Fish Sci (2011) 77:199–205
DOI 10.1007/s12562-010-0318-3

ORIGINAL ARTICLE

Biology

First capture of post-spawning female of the Japanese eel *Anguilla japonica* at the southern West Mariana Ridge

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Received: 27 July 2010 / Accepted: 5 December 2010 / Published online: 7 January 2011

Abstract Two adult male freshwater eels, *Anguilla japonica*, were captured in June 2008 in the West Mariana Ridge (13°N, 142°E) in the North Pacific, but collections of females have yet to be reported. In September 2008, we successfully caught two adult female *A. japonica*, 55.5 and 66.2 cm in total length, in the adjacent but northern area (14°N, 143°E). Six newly hatched eel larvae (pre-leptocephali) were also collected by subsequent plankton sampling conducted near the female catch area. Female adults appeared to be in the post-spawning state, probably a considerable time after spawning, since a small number of remarkably regressed oocytes (50–250 μm in diameter) were observed in the ovaries. Capture of post-spawning female eels and newly hatched larvae near the Suruga Seamount (14°N) together with the previous collection of mature males in the southern area (13°N) corroborates that the area along the West Mariana Ridge is the spawning area of this species, but suggests that the eel spawning may occur over a wider area than previously expected.

Keywords *Anguilla* · Japanese eel · Female · Adult · Spawning area · Pre-leptocephali · West Mariana Ridge

Introduction

Freshwater eels of the genus *Anguilla* are catadromous and undergo migrations to their tropical offshore spawning areas

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after years of growth in freshwater and near-shore habitats [1]. Long-distance migrations are made by temperate anguillid species such as the European eel *A. anguilla* and the American eel *A. rostrata* in the Atlantic and the Japanese eel *A. japonica* in the Pacific [2], but migrating adults have historically been collected only near continental margins [1]. By means of searches for the small larvae called leptocephali, the spawning areas have been determined to be in the Sargasso Sea of western North Atlantic for European and American eels [3] and in the southern part of the West Mariana Ridge in the western North Pacific for the Japanese eel [4, 5]. In addition, age determination of leptocephali has indicated that the Japanese eel appears to only spawn during new moon periods of the spawning season [1, 6, 7].

Although the spawning areas have been determined for the Japanese eel by collecting recently hatched larvae (2–5 days after hatching) [1, 5], no adult eels had been observed or captured in the spawning areas of any freshwater eels until two mature male Japanese eels were caught in the southern part of the West Mariana Ridge (13°N, 142°E) in June 2008 [8]. No adult females of any freshwater eel species have been caught in their spawning areas. Here we report the first capture of female freshwater eels in a post-spawning state approximately 100 km north of the location of the previous catch of mature male Japanese eels.

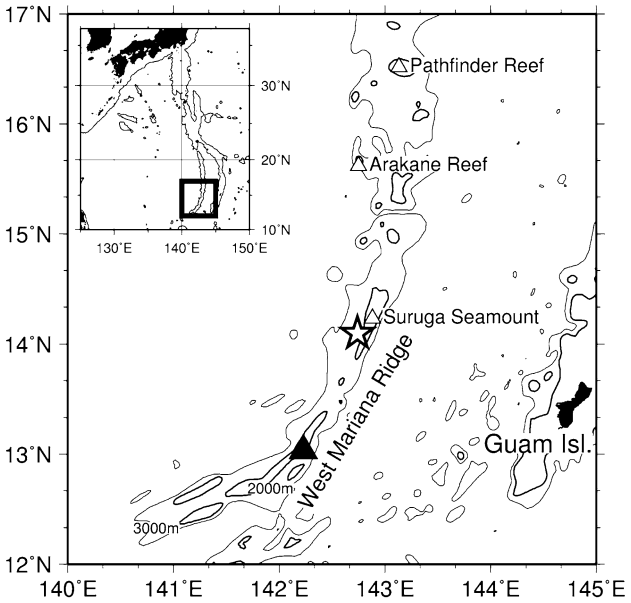

Fig. 1 Southern part of the West Mariana Ridge where adult female eels and newly hatched larvae of *Anguilla japonica* were caught. *Open star* indicates the middle position on the net towing course where adult female eels were caught, and the larvae were also collected near this point. *Closed triangle* indicates the location where matured male eels were caught [8]. Out of a total of seven tows performed, six took place around 13°N, 142°E where the males were previously caught

Materials and methods

Sampling survey

The research cruise of the *R/V Kaiyo Maru* (Fisheries Agency, Japan) took place in the southern area of the West Mariana Ridge (Fig. 1) from 25 August to 3 September 2008. Adult eel sampling was performed using a large mid-water trawl net [maximum mouth opening 60 m wide and 50 m high with 7 mm mesh cod end and a graded series of mesh sizes (60–2,010 mm) in the main body of the net; Nichimo, Tokyo]. The net was towed for 5 h during each deployment at a range of depths from 180 to 500 m. Out of a total of seven nighttime tows performed, six occurred around 13°N, 142°E, where the males were previously caught [8], and a final tow occurred around 14°N, 143°E, about 30 km south of the Suruga Seamount (Fig. 1). For larval collection, a multi-layer plankton net (Ioness; Sea, Chiba, Japan) with 1 m^2 mouth opening and 0.33 mm mesh was used and was towed for 10–15 min at six depth layers at 50 m intervals from a depth of 300 m to the surface. Anguillid larvae were sorted onboard, and total length (TL) to the nearest 0.1 mm and total number of myomeres were determined using a binocular microscope.

During the cruise, hydrographic observations consisting of conductivity, temperature, and depth measurements (CTD) were made through depths of 1,000 m for analysis of hydrographic structure.

Results

Capture of female Japanese eels

Two freshwater eels of the genus *Anguilla* (Fig. 2) were caught in the final tow conducted during the night of 31 August (new moon) to early morning of 1 September 2008 (21:00–02:00) in the northern area near the Suruga Seamount (approximately 14°N), while the six other tows conducted in the southern locations (approximately 13°N) failed to catch any freshwater eels. The successful trawl towing occurred along the course of 14°00′N–14°14′N, 142°52′E–142°36′E, at a depth from 270 to 320 m for the first 2 h 30 min followed by a shallower trawl from 180 to 230 m for the next 2 h 30 min. The two specimens collected were morphologically identified onboard to be Japanese eels. This was confirmed in the laboratory by analyzing mitochondrial DNA fragments of approximately 550 bps containing the 16S ribosomal RNA region (DDBJ/EMBL/GenBank accession numbers AB516443 and AB516444). The two eels were not captured in the cod end but were trapped in the mesh of the main body of the net

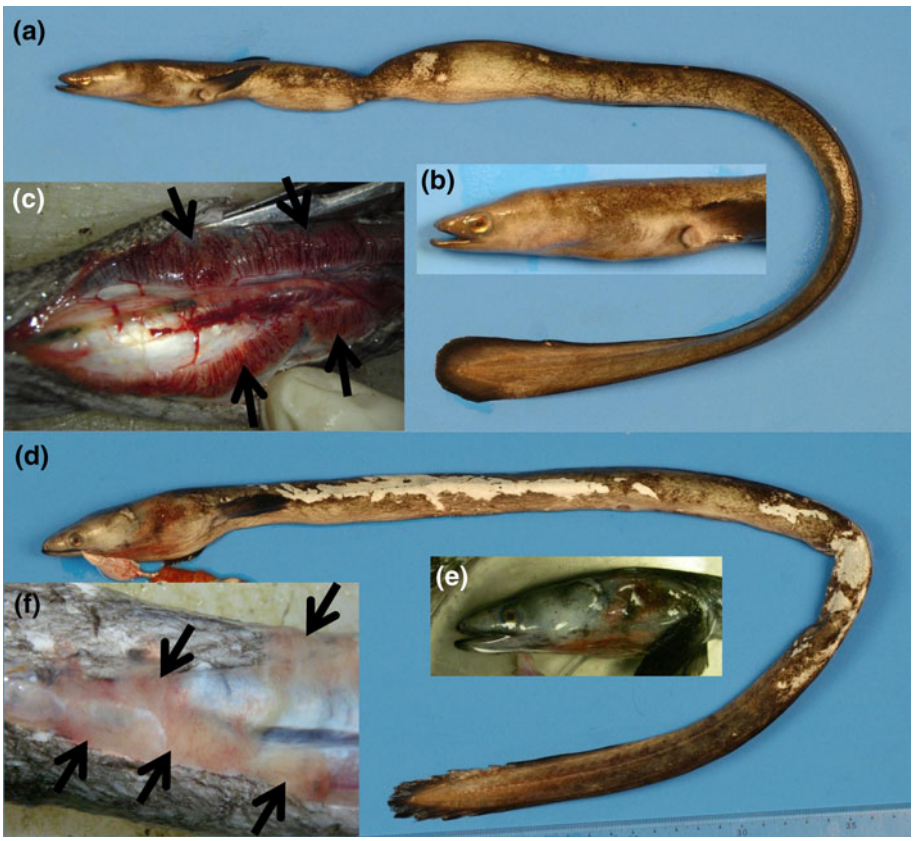

Fig. 2 Two female Japanese eels *Anguilla japonica* caught in the West Mariana Ridge. **a** Specimen no. 1: 55.5 cm total length, 90.5 g body weight. **b** Head of specimen no. 1. **c** Dissected specimen no. 1 showing shrunken ovaries with prominent blood vessels (*arrow*). **d** Specimen no. 2: 66.2 cm total length, 117.5 g body weight. **e** Head of specimen no. 2. **f** Dissected specimen no. 2 showing shrunken, membranous ovaries (*arrow*)

(1050 mm mesh). CTD profile (Fig. 3) indicated that the survey areas were in the North Pacific Tropical Water (NPTW), which showed a subsurface salinity maximum (>35.0 PSU) around the 150–175 m depth layer. The surface salinity at the capture point of the eels (14°N) was about 34.54 PSU (Fig. 3), slightly higher than the value of the salinity front ($\approx$34.5 PSU) that typically forms in this region, whereas lower surface salinities (ca. 34.3 PSU, Fig. 3) were observed in the southern area (13°N). Water temperature at net deployment depths of the eel capture (180–320 m) ranged approximately from 13 to 23°C.

The body color was dark brown, and the skin was abraded and considerably damaged by the netting (Fig. 2). Sizes of specimens 1 and 2 (Fig. 2a, d) were 55.5 and 66.2 cm in total length (TL), and 90.5 and 117.5 g in body weight (BW), respectively. The eye indexes [9] ($\{[(A + B)/4]^2 \times \pi/\mathrm{TL}\} \times 100$, where A and B are horizontal and vertical eye diameters, respectively) were 5.8 and 5.4. The alimentary tracts were degenerated (not shown). The gonads of the two eels were vestigial (Fig. 2c, f) but featured a structure that appeared similar to an ovigerous lamella, a typical structure in this species (Fig. 2c). Furthermore, a small number of oocyte-like structures (100–250 mm in diameter) were observed in both eels when observed under a binocular microscope onboard (Fig. 4a). In histological observations of the gonads (Fig. 4b–e), the oocyte-like structure (50–150 mm in diameter) was very eosinophilic, and without cytoplasm and nucleus (Fig. 4c, e). In eel gonads, eosinophilic components are limited, thus only yolk globules, erythrocytes, and connective tissues are typically stained well with eosin. Furthermore, this structure was enclosed with a flattened cell layer, presumably vestigial ovarian follicular cells. These observations strongly support that the structure is a remarkably regressed oocyte and the gonads are ovaries, and that both eels are female specimens.

Collection of newly hatched larvae of Japanese eel

A total of six newly hatched larvae (pre-leptocephali) (Fig. 5) were collected by two tows conducted on 3 September 2008 (start times 03:19 and 12:21, respectively) at 14°16′N–14°19′N, 142°42′E–142°43′E, near the female catch area (Fig. 1), approximately 10 km north, away from the center of the mid-water trawl net towing course. The surface salinity at the survey area was about 34.51 PSU. All larvae were determined to be the Japanese eel by analyzing mitochondrial DNA (mtDNA) fragments of approximately 550 bps containing the 16S ribosomal RNA region (DDBJ/EMBL/GenBank accession numbers AB550074 to AB550079). The six specimens ranged from 4.5 to 5.7 mm in total length and had no teeth and jaw with

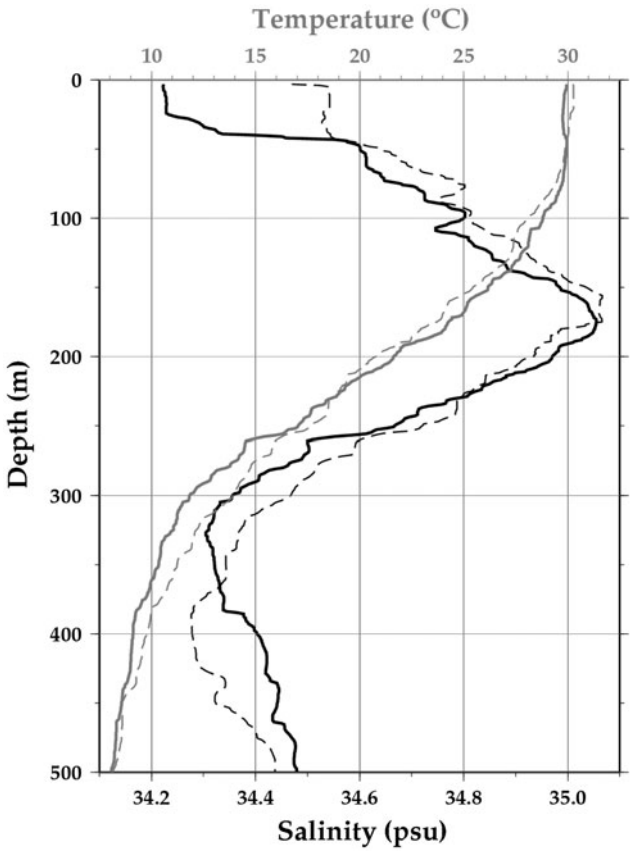

Fig. 3 Vertical distribution of salinity (PSU) (*black line*) and temperature (°C) (*gray line*) in the survey area between the surface and 500 m. *Solid* and *dotted lines* indicate the profiles at 13°N and 14°N, respectively

a dispersed oil globule. Four had unpigmented eyes (Fig. 5a), and the other two showed early eye pigmentation (Fig. 5b). The six specimens were collected at depths of 100–200 m. Five of six specimens were in the 100–150 m depth layer and the other was in the 150–200 m layer (Fig. 6). Water temperature and salinity ranged from 21.7 to 27.7°C and 34.81 to 35.07 PSU in the 100–200 m depth layer and were generally 25.9–27.7°C and 34.81–35.03 PSU in the 100–150 m depth layer.

Discussion

Spawning area along the West Mariana Ridge

The capture during the new moon on 31 August 2008 of post-spawning females together with newly hatched larvae of the Japanese eel strongly suggests that the eel spawning occurred in our survey area (14°N) along the West Mariana Ridge. There appeared to be a deep commitment to the new moon as suggested by previous reports [5, 7, 8]. The larvae caught on 3 September 2008 are estimated to have hatched 2–3 days before based on previous analyses of eye pigmentation state and otoliths [5]. This indicates the larvae hatched during the new moon or 1 day before the new moon and the spawning occurred 1–3 days before the new moon, because in the laboratory, eggs of the Japanese eel incubated at 23 and 25°C hatched 41 and 28 h after fertilization, respectively [10, 11].

This historic first capture of adult female freshwater eels near the Suruga Seamount together with the previous collection of mature males in the southern area (13°N) of the West Mariana Ridge [8] corroborates that the area along the West Mariana Ridge including seamounts [7, 12] is the spawning area of this species. Considering that the adult males and newly hatched larvae were caught over 130 km south of the Suruga Seamount in June 2008 [8], the Japanese eels also use the part of the West Mariana Ridge further south as a spawning area, indicating that eel spawning may occur at multiple locations where the environmental conditions are appropriate. Or, the spawning area might shift monthly or annually according to the changes in oceanographic conditions, such as the position of a salinity front that typically forms in this region [13]. The CTD observation indicated that this spawning area (14°N) was located on the salinity front (ca. 34.5 PSU) between the southern area of low salinity water diluted by precipitation (<34.2 PSU) and the northern area of high salinity water resulting from high evaporation (>34.8 PSU) [14], indicating the intimate relationships between the salinity front and the formation of spawning areas of the Japanese eel [13].

Since the eels were caught by a relatively shallow tow (180–320 m) over deep waters (1,600–3,500 m deep) similar to the fishing depths of the previous catch of two matured males in June (230–280 m) [8], eel spawning appears to occur in the pelagic environment similar to the spawning migration indicated for *A. anguilla* [15]. This suggests that spawning freshwater eels do not require extremely shallow seamounts (e.g., 10–40 m deep from the sea surface) as has been proposed for Japanese eel [5, 7], and spawning may occur if appropriate oceanographic conditions along the ridge of a chain of seamounts provide suitable cues for adult eels to aggregate prior to spawning.

Reproductive and migratory state of the eels

In spite of the evidence for the spawning provided by the collection of newly hatched larvae in nearly the same area as the adults, the histological condition of the ovaries, which had a small number of remarkably regressed oocytes, indicated that these females had spawned well before collection. Considering the new moon spawning tendencies of the Japanese eel [5, 7, 8], it is possible that the present females had spawned during the previous new

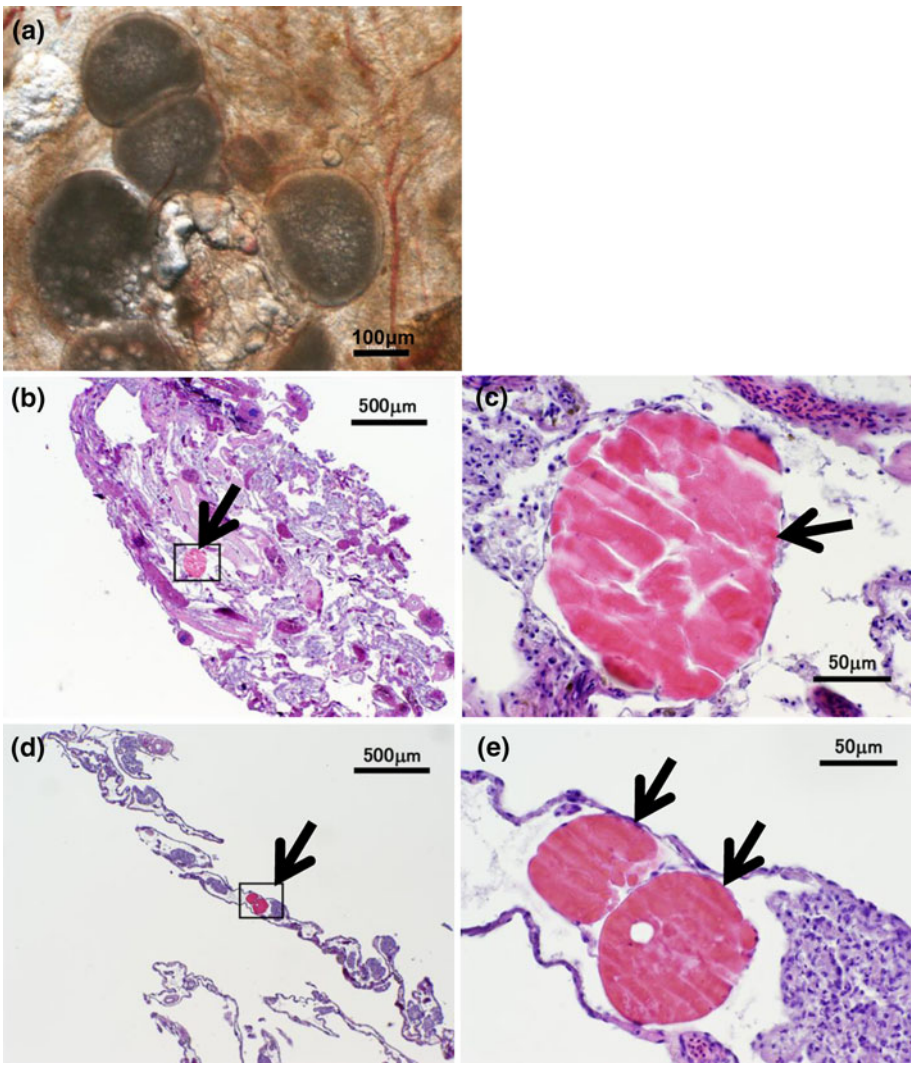

Fig. 4 Microscopic observation of ovaries. **a** Oocytes observed under a binocular microscope in fresh gonad of specimen no. 2 dissected onboard. **b**–**e** Cross-sections of ovaries of specimen no. 1 (**b**, **c**) and no. 2 (**d**, **e**) showing remarkably regressed oocytes (*arrow*)

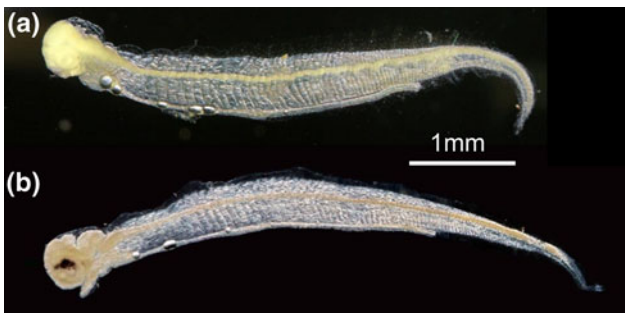

Fig. 5 *Anguilla japonica* pre-leptocephali with no teeth and jaw with a dispersed oil globule. **a** Early-stage larvae with unpigmented eyes. **b** Slightly developed from **a** with early eye pigmentation. Total lengths in **a** and **b** are 4.7 and 5.6 mm, respectively

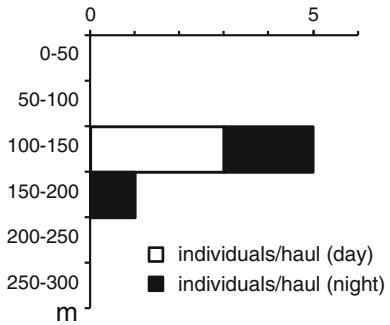

Fig. 6 Vertical distribution of catch per haul number of *Anguilla japonica* pre-leptocephali individuals by day (*open bar*) and by night (*solid bar*) collected by a multi-layer plankton net in six depths at 50 m intervals from 300 m to the surface

moon period, more than 4 weeks before collection. It seems that the eels remained in the spawning area for a period of time after their spawning event.

The eye index of the studied females (5.8 and 5.4) was smaller than that of the matured male eels (7.5, 8.7) collected along the West Mariana Ridge in June 2008 [8]. These values are comparable with those of female silver eels (4.4–8.6) in coastal waters, but apparently larger than those of female yellow eels (2.1–4.8) [16], indicating that

the eels had prepared their body condition for spawning migration during the initial phase of the migration.

Their alimentary tracts had degenerated as has been observed in silver eels of anguillid species collected in coastal areas [17–20], indicating a non-feeding condition during their spawning migration as evidenced by stable isotope analysis [21]. The body weight of the females (90.5 and 117.5 g) was much smaller than those of female silver eels of similar body length caught along the coast of Japan (129–1,843 g) [16, 22] and in the East China Sea (82.7–1,961 g; including male data) [23]. Considering the remarkably high swimming efficiency and low energy costs during their spawning migration, as has been shown theoretically and experimentally in European eels [24–26], this reduction in body weight is likely the result of not only reduced energy consumption during their long-distance migration without feeding, but also of gonadal development for the production of eggs. The severe body weight loss, degenerated organs, and poor body condition of the studied females indicated that they were in the process of dying after spawning.

Vertical distribution of newly hatched larvae

Collection depth of the eel larvae (4.5–5.7 mm in total length) ranged from 100 to 200 m, mainly at a depth of 100–150 m, with indeterminate diurnal vertical migration. These depths were deeper than those of larger eel larvae of 10–22 mm in total length, which showed diurnal vertical migration between 85 m at night and 170 m during daytime [27], suggesting the eel larvae tend towards shallower depths at night and have acquired the behavior of diurnal vertical migration. The present newly hatched eel larvae, which were in the early stage, have little or no ability to migrate vertically and would aggregate at the upper end of the thermocline in the 100–150 or 200 m depth strata where they can maintain neutral buoyancy as they have the identical specific gravity as the environmental seawater. Further investigation with more adult eel sampling including females with eggs is needed to ascertain the spawning ecology of freshwater eels, including the precise depth of spawning as well as the correlation between development from fertilized eggs into larvae and their movement to the appropriate depth layer. This information will contribute to determining the rearing conditions for artificial grass-eel production [11, 28].

Acknowledgments The authors thank Captain Nobuyuki Nagai and his crew members of the *R/V Kaiyo Maru* for supporting sample collection; Mr. Kyoichi Kawaguchi and Drs. Tokio Wada and Misao Arimoto for encouraging this research cruise; Dr. Michael J. Miller for comments on the manuscript; and Mss. Mayumi Sato, Tomoko Kawashima, and Chie Takahashi for assisting with DNA analysis. We gratefully acknowledge the financial support of the Fishery Agency of Japan and the Fisheries Research Agency.

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