

Production of leptocephali of Japanese eel (*Anguilla japonica*) in captivity

H. Tanaka*, H. Kagawa, H. Ohta

National Research Institute of Aquaculture, Nansei, Mie 516-0193, Japan

Received 21 July 2000; accepted 12 January 2001

Abstract

Despite intensive research on wild and captive eels, no resource has so far provided access to all life cycle stages of the Japanese eel *Anguilla japonica*. The transition from the preleptocephalus (newly hatched larva) to the leptocephalus stage (typical leaf-like eel larva) has, therefore, remained the missing link in the eel life cycle. We recently found that a slurry-type diet made from shark egg powder is suitable feed for captive-bred eel larvae. The larvae were successfully reared with this diet in aquaria for 100 days and raised to 22.8 mm in total length (TL). Age, TL, and body proportions of the reared specimens overlapped with those of wild leptocephali. We revealed for the first time the transition from the preleptocephalus to the leptocephalus stage of the eel. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Japanese eel; *Anguilla japonica*; Preleptocephalus; Leptocephalus; Larval culture

1. Introduction

The life cycle of freshwater eels is complex, and has not yet been completely clarified. Tsukamoto (1992) recently described the spawning area of the Japanese eel *Anguilla japonica* as being west of the Mariana Islands, where many small young leaf-shaped leptocephalus stage larvae (7.9 to 24.5 mm TL, 10 to 40 days after fertilization) could be collected in summer. Towards the end of a half-year oceanic

* Corresponding author. Tel.: +81-599-66-1830; fax: +81-599-66-1962.
E-mail address: htanaka@nria.affrc.go.jp (H. Tanaka).

migration, the leptocephali metamorphose into glass eels at about 100 to 140 days of age, having reached 60 mm TL (Tsukamoto and Umezawa, 1990; Kawakami et al., 1998). The juvenile eels reach the estuaries of East Asia in winter–spring at about 55 to 60 mm TL (Tsukamoto, 1990; Kawakami et al., 1998). After growing in freshwater habitats for 5 to 12 years, sexual development is initiated and eels start their return journey to the breeding place in autumn. There are no details available on the successive stages, i.e. fully matured eels, fertilized eggs, and newly hatched larvae, since eels in these stages have never been observed in the field.

Attempts in Japan to induce artificial maturation of Japanese eel started in the 1960s. Yamamoto and Yamauchi (1974) first succeeded in obtaining fertilized eggs and larvae of the Japanese eel by hormone treatments, and preleptocephalus larvae were reared for 2 weeks, reaching 7 mm TL (Yamauchi et al., 1976). Thereafter, many researchers succeeded in obtaining eel larvae (Satoh, 1979; Wang et al., 1980); however, suitable larval feeds were not identified. As a result, preleptocephalus larvae could not survive beyond the depletion of their yolk and oil droplet stores. They remained long and slender and never developed into compressed and deep leptocephali. The number of preanal myomeres, teeth, and tail melanophores were also markedly different between captive-bred preleptocephali and wild leptocephali (Mochioka, 1996). In other eel species, for example, the European eel *A. anguilla* (Prokhorchik, 1986) and the New Zealand freshwater eels *A. dieffenbachii* and *A. australis* (Lokman and Young, 2000), experimentally produced larvae survived only for a few days and, like the Japanese eel, did not develop into leptocephali. Thus, the transition from preleptocephalus, just before first feeding, into the classical willow leaf-shaped leptocephalus stage has remained a mystery in the eel life cycle.

We attempted to rear captive-bred eel larvae in aquaria to observe this transition. We recently discovered that a slurry-type diet made from shark egg powder is a suitable feed for captive-bred eel larvae. Larvae were reared on this diet for 100 days and raised to 20 mm in TL. We report here for the first time the development of reared specimens in comparison with wild leptocephali.

2. Materials and methods

2.1. Animals

Japanese eel larvae were hatched from artificially fertilized eggs obtained from cultured eels that had been induced to mature by repeated injections of salmon pituitary extract, followed by injection of 17,20 β -dihydroxy-4-pregnen-3-one to induce ovulation in the females and by repeated injections of human chorionic gonadotropin into the males (Ohta et al., 1997). Larvae were first kept in 500-l polycarbonate tanks supplied with filtered seawater (cartridge filter pore size: 10 μ m, Toyo Roshi Kaisha) at 22°C to 23°C. The jaws and pigmentation of the eyes were well developed by Day 8 after hatching, and the yolk sacs of the larvae had been absorbed and the oil droplets greatly

reduced in volume. At this stage, the larvae can produce digestive enzymes and, hence, exogenous feeding may start (Kurokawa et al., 1995).

2.2. Diets and feeding trial

We recently identified a food item that is palatable to eel larvae, freeze-dried shark egg yolk (Aquaran, BASF Japan). This is a commercially available product used to improve the nutritional value of food organisms for the culture of marine fish larvae. We confirmed growth (up to 7.9 mm TL) and prolonged survival (for 24 days after hatching) of eel larvae with this feed in preliminary feeding trials in petri dishes, warranting its use for successive experiments. Thereafter, we designed two kinds of nutritionally improved diets (Diets A and B, Table 1) and conducted rearing experiments. Each mixture was homogenized with distilled water or krill extract. The feeding trial was carried out in 5-l round acrylic resin tanks, continuously supplied (400 ml/min) with filtered seawater at 21°C to 22°C except during feeding. Salinity ranged between 32 to 35 ppt and oxygen levels were close to saturation during the whole experiment. Water was drained through a 0.35 mm nylon filter to retain the larvae in the tank. Four tanks were used in this trial, each stocked with 1000 larvae at Day 6 after hatching. The larvae were offered Diet A on Day 8 after hatching, followed by Diet B from Day 16 onward. Five ml of the slurry diet were pipetted onto the bottom of each tank five times a day, at 0900, 1100, 1300, 1500, and 1700 h. The water supply was stopped just before feeding and restarted 1 h later to flush out the uneaten feed. At 1900 h, when flushing of the last feed of the day was completed, each rearing tank was connected to a clean tank by a vinyl chloride tube (inner diameter, 9 mm) to transfer the living larvae by siphoning. Siphoning was not

Table 1
Composition of slurry type diets

Component	Diet A	Diet B
Shark egg powder ^a (g)	80	80
Soybean peptide ^b (g)	20	20
Vitamin mixture ^c (g)	–	3.5
Mineral mixture ^d (g)	–	1.5
Distilled water (ml)	250	–
Krill extract ^e (ml)	–	270

^aAquaran, BASF Japan (Japan).

^bHinute-R, Fuji Oil (Japan).

^cVitamin mixture supplied (per 100 g dry matter): vitamin A, 4500 IU; D₃, 900 IU; E, 60 mg; K₃, 6 mg; B₁, 9 mg; B₂, 30 mg; B₆, 6 mg; nicotinamide, 120 mg; Ca-pantothenate, 42 mg; choline chloride, 1200 mg; folic acid, 2.25 mg; B₁₂, 0.0135 mg; biotin, 0.9 mg; inositol, 600 mg; p-aminobenzoic acid, 60 mg; C, 726 mg.

^dMineral mixture supplied (per 100 g dry matter): Na, 45 mg; K, 115 mg; Mg, 20 mg; P, 175 mg; Ca, 135 mg; Cl, 70 mg; Fe, 8 mg; Zn, 1.3 mg; Mn, 0.325 mg; Cu, 0.115 mg; Co, 0.745 mg; Al, 0.045 mg.

^eKrill (500 g) was homogenized with distilled water (1000 ml) and filtered through nylon plankton net (mesh opening: 0.2 mm). The extract was incubated at 60°C for 30 min to reduce proteolytic activity and stored at –20°C until preparation of diet.

observed to cause noticeable mortality. The light intensity at the surface of the tanks was maintained at 40 to 100 lux during the feeding periods; otherwise, it was below 5 lux.

2.3. Sampling and measurements

Three to ten larvae were randomly sampled in total from the four tanks on Days 0, 7, 10, 15, 20, 25, 30, 35, 40, 50, 70 and 100 after hatching. However, the two last sampling occasions, Days 70 and 100 after hatching, the remaining populations of larvae were below 20 in each tank. Therefore, the mean sizes estimated for these days may be biased. The total length, head length, body depth, and preanal myomeres were measured and counted under a binocular microscope within 1 week after fixation in 5% formalin in 33% seawater. Deformed larvae were eliminated from the measurements. Photographs of the larvae were taken with a binocular microscope equipped with a digital still camera (FUJIX HC-300, Fuji Photo Film, Japan).

3. Results

3.1. Growth and survival

The larvae started feeding as soon as Diet A had been placed onto the bottom of the tank (Fig. 1). A large number of larvae that did not ingest the feed died between 10 and 15 days after hatching. On Day 50 after hatching, the survival rate was approximately

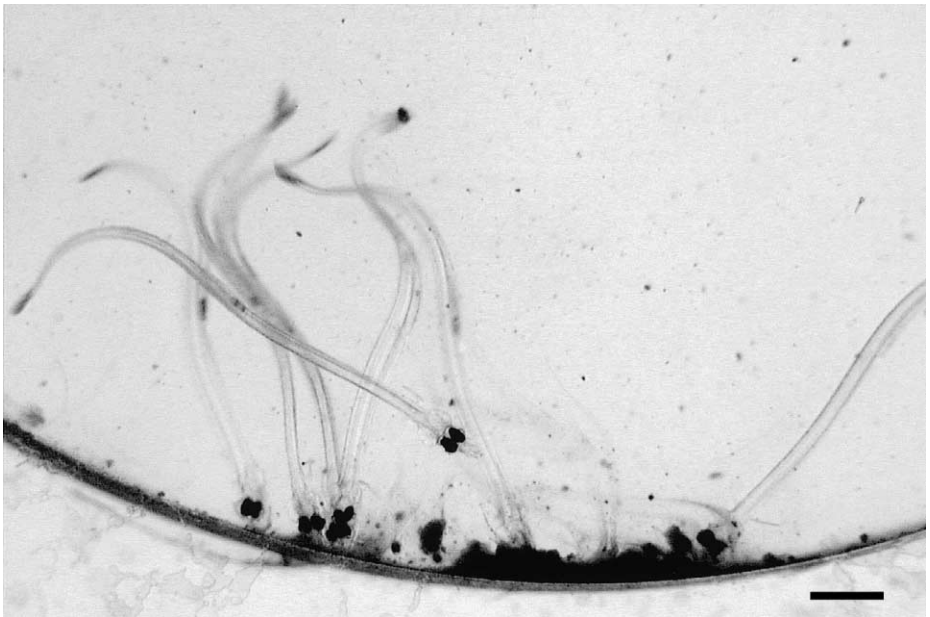


Fig. 1. Feeding behavior of *A. japonica* preleptocephali in the aquarium (15 days after hatching). Scale bar = 1 mm.

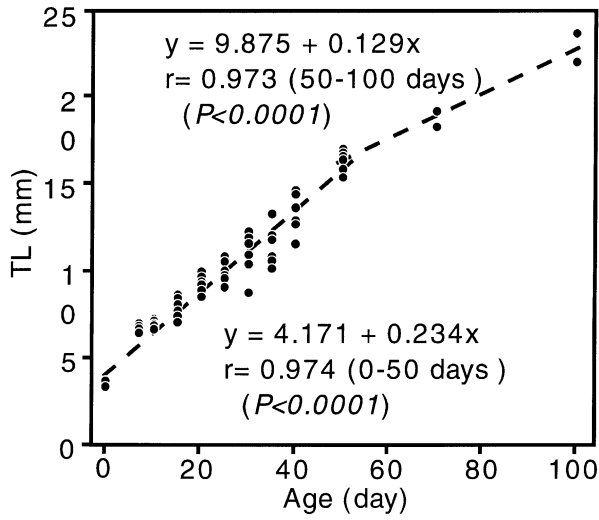


Fig. 2. Growth in total length (TL) of captive-bred *A. japonica* larvae reared in aquaria.

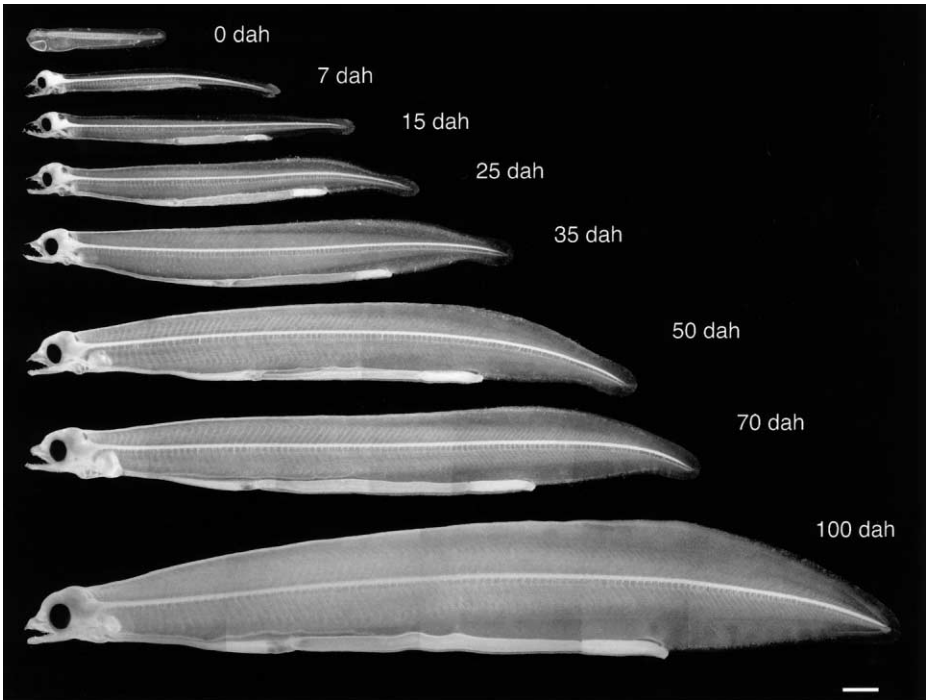


Fig. 3. Transition of captive-bred *A. japonica* preleptocephalus into leptocephalus. Age in days after hatching (dah), total length: 0 dah, 3.6 mm; 7 dah, 6.9 mm; 15 dah, 8.7 mm; 25 dah, 10.6 mm; 35 dah, 13.4 mm; 50 dah, 16.7 mm; 70 dah, 18.4 mm; and 100 dah, 23.7 mm, respectively. Scale bar = 1 mm.

3% to 5% in each tank, which decreased to 0.5% to 2% by 100 days after hatching. The larvae grew linearly up to 50 days after hatching (mean TL 16.4 mm) with a growth rate of 0.234 mm/day (Figs. 2, 3). The growth rate then decreased to 0.129 mm/day, resulting in a mean TL of 22.8 mm by Day 100 after hatching (Fig. 2).

3.2. Physical development

On Day 7 after hatching, the bodies of the reared larvae were elongated, compressed, but not particularly deep (Fig. 3). Thereafter, body depth gradually increased to yield the

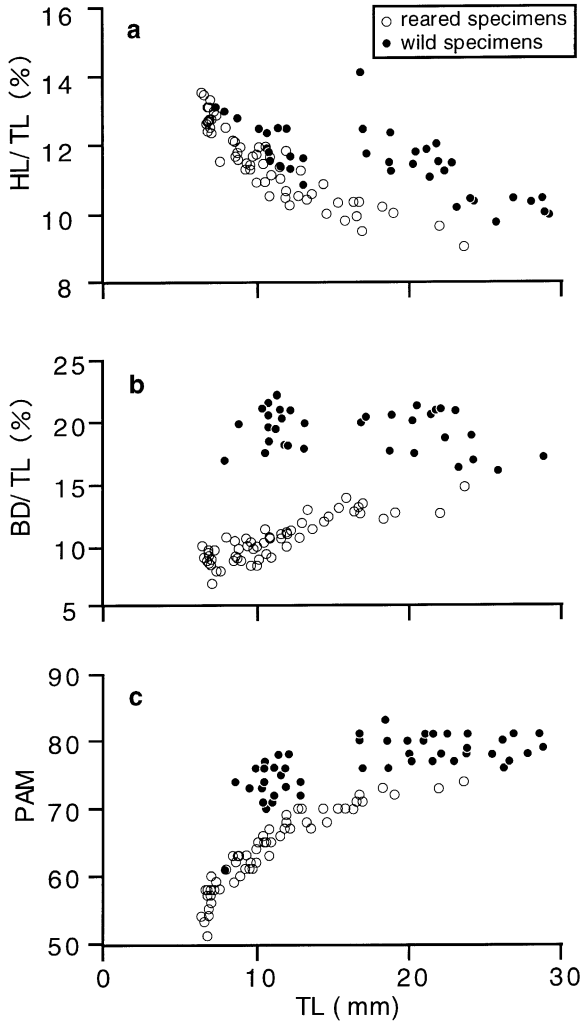


Fig. 4. Changes in *A. japonica* larval characteristics with growth in reared (present study) and wild (Mochioka, 1996) leptocephali. (a) The head length (HL) to TL ratio. (b) The body depth (BD) to TL ratio. (c) The numbers of preanal myomeres (PAM).

willow leaf-like form typical of wild eel leptocephali (Fig. 3). Melanophores at the tip of tail were well developed and densely branched during early ontogeny (Days 7 to 25, Fig. 3). They decreased with the growth of the larvae to only a few dots by Day 100 after hatching. The number of teeth increased with growth from 4 (7 days after hatching) to 7–9 (100 days after hatching) in each jaw. Changes in the body proportions of the reared specimens with growth were compared with those of wild specimens reported by Mochioka (1996) (Fig. 4). The head length to TL ratio decreased with growth in both the reared and wild specimens (Fig. 4a). Although the body depth/TL of the reared specimens gradually increased beyond 10 mm TL and approached the ratios seen in wild specimens, the ratios were distinctly different in the smaller larvae (Fig. 4b). The numbers of preanal myomeres of the reared specimens rapidly increased between 7 to 15 mm TL and also closely matched those of wild specimens (Fig. 4c).

4. Discussion

Little is presently known about the natural diet of leptocephali. Detrital aggregates, fecal pellets of zooplankton (Otake et al., 1993), and larvacean houses (Mochioka and Iwamizu, 1996) have been found in the gut contents of wild *anguilloid* larvae and gelatinous zooplankton has been suggested to be their main food by their peculiar dental configuration (Westerberg, 1990). However, it is impractical to prepare these diet items as feed for larval rearing in aquaria. We, thus, tried to feed the artificially hatched eel larvae on various food items. Tanaka et al. (1995) first confirmed that eel larvae 7 to 13 days after hatching ate rotifers, the most common initial feed used in the production of marine fish fry. It was subsequently proven that the larvae that ate rotifers absorbed the proteins in their intestines, indicating that artificially hatched eel larvae can digest and absorb rotifers (Kurokawa et al., 1995, 1996). These larvae also ate immovable feed, such as a microdiet and boiled egg yolk. However, even the larvae that ate these kinds of feed did not grow much larger and their survival periods were not extended.

We recently found that a slurry-type diet made from freeze-dried shark egg yolk is suitable feed for eel larvae and designed two kinds of diets (Diet A and B). Diet B, which was supplemented with vitamins, minerals, and krill extract, was nutritionally richer than Diet A. However, in the first feeding, the larvae ingested Diet A more than Diet B. Therefore, we offered Diet A as the starter diet and changed to Diet B later. Although the shift from Diet A to B led to the best result so far, it was still incomplete because a large number of larvae died between 10 and 15 days after hatching and a considerable mortality lasted from Day 16 onward. Detailed studies to elucidate nutritional requirements of cultured eel larvae are needed for further developments of larval rearing techniques.

Since the larvae showed negative phototaxis, the intensity of illumination on the top of the tanks during feeding impelled them to go to the bottom of the tank and helped them to locate the food. Stopping the water supply during feeding was essential for the feeding trials in a small tank, because it enabled the larvae to feed on the slurry-type diet at the bottom of the tank. However, stopping the water supply brought bacterial proliferation in the tank. As a result, it became necessary to flush out the uneaten feed

every 2 h and transfer the living larvae to a clean tank by siphoning every night. The feeding period of the larvae in this rearing procedure was limited to 5 h a day. Heavy mortality was observed between 10 and 15 days after hatching when most of starved larvae usually died in any batches of larvae. The intestines of the trial fish that died during this period were not filled with diet. Any incidences of pathogens, diseases and physiological/developmental abnormalities were not evident. So, we presumed that the mortality during this period was mainly associated with a failure in first feeding. While feeding on these diets, the surviving larvae grew linearly up to 50 days after hatching as shown in field collected developing leptocephali (10.2 to 30.5 mm TL, 10 to 55 days after hatching; Tsukamoto et al., 1994). However, the growth rate (0.234 mm/day) was nearly half that of wild Japanese eel leptocephali at the same days after hatching, estimated on the basis of otolith examinations (0.434 mm/day; Tsukamoto et al., 1994). The reared larvae survived more than 100 days, resulting in over 20 mm TL, though the growth rate of the reared larvae further decreased to 0.129 mm/day (50 to 100 days after hatching). The body depth obviously increased to yield the willow leaf-like form and the head length to TL ratio and the number of preanal myomeres also rapidly changed and closely matched those of wild specimens in the TL range from 7 to 15 mm. However, the reared larvae had smaller heads, shallower bodies, and fewer preanal myomeres than the wild larvae. This may imply a nutritional deficiency at an early stage of development in the reared larvae and/or shrinkage in bodies of wild collected larvae, and also suggest that there might be a great difference of body depth between reared and wild larvae just after hatching. For example, marked differences in body proportions and developments between reared and wild larvae have been observed in milkfish (Liao et al., 1977, 1979). The lower water temperature (21–22°C) in the rearing condition than the ambient temperature recorded for wild leptocephali caught at night (27–28°C; Otake et al., 1998) may have contributed to the observed differences in growth rates and in body proportions. The melanophores at the tip of the tail decreased to only a few dots and the number of teeth increased from 4 to 7–9 with the growth of the larvae, comparable to that seen in wild specimens of similar size (Ozawa et al., 1991).

5. Conclusion

We have documented for the first time the changes associated with the transition of eel larvae from preleptocephali to leptocephali, which completes the life cycle of the Japanese eel. The slow growth rate and the differences in body proportions of reared larvae compared to those from the wild may be partly due to an incomplete diet, limited feeding period, and lower water temperature relative to their natural habitats. However, the rapid changes in three morphological indices between 7 and 15 mm TL seem to reflect development of the preleptocephalus larvae into the leptocephalus stage. Progress towards the goal of artificial propagation of eel has, despite intensive research, been at a virtual standstill since the production of preleptocephali 25 years ago. A major obstacle in the pursuit of that goal has now been overcome with the production of eel leptocephali in captivity. Future studies should be focused on the nutritional adequacy of diets, lighting and feeding regimes, and optimum temperature in the next stage of getting cultured leptocephali through to metamorphosis into the glass eel stage.

Acknowledgements

We thank N. Iinuma and J. Kawaguchi for their assistance with larval rearing, T. Unuma and M. Tsuji for supporting maturation induction and artificial fertilization, BASF Japan for providing Aquaran, vitamin mixture, and mineral mixture, Fuji Oil for providing Hinute-R, and Dr. K. Fukusho, Dr. H. Ishioka and Dr. K. Hirose for their helpful suggestions and encouragement. We also thank Professor G. Young, Dr. P.M. Lokman, and Dr. B.H. Pedersen for critical reading of the manuscript and for giving excellent comments.

References

- Kawakami, Y., Mochioka, N., Nakazono, A., 1998. Immigration period and age of *Anguilla japonica* glass-eels entering in northern Kyushu, Japan during 1994. *Fish. Sci.* 64, 235–239.
- Kurokawa, T., Kagawa, H., Ohta, H., Tanaka, H., Okuzawa, K., Hirose, K., 1995. Development of digestive organs and feeding ability in larvae of Japanese eel (*Anguilla japonica*). *Can. J. Fish. Aquat. Sci.* 52, 1030–1036.
- Kurokawa, T., Tanaka, H., Kagawa, H., Ohta, H., 1996. Absorption of protein molecules by the rectal cells in eel larvae *Anguilla japonica*. *Fish. Sci.* 62, 832–833.
- Liao, I.C., Yan, H.Y., Su, M.S., 1977. On morphology and its related problems of milkfish fry from the coast of Tungkang, Taiwan. *J. Fish. Soc. Taiwan* 6, 73–83 (in Chinese with English abstract).
- Liao, I.C., Juario, J.V., Kumagai, S., Nakajima, H., Natividad, M., Buri, P., 1979. On the induced spawning and larval rearing of milkfish *Chanos chanos* (Forsk.). *Aquaculture* 18, 75–93.
- Lokman, P.M., Young, G., 2000. Induced spawning and early ontogeny of New Zealand freshwater eels (*Anguilla dieffenbachii* and *A. australis*). *N. Z. J. Mar. Freshwater Res.* 34, 135–145.
- Mochioka, N., 1996. Morphology and growth of Japanese eel larvae. In: Tabeta, O. (Ed.), *Early Life History and Prospects of Seed Production of Japanese Eel Anguilla Japonica*. Kouseisha-Kouseikaku, Tokyo, pp. 22–32 (in Japanese).
- Mochioka, N., Iwamizu, M., 1996. Diet of anguilloid larvae: leptocephali feed selectively on larvacean houses and fecal pellets. *Mar. Biol.* 125, 447–452.
- Ohta, H., Kagawa, H., Tanaka, H., Okuzawa, K., Iinuma, N., Hirose, K., 1997. Artificial induction of maturation and fertilization in the Japanese eel, *Anguilla japonica*. *Fish. Physiol. Biochem.* 17, 163–169.
- Otake, T., Nogami, K., Maruyama, K., 1993. Dissolved and particulate organic matter as possible food source for eel leptocephali. *Mar. Ecol.: Prog. Ser.* 92, 27–34.
- Otake, T., Inagaki, T., Hasumoto, H., Mochioka, N., Tsukamoto, K., 1998. Diel vertical distribution of *Anguilla japonica* leptocephali. *Ichthyol. Res.* 45, 208–211.
- Ozawa, T., Kakizoe, F., Tabeta, O., Maeda, T., Yuwaki, Y., 1991. Japanese eel leptocephali from three cruise in the western North Pacific. *Nippon Suisan Gakkaishi* 57, 1877–1881.
- Prokhorchik, G.A., 1986. Postembryonic development of European eel, *Anguilla anguilla*, under experimental conditions. *J. Ichthyol.* 26, 121–127.
- Satoh, H., 1979. Try for perfect culture of the Japanese eel. *Iden* 33, 23–30 (in Japanese).
- Tanaka, H., Kagawa, H., Ohta, H., Okuzawa, K., Hirose, K., 1995. The first report of eel larvae ingesting rotifers. *Fish. Sci.* 61, 171–172.
- Tsukamoto, K., 1990. Recruitment mechanism of the eel, *Anguilla japonica*, to the Japanese coast. *J. Fish. Biol.* 36, 659–671.
- Tsukamoto, K., 1992. Discovery of the spawning area for Japanese eel. *Nature* 356, 789–791.
- Tsukamoto, K., Umezawa, A., 1990. Early life history and oceanic migration of the eel, *Anguilla japonica*. *La Mer* 28, 188–198.
- Tsukamoto, K., Lee, T.W., Mochioka, M., 1994. Age and growth of Japanese eel leptocephali. In: Otake, T., Tsukamoto, K. (Eds.), *Preliminary Report of the Hakuho Maru Cruise KH-91-4*. Ocean Research Institute, University of Tokyo, Tokyo, pp. 50–54.

- Wang, Y., Zhao, C., Shin, Z., Ten, Y., Zhang, K., Li, Y., Yang, Y., Hong, Y., 1980. Studies on the artificial inducement of reproduction in common eel. *J. Fish. China* 4, 147–158 (in Chinese, with English abstract).
- Westerberg, H., 1990. A proposal regarding the nutrition of leptocephalus larvae. *Int. Rev. Hydrobiol.* 75, 863–864.
- Yamamoto, K., Yamauchi, K., 1974. Sexual maturation of Japanese eel and production of eel larvae in the aquarium. *Nature* 251, 220–222.
- Yamauchi, K., Nakamura, M., Takahashi, H., Takano, K., 1976. Cultivation of larvae of Japanese eel. *Nature* 263, 412.