

[SHORT COMMUNICATION]

Embryonic Development of the Pacific Lamprey, *Entosphenus tridentatus*

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ABSTRACT—Embryonic development of the Pacific lamprey, *Entosphenus tridentatus*, from Japan is described. Egg sizes averaged 1.249 mm (longest axis) and 1.145 mm (shortest axis), the time required for hatching being 11 days at 18°C, shorter than previously reported for a lower water temperature (19 days at 15°C). Early development in *E. tridentatus* proceeded at a similar rate to that in other lampreys, in spite of different rearing water temperatures for the latter, indicating possible specific differences in basic developmental rates.

Key words: egg size, developmental rate, diadromous, speciation

INTRODUCTION

Although the embryonic development of lampreys is worthy of note considering lamprey speciation and their position in the evolution of vertebrates (Piavis, 1971), such patterns have received relatively little attention (e.g., Piavis, 1971; Tahara, 1988).

The Pacific lamprey, *Entosphenus tridentatus* (Gairdner), is characterised by a parasitic, diadromous life-style, usually moving down stream to the sea after metamorphosis, although the species has also been reported as remaining in freshwater on occasion (McPhail and Lindsey, 1970). The species is distributed along the Pacific coast and off coastal islands of North America, from Unalaska Island (Aleutians) to Baja California (McPhail and Lindsey, 1970; Scott and Crossman, 1973). In the Japanese Archipelago, Okada and Ikeda (1938) first described the species from the Yufutsu River, Hokkaido Island. However, because only six specimens in total had been reported up to the time of a report by Honma and Katoh (1987), the presence of *E. tri-*

dentatus in Japanese waters was considered an exploratory or extirpation migration (e.g., Yamazaki and Goto, 2000). Recently, Fukutomi *et al.* (2002) reported natural reproduction of *E. tridentatus* in the Naka River, eastern Honshu Island, Japan. Early developmental features of the species being almost unknown, except for brief descriptions of egg size and time of hatching (e.g., Scott and Crossman, 1973), embryonic development in *E. tridentatus* collected from the Naka River was investigated under experimental conditions and compared with that of other lamprey species.

MATERIALS AND METHODS

Sexually mature adults of *E. tridentatus* were collected in the Yusaka stream, a tributary of the Naka River, Tochigi Prefecture, eastern Honshu Island, Japan, on 1 May 2000. These adults were consistent with those reported by Fukutomi *et al.* (2002) (NSMT-P59933). These individuals were distinctly identified with *E. tridentatus* based on Iwata (2000), because of having large size at maturity (Total length 520 mm and body weight 335 g at male, 527 mm and 386 g at female), a developed supraoral lamina with three sharp cusps, and four series of lateral teeth on each side of the disc. Fertilized eggs were obtained by artificial insemination following Fukutomi *et al.* (2002). Embryos were tank-reared in flowing natural ground water maintained at 18.0°C.

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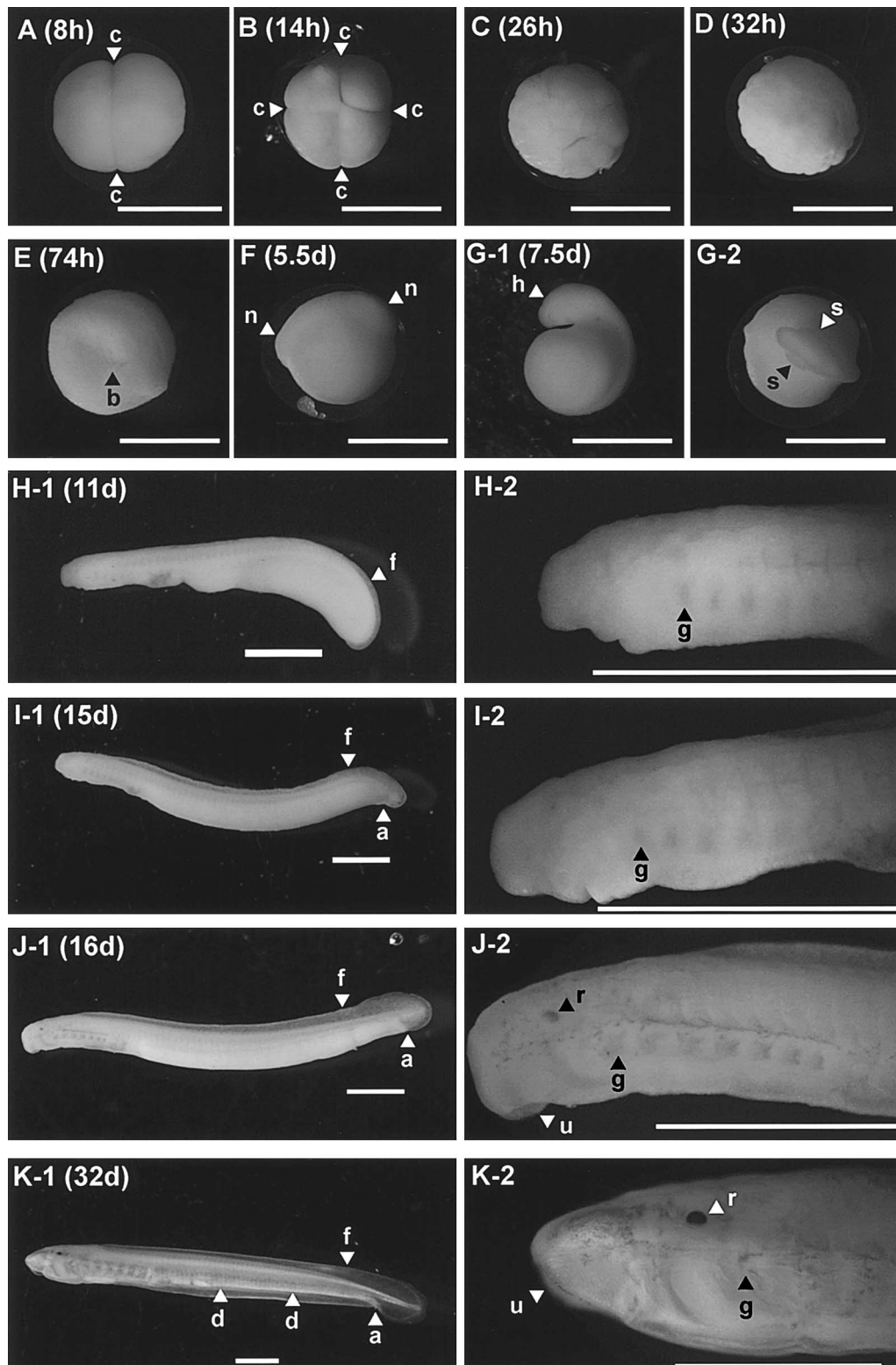


Fig. 1. External view of embryonic development of *Entosphenus tridentatus*. A: two-cell stage, B: eight-cell, C: morula, D: early blastula, E: early gastrula, F: early neurula, G-1: lateral view of head protrusion, G-2: dorsal view of G-1, H-1: hatching, H-2: enlargement of H-1, I-1: melanophore, I-2: enlargement of I-1, J-1: eye spots, J-2: enlargement of J-1, K-1: completion of digestive tract, K-2: enlargement of K-1. Numerals in parenthesis indicate time (day) after fertilization. a, anus; b, blastopore groove; c, cleavage furrow; d, digestive tract; f, dorsal fin; g, 1st gill pore; h, head protrude; n, neural fold; r, pigmented retinae; s, cheek-like swellings; u, upper lip. Scale bars indicate 1 mm.

A time-series of normally developed embryos was preserved in 10% formalin and later examined under a dissecting microscope. Developmental stages were determined mainly from external characteristics, following Tahara (1988).

RESULTS

The developmental sequence was as follows:

0 hr. Ovulated, unfertilized egg. Egg sizes (mm) varied between 1.148–1.384 (average \pm SD: 1.249 \pm 0.062) and 1.037–1.274 (1.145 \pm 0.053) for longest and shortest axes, respectively.

2 hr after fertilization (AF). Polar spot observed at animal pole region. Under absorbed polar cone apparent on polar spot.

8 hr AF. Two-cell stage. First cleavage divides egg meridionally into two blastomeres of approximately equal size (Fig. 1A).

14 hr AF. Four to Eight-cell stage. Second and third cleavage furrows appear meridionally and horizontally, respectively (Fig. 1B).

20 hr AF. Twenty-four to thirty-two-cell stage. More cleavage furrows appear in each hemisphere.

26 hr AF. Morula. New cleavage furrows appear in blastomeres of animal hemisphere (Fig. 1C).

32 to 44 hr AF. Early blastula. Animal hemisphere blastomeres with smooth surface, vegetal half remaining rough (Fig. 1D).

50 to 62 hr AF. Late blastula. Embryo spherical in shape with smooth surface.

74 to 98 hr AF. Early gastrula. Blastopore groove appears above dorsal cone. Embryo shape spherical with a

smooth surface (Fig. 1E).

4.5 days AF. Late gastrula. Blastopore groove becomes elliptical in shape with flat blastopore lip on dorsal surface.

5.5 d AF. Early neurula. Neural folds elevate to contain a neural groove (Fig. 1F).

6.5 d AF. Late neurula. Neural folds contact and fuse in dorsal midline. Anterior end of embryo begins to protrude.

7.5 d AF. Head protrusion. Head protrudes making an acute angle against yolk mass (Fig. 1G-1). Appearance of cheek-like swellings on both sides of head (Fig. 1G-2).

8.5 d AF. Stomodaeum. Stomodaeum appears as a longitudinal slit-like invagination. Cheek-like swellings fuse in ventral midline.

11 d AF. Hatching. Head and neck elongate (Fig. 1H-1, 2). Elevation of dorsal fin. Embryos start hatching.

12 to 13 d AF. Early tailbud. Heart starts beating. Stomodaeum becomes a transverse slit-like invagination. Elongation of trunk. Tailbud appears.

15 d AF. Middle tailbud. Trunk becomes straight (Fig. 1I-1). Melanophores appear in head and trunk regions only at living condition (Fig. 1I-2). Anus directed ventrally. Larvae begin swimming.

16 d AF. Late tailbud. Pigmentation occurs in retinae (Fig. 1J-1). Upper lip expands anteriorly and laterally (Fig. 1J-2). External naris shifts anteriorly.

17 to 18 d AF. Upper lip further expands forming oral hood. External naris continues anterodorsal shift. Trunk melanophore numbers increase. Tail tip pointed backwards. Anal tube elongates.

22 to 24 d AF. Oral hood expands. External naris finally

Table 1. Post-fertilization times to reach successive developmental stages in *Entosphenus tridentatus* and other lamprey species

Stages	<i>E. tridentatus</i>	<i>Petromyzon marinus</i> ¹⁾	<i>Lethenteron reissneri</i> ²⁾
Two-cell	8 hr	2 hr	6.5 hr
Eight-cell	14 hr	10 hr	15.5 hr
Morula	26 hr	19 hr	28 hr
Blastula	32 hr	24 hr	48 hr
Gastrula	74 hr	64 hr	78 hr
Neural plate	5.5 d	4 d	5 d
Head protrusion	7.5 d	6 d	7.5 d
Hatching	11 d	10 d	11 d
Melanophore	15 d	13 d	16 d
Eye spots	16 d	15 d	18 d
Gall bladder	--- ³⁾	17 d	24 d
Completion of digestive tract	32 d	33 d	31 d
Temperature condition	18.0°C	18.4°C	15°C

¹⁾ after Piavis (1971)

²⁾ after Tahara (1988)

³⁾ no data

positioned dorsally. Seven pairs of external gill pores open.

32 d AF. Earliest stage of ammocoete larvae. Formation of digestive tract completed (Fig. 1K-1, 2). First observation of feeding.

DISCUSSION

The early development of *E. tridentatus* proceeded at a rate more or less similar to those reported for other lamprey species (Table 1). Piavis (1971) noted that embryonic development in some lamprey genera was characterized by similar morphological and physiological end points, with only minor deviations.

Regarding *E. tridentatus*, the time required for hatching (11 days at 18°C) was shorter in the present study compared with that (19 days at 15°C) described by Scott and Crossman (1973). This inconsistency should result from an effect of the different water temperatures in the two studies, indicating that in lamprey species developmental rate might depend on the temperature. On the other hand, despite the temperature differences in which the embryos of different species have been reared experimentally, the post-fertilization time required for hatching and subsequent stages was not dissimilar between them (Table 1). These results were expected that sensitivity of developmental processes to temperature varies among species. Differences in basic developmental rate, possibly effected by egg size, have been treated as valuable traits for discussing the speciation process among related fish species with differing life-histories, such as diadromous and fluvial (e.g., Katoh and Nishida, 1994). In lampreys, differences in egg size have also been reported among related lamprey species, for example *Lethenteron* complex comprised by anadromous *L. japonicum* having smaller eggs and fluvial *L. kessleri* and *L. sp. N* having larger ones (Yamazaki *et al.*, 2001). In order to further clarify the speciation process in lampreys, comparative studies should be made of early development among related lamprey species reared under similar conditions.

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