

## Artificial fertilization and time course observations of embryonic development of round herring, *Etrumeus teres*, off the Pacific coast of Japan

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**Abstract** Time course data of the embryonic development of the round herring, *Etrumeus teres*, was described from fertilization to hatching at 20.0°C for artificially fertilized eggs from adults sampled in Tosa Bay, southern Japan. The early blastula stage occurred at 4.3 h. The blastoderm covered more than half of the yolk at 15.1 h, the blastopore closed at 21.9 h and the embryo began to hatch at 64.2 h after fertilization.

**Key words:** *Etrumeus teres*, round herring, development, embryo

*Etrumeus* is a clupeid genus consisting of two species, *E. teres* and *E. whiteheadi*. The former species has a worldwide distribution; in the Indo-West Pacific including off southern African coast, western Atlantic, and eastern Pacific, and the distribution of the latter is restricted to off the southern African coast and in the southeastern Atlantic (Whitehead, 1985). Around Japan, *E. teres* inhabits in the western coastal waters and is an important resource for commercial fisheries.

There are some papers dealing with the early life history of this species around Japan reflecting the importance of this species to fisheries. The distributions of eggs and larvae were reported by Konishi (1980), Hayashi (1990) and Uehara and Mitani (2002). Hayashi and Kawaguchi (1994) investigated the larval growth by examination of the otolith microstructure.

As for the early development, despite the larval development being reported in some detail (Uchida, 1958; Takita, 1988; Watson and Sandknop, 1996), the embryonic development (Mito, 1961; Ahlstorm and Moser, 1980) is still requires clarification, i.e. time course data of the embryonic development from

fertilization to hatching. Time course data of the embryonic development is essential for ageing eggs. Given the egg ages, it is possible to estimate not only spawning time but also egg mortality. O' Toole and King (1974) described a series of embryos from the blastodermal cap stage and the larval development of *E. teres* collected off South Africa but without time course information. However, after their publication in 1974, Wongratana (1983) found a new species *E. whiteheadi* off South Africa. We are not able to categorically state which species was described by O' Toole and King (1974) at this point.

A simple description of embryonic development is not the scope of this study. The focus of this study is describing a detailed time course series of observations of the embryonic development from fertilization to hatching, of *E. teres* off the Pacific coast of Japan and comparisons are made with available information in the literature.

Adult *E. teres* were caught with a midwater trawl (67 m long, 23 m vertical mouth opening) in Tosa Bay off southern Shikoku, Japan (33° 22' N, 133° 48' E) at 19:52-20:22 hours on 27 November 1998. Estimated trawl depth was 32 m at the centre

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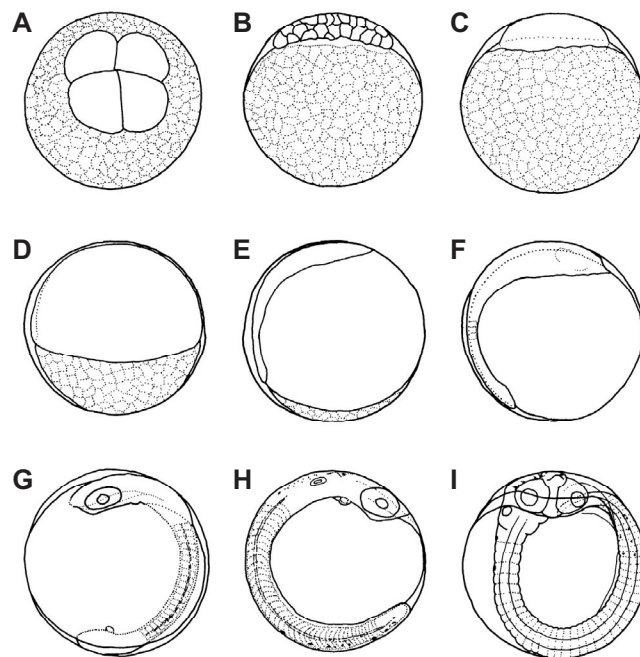
of mouth opening. Sea surface temperature at collection was 22.6°C. Ripe fishes were immediately sorted as parental stock for artificial fertilization. Eggs of two females (184 and 186 mm standard length [SL] after 10% formalin fixation) were collected in a dry stainless bowl by pressing abdomens of the females, then milt of four males (172 to 188 mm SL after 10% formalin fixation) was added over them in the same manner. Fertilization was ensured by mixing eggs, sperm, and field sea water at 20:38 hours. After several minutes the eggs were washed and fragments of tissues were removed.

Approximately 200 floating eggs were transferred to a 1-L polyvinyl chloride bottle (97 mm diameter, 167 mm height) containing filtered sea water. Then, the bottles were placed in a temperature-controlled bath (Thermostatic Water Bath T-2; Thomas Kagaku, Tokyo, Japan) equipped with a cooler

(Handy Cooler TRL-107NH; Thomas Kagaku, Tokyo, Japan). Water temperature was maintained at 20.0°C, which approximated the temperature in the field during the period of peak spawning. Removal of dead eggs and exchange of incubation water were arbitrarily done.

Embryonic development was checked under binocular microscopes (SMZ-10 and OPTIPHOTO; Nikon, Tokyo, Japan) at intervals and color photographs of each stage were taken in life with combinations of various focal lengths and lighting conditions on board. After observations, some of the specimens were fixed in 5% formalin for additional observations. All measurements were from fresh specimens using a calibrated ocular micrometer. Myomere counts followed the definitions of Leis and Trnski (1989).

The newly fertilized eggs were spherical, transparent, buoyant and unpigmented. Eggs ranged



**Fig. 1.** Embryonic development of eggs of *Etrumeus teres*. **A** 4-cell stage, 1.6 h; **B** morula stage, 3.6 h; **C** late blastula stage, 6.0 h; **D** blastoderm covered more than half of the yolk surface, 15.1 h; **E** appearance of embryo, 18.2 h; **F** 4 myomeres, 22.3 h; **G** formation of optic lens, 24 myomeres, 30.4 h; **H** beginning of embryonic movement, beginning of heart pulsation, 43 myomeres, 40.9 h; **I** embryo encircled the yolk sac, 61.1 h. Drawing of yolk segmentation is omitted after blastula stage to make the epiboly clear, although the segmentation is visible all through the embryonic development.

from 1.38 to 1.45 mm in diameter ( $1.41 \pm 0.02$  mm,  $n = 13$ ) (mean  $\pm$  SD) and had no oil globule, a clear and unsculptured chorion, narrow perivitelline space, and segmented yolk. The average ( $\pm$  SD) major diameter of yolk segments was  $0.14 \pm 0.03$  mm ( $n = 17$ ).

Fertilized eggs reached the 2-cell stage by 1.4 h, the 4-cell stage at 1.6 h (Fig. 1A), the 8-cell stage at 2.4 h, the 16-cell stage at 2.5 h, and the morula stage at 3.6 h after fertilization (Fig. 1B). Eggs reached the early blastula stage at 4.3 h, the late blastula stage at 6.0 h (Fig. 1C), and the gastrula stage by 12.0 h. After 15.1 h, the blastoderm covered more than half of the yolk surface (Fig. 1D), the embryonic body appeared at 18.2 h (Fig. 1E), and the blastopore closed at 21.9 h. Four myomeres appeared and differentiation of optic vesicles commenced at 22.3 h (Fig. 1F). Optic and Kupffer's vesicles were clearly visible at 23.4 h and 12 myomeres appeared at 24.8 h. Optic lens formed and 24 myomeres appeared at 30.4 h (Fig. 1G). Otic capsules were observed at 33.1 h. After 36.3 h, Kupffer's vesicle disappeared and the posterior tip of the body became separate from the yolk. The embryo, with 43 myomeres, began to move and the heart began to pulsate at 40.9 h (Fig. 1H). By this stage, melanophores appeared on the head and dorsal part of the posterior body. Otoliths were visible in each otic capsule at 42.2 h. Between 42.2 h and 61.1 h, eggs floating in the upper layer in a bottle began to be distributed in the middle to bottom layer. By 61.1 h, embryo had encircled the yolk sac, the posterior tip of the body reached beyond anterior tip of the head, and melanophores

increased on the ventral part of the posterior body (Fig. 1I).

The embryos began to hatch at 64.2 h after fertilization. Newly hatched larvae ranged from 4.09 to 4.82 mm in total length ( $4.47 \pm 0.33$  mm,  $n = 5$ ) (mean  $\pm$  SD), 3.95 to 4.64 mm in notochord length ( $4.30 \pm 0.30$  mm,  $n = 5$ ) (mean  $\pm$  SD), and had 54 myomeres (Fig. 2). Melanophores were scattered dorsally from midbrain to the caudal tip. Patch of melanophores was observed on the posterior part of intestine along with ventral finfold (approximately 10 myomeres ahead of the anus). A few melanophores were found on the lateral side of the body and just behind of the anus. Eyes were unpigmented. Pigmentation patterns, especially the number of dorsally scattered melanophores, varied individually.

This study firstly details the entire embryonic development of genus *Etrumeus* from fertilization to hatching with time course information. Egg size is similar to that of an *Etrumeus* species (described as *E. teres* in the original article) off South Africa examined by O' Toole and King (1974).

No reports are available on the time course of egg development in *Etrumeus*. O' Toole and King (1974) described and drew some stages of the *Etrumeus* species without time data. Optic vesicles and lens were drawn on the figure prior to the blastopore closure. However, our results show that the optic vesicles and lens were formed at 1.5 h and 8.5 h after the blastopore closure, respectively. Thus, the blastopore closure in *E. teres* off the Pacific coast of Japan occurs at an earlier embryo stage than that in

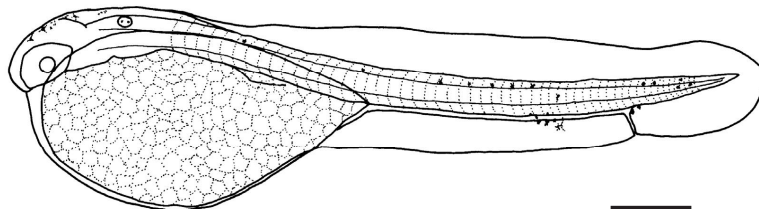


Fig. 2. Newly hatched larva of *Etrumeus teres*. Scale bar = 0.5 mm.

the *Etrumeus* species off South Africa.

Hatching of *E. teres* off the Pacific coast of Japan began at 64.2 h after fertilization at 20°C. This is slightly longer than approximately 2.1 days at 21-22 °C in the Gulf of Mexico (Houde, 1977). Considering the slightly low temperature in this study, it seems that both times required from fertilization to hatching are nearly identical. Incubation time in the *Etrumeus* species off South Africa was estimated 38.4 h at 20°C by the formula (O' Toole and King, 1974). This is considerably shorter than our results. Embryos of *E. teres* off the Pacific coast of Japan and in the Gulf of Mexico developed more slowly at the same temperature.

Vertical shifts in the positions of late stage eggs were observed in a bottle, i.e. from upper layer to middle-bottom. This phenomenon was observed in several fish species and was caused by the increase in specific gravity of eggs (e.g., Coombs, 1981; Coombs *et al.*, 1985; Tanaka, 1990). Thus, the change of specific gravity before hatching would cause the sinking of *E. teres* eggs as well as other species. Deeper distribution of late stage eggs has been suggested in the field study in the coastal area off southwestern Japan (Konishi, 1980).

The size range of newly hatched larvae in this study (4.09-4.82 mm in total length) is similar to those in previous reports for *Etrumeus* (Mito, 1961; O' Toole and King, 1974). Patch of melanophores on the posterior part of intestine, which is a diagnostic feature in larval *E. teres* (Takita, 1988), were also observed in newly hatched larvae of this study, although no melanophores on that part of the body were seen in the *Etrumeus* species off South Africa (O' Toole and King, 1974).

As stated above, some remarkable differences in developments of eggs and newly hatched larvae are shown between *E. teres* off the Pacific coast of Japan and the *Etrumeus* species off South Africa (O' Toole and King, 1974). Also, there is obvious difference in incubation time between the Gulf of Mexico (Houde, 1977) and South Africa (O' Toole and King, 1974). The differences might be due to different species; i.e., the *Etrumeus* species off South Africa was not *E. teres* but *E. whiteheadi*. Even if the species described by O' Toole and King (1974) was *E. teres*, the early development described

here suggests significant geographical differences within species. It is known that *E. teres* in the Gulf of Mexico does not spawn at the low temperatures observed in South African waters (Houde, 1977). Also, *E. teres* off the Pacific coast of Japan does not spawn at such low temperature (Uehara and Mitani, unpubl.). *Etrumeus* off the Pacific coast of Japan and in the Gulf of Mexico likely to be adapted to warm temperatures.

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