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Morphological and physiological characteristics of an oceanic-migrating Japanese eel *Anguilla japonica* off Fukushima, Japan

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Abstract — A female silver Japanese eel *Anguilla japonica* (681 mm TL) was captured by bottom trawl off Fukushima on 18 October 2013. This is the first report of an oceanic-migrating Japanese eel in the western North Pacific Ocean off northern Japan. We described the status of the silvering stage, morphological characteristics, age, developmental stage of the ovaries, and the serum levels of sex steroids for the eel. The GSI of the eel was 2.33 and the mean diameter of the largest group of oocytes was 269.9 μm and these were at the early vitellogenic stage. The serum E_2 level was 3.9 ng/ml and 11-KT was 17.9 ng/ml. These steroid levels were higher than the mean steroid levels of female silver eels captured in Hamana Lake, but other morphological and physiological characteristics of the eel were not much different from those of silver eels captured in river, estuary, and coastal waters.

Key words: *Anguilla japonica*, spawning migration, physiology, morphology, oocyte development, sex steroids

Introduction

The freshwater eels of the genus *Anguilla* are famous for their long spawning migrations to the open ocean, and these migrations take place after a transformation from yellow eel to silver eel (Tesch 1977, Aoyama and Miller 2003). A variety of morphological and physiological changes in relation to silvering transformation have been reported in anguillid eels, including changes in the enlargement of the eyes and pectoral fins, degeneration of the digestive tract, and swim bladder modifications (reviewed in Aoyama and Miller 2003). These changes may be adaptations to the environments of the open ocean that are drastically different from their typical habitats in freshwater or coastal areas. At the same time as those changes, gonadal development begins in anguillid species, for example, the oocytes of most silver Japanese eels *Anguilla japonica* develop from the oil droplet stage to the early vitellogenic stage (e.g., Utoh et al. 2004, Sudo et al. 2011). In addition, the levels of the sex steroids, estrogen (estradiol-17β, E_2) and androgen (11-ketotestostrerone, 11-KT), in blood increase during silvering (e.g., Lokman et al. 1998, Sudo et al. 2011).

Recently, mature adult Japanese eels and giant mottled eels *Anguilla marmorata* have been caught in their spawning ground near the West Mariana Ridge, and these adult eels provided new insights into their reproductive ecology and physiology (Chow et al. 2009, Chow et al. 2010, Kurogi et al. 2011, Tsukamoto et al. 2011). However, the natural reproductive ecology and physiology of freshwater eels remain a mystery because oceanic-migrating eels have been caught only incidentally along continental margins (e.g., Matsui 1957; captured in the East China Sea) and have never been collected in the open ocean (Sasai et al. 2001, Tsukamoto et al. 2009).

In this study, we described the silvering stage, morphometric characteristics, age, developmental stage of ovaries, and serum level of sex steroids of an oceanic-migrating eel captured by bottom trawl fishery in the waters off Fukushima.

Materials and methods

A Japanese eel was captured on 18 October 2013 off Fukushima, Japan by a bottom trawl (Fig. 1). The latitude, longitude, depth, and surface water temperature of the towing point were recorded. The eel was kept in captivity for 2 days and then anesthetized until overdosed to be comatose with approximately 0.8‰ 2-phenoxyethanol before being examined at Soma branch of the Fukushima Prefectural Fisheries Experimental Station. The silvering stage of the eel was determined by the examination of the color of the pectoral fins and the body coloration according to the silvering index stages (Okamura et al. 2007). The total length (TL, mm) was

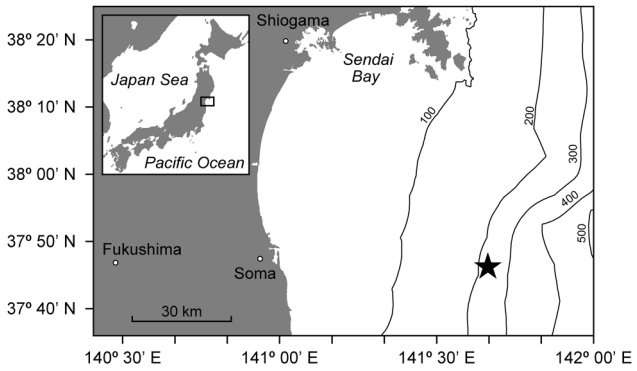

Fig. 1. Map of the collection area and its location in Japan (inset). The solid star indicates the towing site where the oceanic-migrating Japanese eel *Anguilla japonica* was captured.

measured to the nearest 1 mm and the body weight (BW, g) was weighed to the nearest 0.1 g. The horizontal (Dh, mm) and vertical (Dv, mm) left eye diameters and the left pectoral fin length (FL, mm) were measured to the nearest 0.01 mm using a slide caliper. Then, three indices were calculated: the condition factor (K), $K=10^6 BW TL^{-3}$; eye index (EI), $EI=100\pi TL^{-1}[0.25(Dh+Dv)]^2$ (Pankhurst 1982); and pectoral fin length index (FI), $FI=100 FL TL^{-1}$. The gonads, liver, digestive tract (including the stomach and intestines), and heart were removed and weighed to the nearest 0.01 g (GW, LW, DW, and HW, respectively) for the calculation of the gonadosomatic index (GSI), $GSI=100 GW BW^{-1}$; hepatosomatic index (HSI), $HSI=100 LW BW^{-1}$; digestive tract somatic index (DSI), $DSI=100 DW BW^{-1}$; and cardiosomatic index (CSI), $CSI=1000 HW BW^{-1}$ (Hagihara et al. 2012). The swim bladder was also removed and the length (RML), width (RMW), and thickness (RMT) of the rete mirabile were measured to the nearest 0.01 mm using a slide caliper for calculation of the rete mirabile length index (RMLI), $RMLI=10 RML TL^{-1}$; rete mirabile width index (RMWI), $RMWI=10 RMW TL^{-1}$; and rete mirabile thickness index (RMTI), $RMTI=10 RMT TL^{-1}$ (Yamada et al. 2001). Genetic identification was carried out using its mitochondrial DNA 16S rRNA sequence following the standard protocol of Aoyama et al. (1999).

The sagittal otoliths were removed from the eel and cleaned by removing tissue fragments. Then otolith was observed under a stereoscopic microscope using reflected light. Slow growth rings appeared dark through the dark transparent background of the container, whereas fast or opaque growth rings appeared light under reflected light. We assumed that the distinct transition check (elver check, Tzeng et al. 2002) thought to be associated with the inshore entrance of freshwater areas corresponded to an age of 0. The age of the eel was determined by counting the slow growth rings outside the elver mark.

The sex was determined as female by visual inspection of the gonad morphology. The removed ovaries were immediately immersed in ice-cold eel Ringer (NaCl 151 mM, KCl 3.3 mM, $CaCl_2$ 4.9 mM, $MgCl_2$ 3.5 mM, HEPES 10 mM; pH 7.4) and were divided into two pieces. Ovarian follicles were isolated from ovarian tissue and digitally photographed under a stereomicroscope, and the diameters of 200 randomly selected oocytes were measured using Image J (Schneider et al. 2012). The mean diameter of the largest group of oocytes (OD) was calculated by averaging the 40 largest oocyte diameters. Another piece of ovarian tissue was fixed in Bouin solution for 24 h and then transferred to 70% ethanol, dehydrated in an ascending series of graded ethanol concentrations, and embedded in paraffin. Sections of 5 μm thickness were prepared and stained with hematoxylin and eosin. Sections were observed using an optical microscope and digitally photographed.

Before the eel was decapitated, a blood sample was taken from the caudal portion using a non-heparinized syringe. After being stored at 4°C for 24 hours, the blood sample was centrifuged, and then serum was collected and stored at −20°C until steroid measurements. The concentrations of E_2 and 11-KT in the serum sample were measured by a time-resolved fluoroimmunoassay (TR-FIA) according to the methods of Yamada et al. (1997).

Results and Discussion

The migrating eel was identified as Japanese eel since determined partial sequence (503 bp) of mitochondrial DNA 16S rRNA had 99–100% identity with deposited data of Japanese eels in DDBJ (DNA Data Bank of Japan).

The time, latitude, longitude, and depth of the net-in point were 07:20 a.m., 37°46′30″N, 141°38′42″E and 258 m, while those of the net-out point were 09:10 a.m., 37°51′30″N, 141°39′48″E and 258 m, respectively, and the surface water temperature was 19.0°C. The Japanese eel was captured with a variety of demersal organisms in the same net, e.g., horsehair crab *Erimacrus isenbeckii*, chestnut octopus *Octopus conispadiceus*, yellow goosefish *Lophius litulon*, Pacific cod *Gadus macrocephalus* and greeneyes *Chlorophthalmus borealis*. The capture of migrating eels in offshore areas has been reported only from the East China Sea (Uchida 1956 cited in Matsui 1957, Matsui 1957, Sasai et al. 2001), thus this is the first report of the Japanese eel in the western North Pacific Ocean off northern Japan.

The eel had a metallic hue at the base of the pectoral fins and melanization at the tip of the fins (Fig. 2a, b), indicating that this eel was in the silver phase (S1 stage, Okamura et al. 2007) and thought to be migrating toward its spawning ground. The migrating eel was 681 mm TL and 500.8 g BW; K was 1.59; EI was 3.55; FI was 5.35; GSI was 2.33; HSI was 1.60; DSI was 0.52; CSI was 0.86; RMLI was 0.13; RMWI was 0.14; and RMTI was 0.05 (Table 1). The eel was

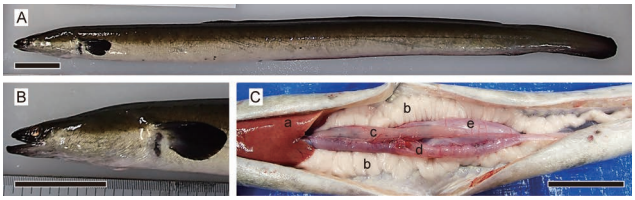

Fig. 2. The oceanic-migrating Japanese eel *Anguilla japonica* captured off Fukushima. A: Lateral view of the eel. B: Anterior part of the eel. C: Ventral dissection of the eel. (a) liver, (b) ovaries, (c) stomach, (d) intestine, (e) swim bladder. All scale bars indicate 50 mm.

Table 1. Values of morphometric and physiological characteristics of the oceanic-migrating Japanese eel *Anguilla japonica*.

	Value
Total length (mm)	681
Body weight (g)	500.8
Age (year)	8+
Condition factor	1.59
Eye index	3.55
Pectoral fin length index	5.35
Gonadosomatic index	2.33
Hepatosomatic index	1.60
Digestive tract somatic index	0.52
Cardiosomatic index	0.86
Rete mirabile length index	0.13
Rete mirabile width index	0.14
Rete mirabile thickness index	0.05
Serum E_2 level (ng/ml)	3.9
Serum 11-KT level (ng/ml)	17.9

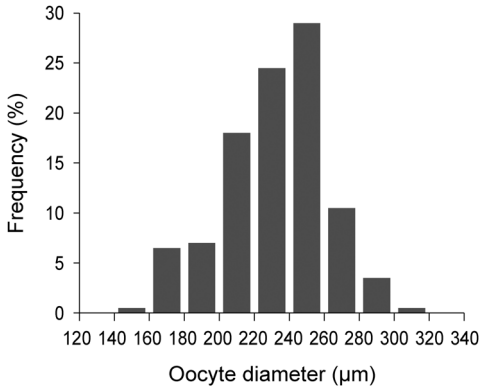

Fig. 3. The frequency distribution of the oocyte diameter of the oceanic-migrating Japanese eel *Anguilla japonica*.

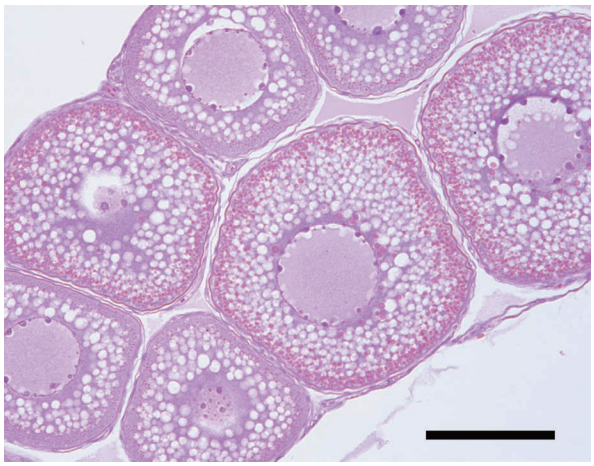

Fig. 4. Light micrograph of ovarian tissue in the oceanic-migrating Japanese eel *Anguilla japonica* showing that the oocytes of the largest group were at the early vitellogenic stage.

determined to be 8 years old based on its otolith. The condition factor (K) of the oceanic-migrating eel was clearly higher than that of adult female Japanese eels caught in the spawning ground (0.65±0.19), and the GSI of the migrating eel was remarkably lower than that of the adult females (11.65±1.74, Tsukamoto et al. 2011). Meanwhile, most indices of the migrating eel were similar to those of female silver eels captured in river, estuary, and coastal areas (Yamada et al. 2001, Okamura et al. 2007, Yokouchi et al. 2009, Sudo et al. 2011).

The macroscopic observation of gonads indicated that the eel was a maturing female (Fig. 2c). The OD was 269.9 μm. The frequency distribution of oocyte diameter showed that oocyte diameters ranged from approximately 140 μm to 320 μm with a single mode peak around 250 μm (Fig. 3). The ovarian tissue of the eel was histologically examined and the observation revealed that the largest group of oocytes was at the early vitellogenic stage (Fig. 4), and we could not find any distinct differences from oocytes of silver eels captured in estuary and coastal areas (Utoh et al. 2004, Sudo et al. 2011). The serum E_2 level was 3.9 ng/ml and 11-KT was 17.9 ng/ml. Those values were slightly higher than the mean steroid levels of female silver eels captured in Hamana Lake (mean±SE; E_2: 1.9±0.2; 11-KT: 12.3±2.8; Sudo et al. 2012), and the E_2 level was similar to the that of post-spawning female Japanese eels caught in the spawning ground (1.6–3.9 ng/ml; n= 3; Tsukamoto et al. 2011).

In this study, we described the morphological and physiological characteristics of the oceanic-migrating eel for the first time. The fact that most of these characteristics of the oceanic-migrating eel were not much different from those of silver eels captured in river, estuary, and coastal waters (e.g., Okamura et al. 2007, Yokouchi et al. 2009) possibly implies that this eel had only just started its oceanic spawning migration, and the oocytes of Japanese eels seem not to drastically develop in the early period of their spawning migration. Further sample collection of oceanic-migrating eels, including far offshore in the open ocean, are needed to gain better insight into the reproductive physiology and migration ecology of anguillid eels.

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