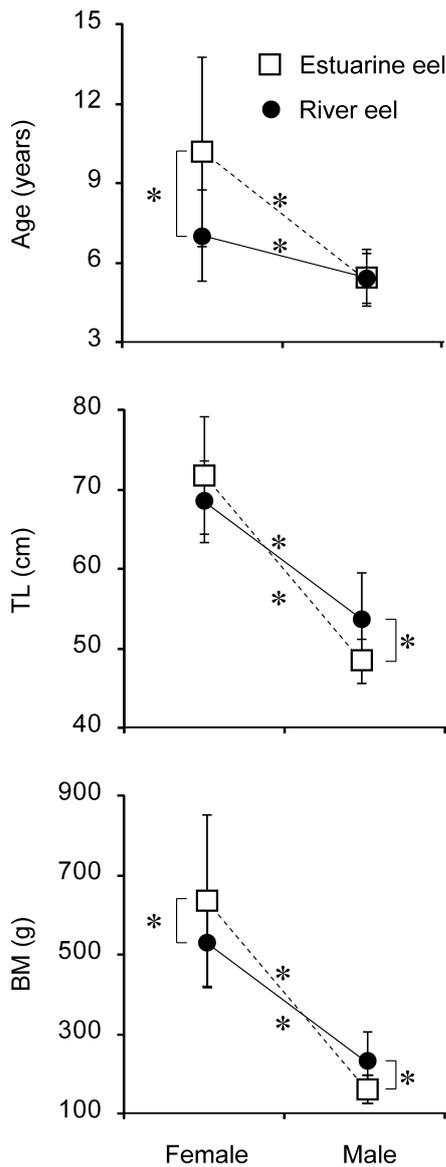
**Results and Discussion**

The body sizes and age of eels used in this study were summarized in Table 2. A total of 252 Japanese eels collected in the Hamana Lake system were classified into 160 yellow eels (125 females and 35 males) and 92 silver eels (61 females and 31 males). In both river and estuarine females, silver eels were significantly older (river eels: 10.2±3.6 years, estuarine eels: 7.0±1.7 years) than yellow eels (river eels: 7.4±2.3 years, estuarine eels: 5.8±1.5 years). In males, silver eels (river eels: 5.5±1.1 years, estuarine eels: 5.4±0.9 years) were also significantly older than yellow eels (estuarine eels: 4.9±1.0 years). As for sizes, female silver eels (TL of river eels: 71.9±7.4 cm, the BM of river eels: 637.9±216.3 g, TL of estuarine eels: 68.5±5.1 cm, the BM of estuarine eels: 529.5±112.2 g) were significantly larger than yellow eels (TL of river eels: 59.7±6.7 cm, BM of river eels: 327.1±148.9 g, TL of estuarine eels: 61.4±7.2 cm, BM of estuarine eels: 358.7±151.3 g) in both habitats, whereas no significant differences in males were observed between yellow eels (TL of estuarine eels: 52.6±4.0 cm, BM of estuarine eels: 213.8±

**Table 2.** Biometrical characteristics of Japanese eel collected in Hamana Lake system.

	Female				Male		
	Estuarine eel		River eel		Estuarine eel	River eel	
	Yellow	Silver	Yellow	Silver	Yellow	Silver	Silver
n	89	23	36	38	35	18	13
Age (years)	5.75±1.48 3-10	7.04±1.72 4-12	7.42±2.27 5-16	10.21±3.59 6-22	4.89±0.99 4-7	5.44±0.92 4-7	5.46±1.05 4-7
TL (cm)	61.4±7.2 46.1-76.5	68.5±5.1 59.8-81.5	59.7±6.7 50.1-76.5	71.9±7.4 57.5-85.6	52.6±4 42.5-59.4	53.8±5.9 45.6-63.4	48.5±2.8 43.7-52.9
BM (g)	358.7±151.3 118.2-696.7	529.5±112.2 359.8-831.5	327.1±148.9 137.5-791.9	637.9±216.3 300-1221.1	213.8±55.7 118.8-359.1	232.1±76.4 117.8-384.3	164.8±29.8 107.7-210.5

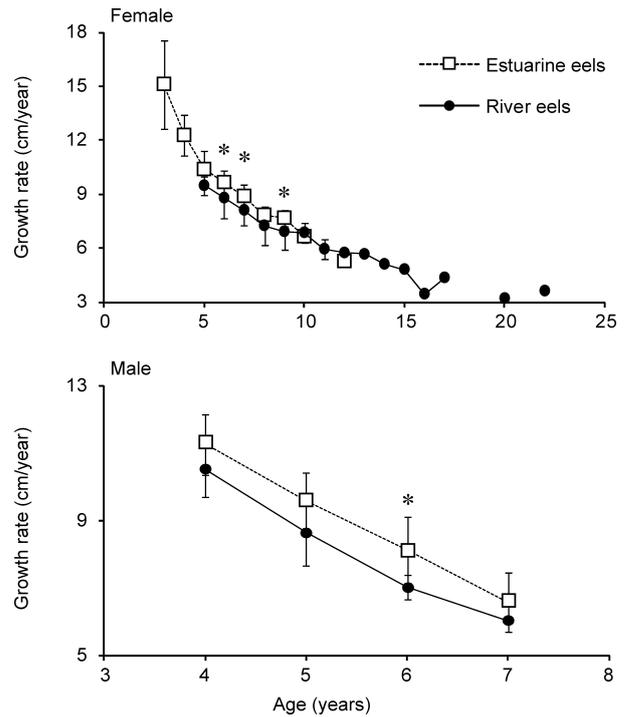
Note: upper numbers: mean±SD; lower: range, \*indicate significant difference between yellow and silver eel.



**Fig. 3.** Comparison of age, TL and BM between estuarine and river eels in both sexes at silver-phase. Solid and dotted lines and brackets show the statistical comparisons, and the asterisks indicate significant differences.

55.7 g) and silver eels (TL of river eels:  $48.5 \pm 2.8$  cm, BM of river eels:  $164.8 \pm 29.8$  g, TL of estuarine eels:  $53.8 \pm 5.9$  cm, BM of estuarine eels:  $232.1 \pm 76.4$  g).

Female silver eels had significantly older ages and larger body sizes (TL and BM) than male silver eels in both estuarine and river eels (Fig. 3), which indicates that males initiated spawning migration at younger age and with smaller body size than females. These sexual dimorphisms were also observed in other temperate eels (e.g. *Anguilla anguilla*: Moriarty 2003, *A. rostarata*: Oliveira 1999, *A. australis*: Todd 1980) and some tropical eels (*A. marmorata*: Robinet et al. 2003, *A. reinhardtii*: Walsh et al. 2003). These differences appear to be a common phenomenon in anguillid eels, and it was hypothesized that male eels use a time-minimizing strat-



**Fig. 4.** Mean growth rates of male and female Japanese eel at each age in the Hamana Lake system, Japan. Asterisks indicate significant differences.

egy and migrate at the earliest possible age, whereas females use ‘a size-maximizing strategy’ to maximize size and migrate at an optimal body size (Helfman et al. 1987). In male fish, the energy cost of spermatogenesis thought to be relatively low. Thus, it is sufficient for male eels to reach the minimum size that is needed to complete their migration to reach the spawning site. In female fish, fecundity depends on body size (Wootton 1984). Female eels use ‘a size-maximizing strategy’ for maximizing fecundity, which is closely related to fitness.

This study showed that the growth rate of estuarine eels was higher than that of river eels in both females and males (Fig. 4), which is similar to studies on other species of anguillid eels. For age 6, 7 and 9 female eels and age 6 male eels, there were significant differences between freshwater and estuarine eels ( $p < 0.001$ ). Eels in other ages could not be compared statistically because it is impossible to detect statistical differences when small sample size is too small ( $n < 5$ ). Growth rates also decreased, as the age increased in both females and males from both habitats (Fig. 4). For temperate eels, there are some studies showing that growth rate was higher in brackish water than in freshwater (Morrison et al. 2003, Tzeng et al. 2003, Arai et al. 2004, Cairns et al. 2009, Lamson et al. 2009, Kaifu et al. *in press*). Thus, higher growth rate in brackish water might be a common feature in temperate eels in the present day conditions of estuaries and rivers after human impacts have occurred in both types of habitats. Decreases in growth rates of eels with age are also

common feature of the Japanese eel in several parts of Japan (Kotake et al. 2007).

In this study, we also investigated the differences in age and body size of silver eels from estuarine and freshwater habitats. In male silver eels, there was no significant difference in age between estuarine and river eels, but the TL and BM of estuarine eels were significantly larger than river eels (Fig. 3). In female silver eels, the age of river eels was significantly older than those of estuarine eels and the BM of river eels was greater than in estuarine eels (Fig. 3), while no significant difference was observed in TL between habitats. These results indicate that effects of the two types of habitats on age and body size at the silver-stage were different between females and males, though the ecological meanings of these differences are unclear.

The Japanese eel is widely distributed from subtropical Taiwan to North of Japan so the environmental influences on its life history may vary. This wide distribution may have effects on its migratory strategy through the various environmental factors such as temperature and day length. In addition, mortality in each habitat was not evaluated in this study, although it could have some effect on migratory strategy. For a better understanding the demographic aspects of eels, habitat mortality in each type of habitat should be considered in future studies.

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