

# ウナギブレブレトケファルス幼生の形態変化と摂餌器官の発達

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# Early Development of Preleptocephalus Larvae of the Japanese Eel in Captivity with Special Reference to the Organs for Larval Feeding

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## Abstract

To investigate the unavailability of *Brachionus* rotifers as an initial food for rearing preleptocephalus larvae of the Japanese eel, the ontogenetic morphological changes during the early developmental stages of eel with special reference to the organs for larval feeding were studied in captivity.

Fertilized eggs were obtained from matured adults induced by hormonal treatments. The eggs were incubated at 21°C and 25°C in 33-34 psu sea water, and the newly hatched larvae were reared until the completion of yolk-absorption. Hatching of larvae began 45.0 h after the fertilization at 21°C and 31.5 h at 25°C, and terminated within 3.5 to 4.0 h after the onset of hatching. The absorption of yolk completed on 10 dph at 21°C (211.7 day-degrees) and 8 dph at 25°C (201.5 day-degrees), and simultaneously four pairs of characteristic sharp-pointed larval teeth fully developed on both of upper and lower jaws. A series of histological sections of oesophageal part from 6 dph to 10 dph specimen showed that eel larvae had a characteristic thicker tissue layer and well-developed circular muscles surrounding larval gullet without mucous cells.

**Key Words:** Japanese eel, larval rearing, early development, oesophagus, mucous cell

## Introduction

The Japanese eel *Anguilla japonica* is naturally distributed in the Far East Asia and a commercially important species. The intensive commercial culture of eel was initiated in Japan more than one hundred years ago, and nowadays the eel culture is an important industry not only in Japan but also in Taiwan and China<sup>1,2,3)</sup>. In spite of its large aquaculture production, the culture of Japanese eel still completely rely on the field-collected wild glass eels as seedlings, but the annual catch of glass eels is reported to be fluctuating with a continuously decreasing trend caused by various reasons<sup>2)</sup>. Therefore the establishment of production techniques of glass eel is highly desired among fish-farmers.

The first captive-bred glass eel was produced in the National Research Institute of Aquaculture (NRIA) in Japan on 2002 using a kind of pasty liquid-form diet made from eggs of spiny dogfish (*Squalus acanthias*) as an initial feed<sup>4,5)</sup>. However, the rearing techniques for mass production have not been established so far in spite of the efforts by many researchers. The survival of eel larvae during its early developmental stage in captivity is very low to date. The main problem associated with the development of seedling production of the eel is the initial feed used for the larviculture during the early developmental stages of the eel. The

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most crucial constraint would be that preleptocephalus larvae of the Japanese eel could not be raised by feeding *Brachionus* rotifers, unlike other many marine teleosts larvae reared in hatcheries<sup>4-8)</sup>.

In this research the early development of preleptocephalus larvae of the Japanese eel with special reference to the larval feeding organs and their functions were studied to clarify the reason why rotifers were not available for the larval rearing of the Japanese eel.

### Materials and Methods

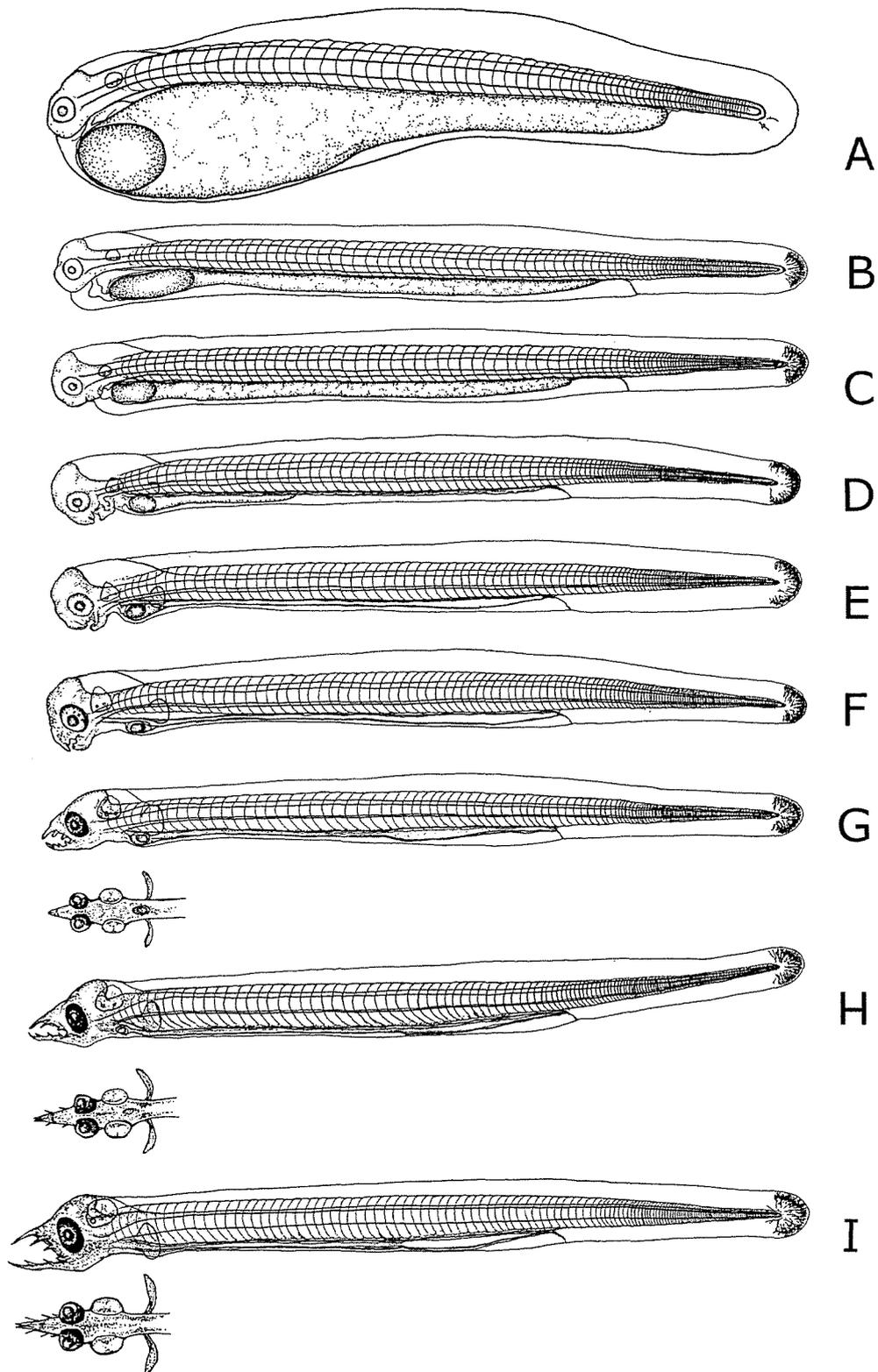
The larval rearing of the Japanese eel was conducted using the facilities of NRIA in 2004-2007. Fertilized eggs were obtained by hand stripping matured adults induced by hormonal treatments using a so-called NRIA protocol<sup>9,10)</sup>. After separation, the floating (fertilized) eggs were stocked to polycarbonate cylindrical tanks and incubated until hatching at two temperatures of 21°C (21.0-21.5°C, 2004-2006) and 25°C (25.0-25.1°C, 2006-2007) in 32-34 psu sand-filtered sea water. The day larvae hatched was defined as 0 dph (day post-hatching). The newly-hatched larvae were reared in a dark condition until the completion of yolk-absorption under the two different temperatures.

The observation of embryonic and larval development until the completion of yolk-absorption was conducted under a microscope using live specimens following the body size measurements (n=10 daily, mean±SD) after anesthetized with a small amount of tricaine methanesulfonate (MS-222). Larvae were preserved in 5% formalin solution and haematoxylin-eosin stained sections (thickness: 6 μm) were supplied for the observation of larval morphology and histological studies of the digestive systems of eel larvae. In addition to that, a scanning electron microscopic (SEM; JSM-6320 F, JEOL Ltd., Tokyo) observation was made on larval teeth development after fixation with glutaraldehyde-tannic acid and post-fixation in osmium tetroxide using 25 dph samples of 2006 with fully developed larval teeth reared on pasty liquid-form diet.

### Results

The diameter of hand-stripped eggs from matured females was  $0.92 \pm 0.05$  mm at 21°C and  $0.98 \pm 0.03$  mm at 25°C. After the formation of the perivitelline space, the egg diameter reached its maximum size of about 1.6-1.7 mm in 3.5 h at 21°C and 2.5 h at 25°C. The normally-developing eggs of the Japanese eel were transparent, non-adhesive, free, pelagic and spherical in shape with a characteristic wide perivitelline space. Initial dispersed small oil globules gradually clustered and eventually formed a single large oil globule during the late embryonic stage. The eggs exhibited a typical meroblastic cleavage after insemination. The sequence of events that occurred during embryonic development was almost identical to that of the previous reports on the Japanese eel<sup>6,7)</sup> and those of marine teleosts with pelagic eggs<sup>11,12)</sup>.

Morphological changes of the early developmental stage of the preleptocephalus larvae of *Anguilla japonica* were shown in Fig. 1. Hatching of eggs began 45.0 h after the fertilization at 21°C and 31.5 h at 25°C, and terminated within 3.5 to 4.0 h after the onset of hatching. The mean total length (TL) of newly-hatched eel larvae was  $2.99 \pm 0.07$  mm at 21°C and  $2.75 \pm 0.08$  mm at 25°C. The emergent larvae had an elongated pear-shaped yolk sac and a single oil-globule situated at the anterior part of yolk sac. Initial rows of branched melanophores on the tip of caudal part increased its number and eventually formed a fan-shaped strong batch of melanophores with larval growth. On 6 to 7 dph at about 7 mm TL, the characteristic oval-shaped eye nearly completed its pigmentation. On 10 dph at 21°C ( $6.82 \pm 0.16$  mm TL, 211.7 day-degrees) and 8 dph at 25°C ( $7.13 \pm 0.14$  mm TL, 201.5 day-degrees), most larvae completed



**Fig. 1** *Anguilla japonica* preleptocephali reared in the laboratory at 25°C: A, 0 dph, 2.75 ± 0.08 mm TL; B, 1 dph, 4.96 ± 0.14 mm TL; C, 2 dph, 5.96 ± 0.22 mm TL; D, 3 dph, 6.58 ± 0.14 mm TL; E, 4 dph, 6.63 ± 0.11 mm TL; F, 5 dph, 6.90 ± 0.19 mm TL; G, 6 dph, 7.18 ± 0.13 mm TL; H, 7 dph, 7.26 ± 0.08 mm TL; I, 8 dph, 7.13 ± 0.14 mm TL.

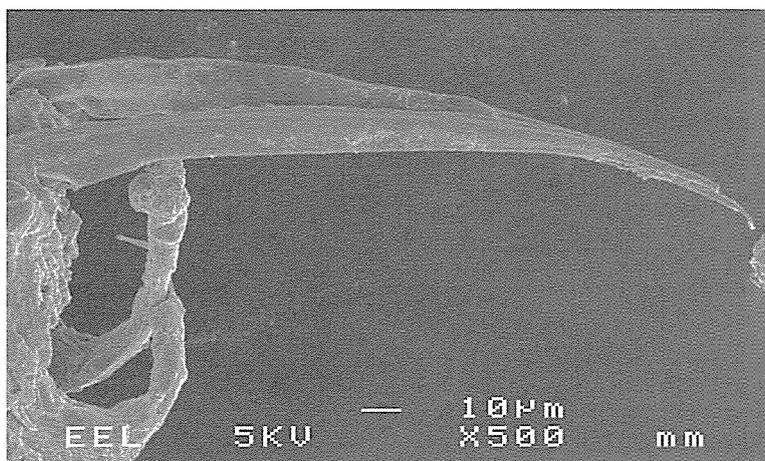
the absorption of yolk-sac. At this stage eel larvae showed a strong negative phototaxis and had a tendency to gather and swim around at the bottom of the rearing tank when light was on. Larvae also had well developed large auditory vesicles as shown in Fig. 1 G-I. After the completion of yolk absorption, eel larvae started to die, and by 11 dph a complete annihilation of larvae occurred at 25°C without feeding.

One of the biggest morphological characteristics of preleptocephalus larvae of eel is sharp and pointed larval teeth, i. e. four pairs on both upper and lower jaws that appeared at 7 to 8 dph (Fig. 1 H, I). Rudimental larval tooth anlagen firstly appeared on the ventral side of larval jaws and they gradually shifted to the tip of snout with growth. When larvae completed yolk absorption, their larval teeth were fully developed with sharp-pointed tip, and the jaw movement became more active than its previous stage. Under a SEM observation for the tip of larval teeth (Fig. 2), it was clarified that these larval teeth had plane and smooth surface without any special structures on them. In the captive condition eel larvae sometimes exhibited S-shaped body position in water column but never showed active and spontaneous attacking behaviour toward prey using the long pointed larval teeth. A series of longitudinal histological sections of the oesophageal part from 6 dph to 10 dph specimen at 25°C (Fig. 3) showed that eel larvae had thicker tissue layer at inside of the epithelium and well-developed circular muscles surrounding the part of larval gullet. Another histological characteristic during this stage of eel larvae was that mucous cells did not develop yet on the inside of the larval gullet from 6 dph (two days before the completion of yolk) till 10 dph (two days after the completion of yolk absorption) under captive condition at 25°C.

### Discussion

The information on the biology of the early larval stages of the present species in the ocean is very limited<sup>13,14)</sup>. Recently the spawning ground of the Japanese eel was successfully pin-pointed to be near a seamount (the Suruga Seamount), west of the Mariana Islands in the Western Pacific by field researchers<sup>15)</sup>. Nevertheless natural preys that preleptocephalus stage eel larvae ingest in their nursery area of the open ocean are still not clarified yet. Therefore, at the moment, we have to develop larval feeds for rearing eel larvae by trial and error without useful information from their natural feeding habit and behaviour.

Marine *Brachionus* rotifers are the most important and initial food item for producing marine fish larvae used commonly all over the world<sup>16,17)</sup>. The use of cultured rotifers as an initial food substantially realized the mass production of marine fish larvae in hatchery<sup>18)</sup>. Tanaka *et al.*<sup>19)</sup> first reported that some of the eel larvae ingested rotifers under captive conditions and Kurokawa *et al.*<sup>20)</sup> showed that eel larvae absorbed proteins derived from rotifers into their larval intestine, by using immuno-histological methods. Nevertheless successful rearing of eel larvae by feeding rotifer as an initial food has not been achieved yet, as most eel larvae did neither accept nor ingest rotifers even though a sufficient number of rotifers was given in various ways<sup>8)</sup>. In some occasions, rotifers could be found in the oral cavity of eel larvae after being fed rotifers. Although eel larvae never showed active feeding behaviour to feed items by using their larval teeth, they sometimes take in rotifers non-spontaneously through half-closed mouth at the bottom layer of the tank when fed with rotifers. Even the larvae were in very healthy and active condition, they just kept on holding rotifers in their oral cavity without swallowing and sending rotifers to larval intestine with peristalsis<sup>8)</sup>. Few years later after the first report on the ingestion of rotifer by eel larvae, Tanaka *et al.*<sup>4,5)</sup> successfully produced the first captive-bred glass eel not by feeding rotifers but a pasty liquid-diet for more than 250 days. Various kinds of solid food items were tested without any success, *i.e.* zooplankton, phytoplankton, micro-formulated diets for marine fish, fish eggs, etc.<sup>4,5)</sup> before they found the availability



**Fig. 2** Scanning Electron Microscope (SEM) photomicrograph of the first pair of larval teeth (upper jaw) of *Anguilla japonica* preleptocephalus at 25 dph. Bar indicates 10  $\mu$ m.  $\times$  500.

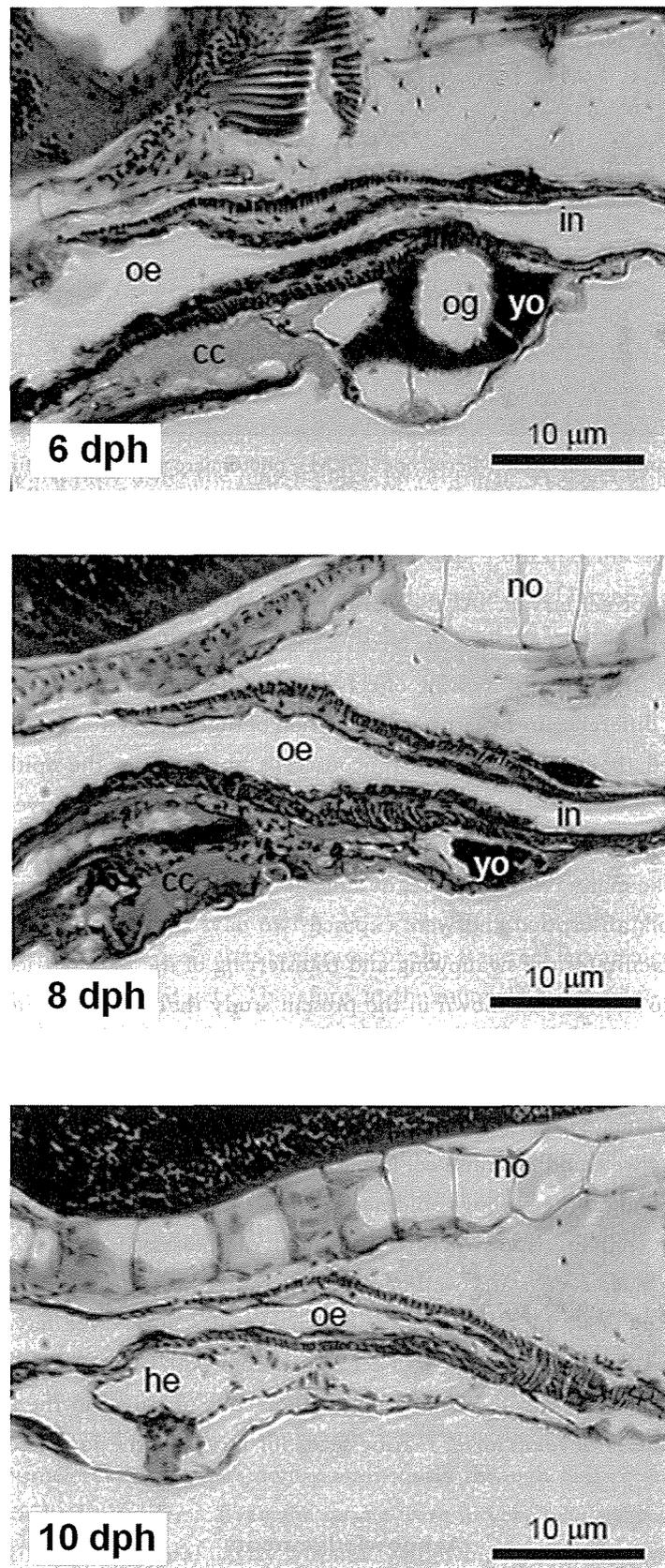
of this pasty liquid-diet for eel larvae. At the present moment, only the soft and pasty liquid-form diet made from spiny dog fish egg is applicable for larval rearing of eel in captivity. On the other hand, there have been no reports on the successful rearing of eel larvae by feeding rotifers so far<sup>8)</sup>.

Tanaka<sup>21)</sup> studied the difference in structure and function of digestive system of the many teleosts larvae. In that study he showed that in most species mucous cells appeared in the epithelium by the time of feeding commencement and those mucous cells started to secrete PAS-positive materials (*i.e.* mucopolysaccharides) into the lumen immediately after their appearance. According to the results obtained in the present study, not like many teleosts larvae, no mucous cells appeared on the inside of the gullet when eel larvae completed yolk absorption and were exposed two days starvation. Mucopolysaccharide from mucous cells probably facilitates the swallowing and transferring of the food particles to fore-gut after the ingestion. In addition to that, it was shown in the present study that larvae had a thicker tissue layer at the inside of epithelium and well-developed circular muscles in their oesophagus as shown in Fig. 3. This well-developed circular muscles surrounding larval gullet might have a role to control the passage of food items by changing its opening-size of the gullet. From these new findings, it might be possible to make a rational explanation why we could not raise eel larvae by feeding rotifers as follows. If eel larvae were fed on solid-form foods including rotifers, they could not swallow them even the particle size was enough small to pass gullet due to the characteristic structure that eel larvae possessed; namely, very thick tissue layer in larval gullet without mucous cells and well-developed circular muscle surrounding it.

The information on the early larval stage of this fish is still scanty. We have to accumulate more information on the early developmental stages of eel larvae from both of wild and captive-bred samples to establish the production technology of grass eels. But the results obtained in the present research would become an important clue to develop novel rearing feeds for larvae of the Japanese eel in future.

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**Fig. 3** Photomicrographs of histological sections (longitudinal) for oesophageal part of *Anguilla japonica* preleptocephali from 6 dph to 10 dph. Bars indicate 10 µm: oe, oesophagus; in, intestine; no, notochord; og, oil globule; yo, yolk; cc, cardiac cavity; he, heart.

on the development of seedling production technologies of the Japanese eel and lobster).

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## ウナギプレプトケファルス幼生の形態変化と摂餌器官の発達

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### 要 約

ホルモン処理によって人工催熟した親魚から搾出した卵及び精子を人工授精し、その受精卵から孵化仔魚を得た。孵化仔魚は卵黄吸収が終了するまで、21℃と25℃、塩分32-34、0-数lxの暗条件下で飼育管理し、日々の連続標本からその外部形態及び摂餌に関わる器官の発達を観察した。孵化仔魚は、水温21℃では日齢10(211.7℃・日)、25℃では日齢8(201.5℃・日)で卵黄吸収を終了し、口には上下顎それぞれ4対の針状幼歯が特徴的となった。この時期、HE染色された組織切片を見ると、咽頭部に食道壁の肥大により狭窄した部位が観察され、またその内壁には粘液細胞もほとんど発達しないため、ワムシのような固形分の嚥下の困難が想像された。