

## Embryonic Growth during Gestation of the Viviparous Eelpout, *Zoarces elongatus*

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The viviparous mode of reproduction in teleosts was categorized as lecithotrophy and matrotrophy on the basis of maternal-fetal trophic relationships (Wourms, 1981). Lecithotrophic embryos derive their nutrition solely from yolk reserves, whereas matrotrophic embryos depend on a supply of maternal nutrients during gestation (Wourms, 1981, 1991). Designation of a particular species as lecithotrophic or matrotrophic has been based on a comparison between the dry weight or total organic weight of the egg and that of the full-term embryo (Wourms et al., 1988).

Within the Zoarcidae, viviparity appears to have evolved only in three species of the genus *Zoarces* (Nelson, 1993). Heretofore, almost all the information available on zoarcid viviparity has been derived from studies of the European species, *Z. viviparus* (e.g. Korsgaard, 1986), with little being known about the other species. *Z. elongatus* is a viviparous teleost, which inhabits the Pacific coast of northern Japan. Embryos of *Z. elongatus* are retained in the ovarian cavity for more than five months after fertilization (Koya et al., 1993). Ripe eggs of *Z. elongatus* are very large, about 4.4 mm in diameter (Koya et al., 1993), indicating that the embryos have access to abundant yolk reserves during early development. In addition, the embryos of this species grow from 29.1 mm at hatching to 61.0 mm by late gestation (Koya et al., 1993). Hence, it is thought that the embryos received some maternal nutrients after yolk resorption. In order to determine whether *Z. elongatus* is lecithotrophic or matrotrophic, it is necessary to examine embryonic growth, especially the change in dry weight during gestation. However, no infor-

mation pertaining to this subject was available.

In the present study, the changes in total length, body weight and dry weight were investigated as criteria for growth during gestation in *Z. elongatus*. In addition, tracer experiments on the mechanism and site of nutrient absorption by the embryo were performed.

## Materials and Methods

Female *Zoarces elongatus* were caught by angling in Akkeshi Bay, eastern Hokkaido, Japan, during May to October 1992. Thirty-four fish were subsequently transferred to Usujiri Fisheries Laboratories, Hokkaido University, and kept in an indoor 1000 liter circular tank with flowing sea water under natural photoperiod conditions. At monthly intervals during the gestation period (September to February, Koya et al., 1993), four to ten females were anesthetized with ethyl 4-aminobenzoate and the ovaries removed. Embryos were removed from the ovaries and the total lengths and wet weights measured, before being dried for 48 hr at 80°C. The dry weight was then determined.

An *in vitro* tracer experiment was carried out in order to determine the site of embryonic nutrient absorption. Isolated from the ovaries, embryos were placed into L-15 medium (pH 7.5, Sigma Chemical Co.) containing 10 mM HEPES, 100 mg/l streptomycin sulfate and 75 mg/l penicillin G potassium. Bovine serum albumin (BSA, fraction V, Sigma Chemical Co.) at a concentration of 1% w/v was used as a tracer. Embryos were incubated for 8–12 hr at 12°C and then fixed with Bouin's solution for 12 hr at 4°C. After washing three times with 0.1 M sodium phosphate buffer (PBS, pH 7.4) containing 10% sucrose for 6 hr at 4°C, they were embedded in paraffin (m.p. 52–54°C) and sectioned at 5 µm thickness. Immunohistochemistry procedures were carried out using the second antibody procedure and the peroxidase-antiperoxidase complex (PAP) technique of Sternberger et al. (1970). Deparaffinized sections were treated with 1% H<sub>2</sub>O<sub>2</sub> in methanol to inhibit endogenous peroxidase activity and then incubated in 10% normal goat serum to avoid non-specific binding of antibodies. After rinsing with distilled water followed by PBS, the sections were subjected to a solution of rabbit anti-BSA antiserum (Wako Pure Chemical Industries, LTD.) diluted 1:5000. After washing with PBS, a solution of goat anti-rabbit IgG antiserum (Wako Pure Chemical Indust-

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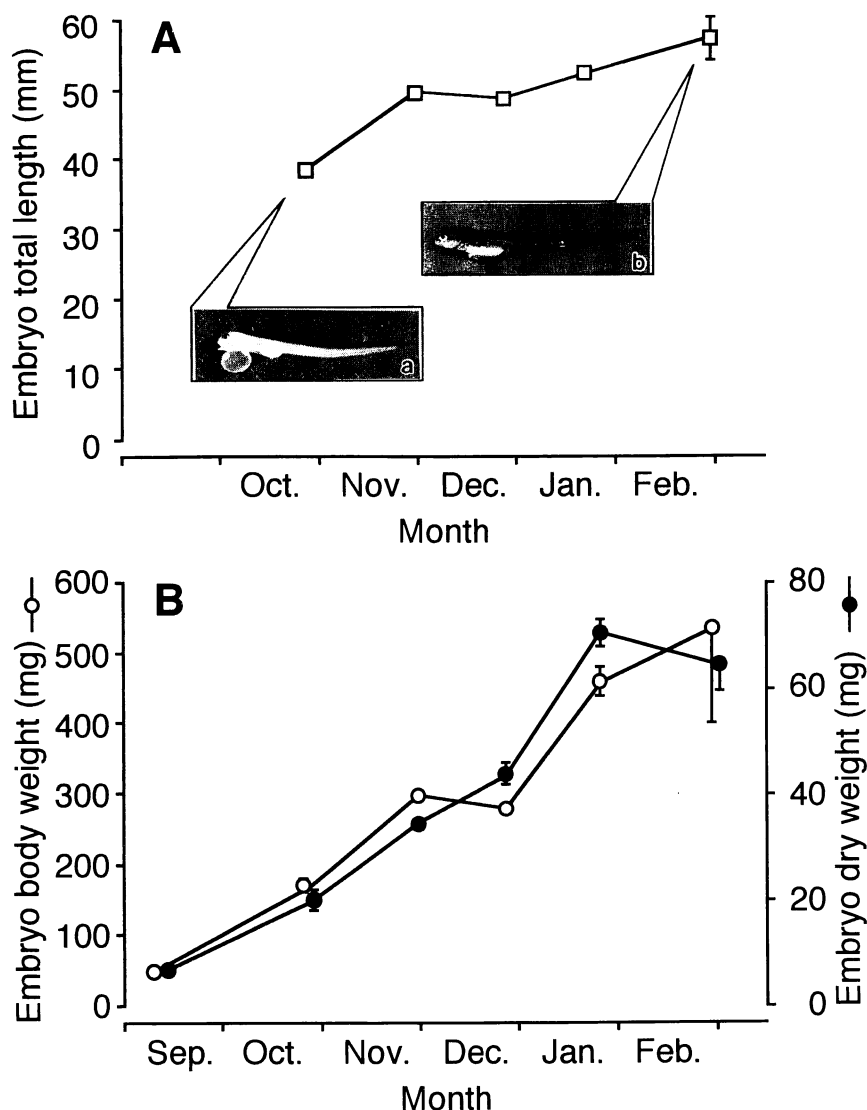


Fig. 1. Growth of embryo in *Zoarces elongatus* during gestation. A) Changes in total length (mean  $\pm$  SE). Inset photographs show yolk-sac embryo in October (a) and juvenile in February (b); B) changes in body weight (○) and dry weight (●) (mean  $\pm$  SE).

ries, LTD.) was applied. The sections were then incubated with PAP (Jackson Immuno Research Laboratories, Inc.) and any end product on the sections indicative of BSA localization made visible with 3,3'-diaminobenzidine. The specificity of immunohistochemical staining was examined in the control sections which were not subjected to rabbit anti-BSA antiserum. For routine histological observations, sections were stained with haematoxylin and eosin (H-E).

## Results

**Embryo growth.**—Figure 1 shows the changes in total length (TL), wet weight and dry weight of embryos during gestation. The ripe eggs of *Zoarces elongatus* were 4.4 mm in diameter, their wet and dry weights being 45 mg and 7 mg, respectively. Fertilization occurred in September after which the embryos developed before hatching in October. Within one month after hatching (Fig. 1A, inset a), the

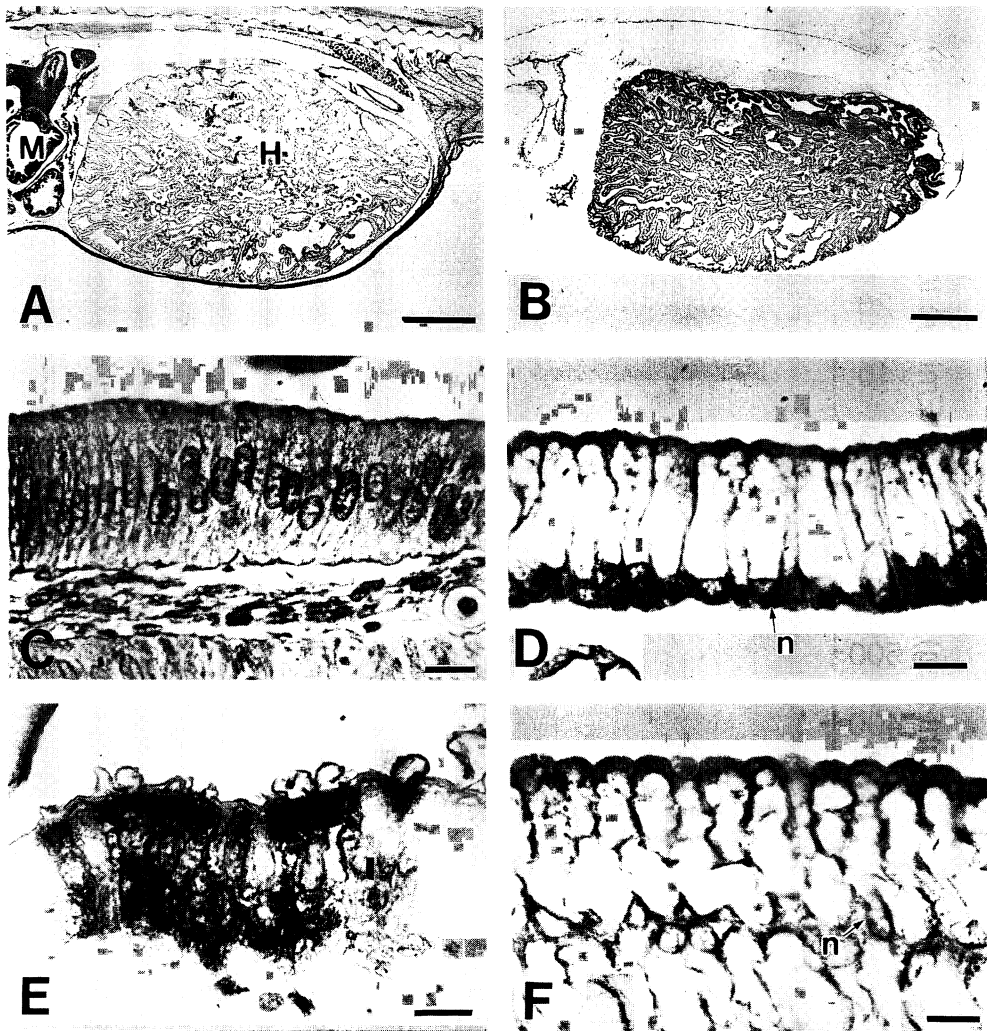


Fig. 2. Photomicrographs of the sections of *Zoarces elongatus* embryos in October. *H*—hind-gut; *M*—mid-gut; *n*—nucleus. A) Sagittal section. Haematoxylin-eosin (H-E) staining. Scale bar = 1 mm; B) sagittal section. Immunostaining of the PAP method using anti serum against BSA. Reaction products were detected in the mid- and hind-gut. Scale bar = 1 mm; C) H-E staining section of the mid-gut epithelium. Scale bar = 10  $\mu$ m; D) H-E staining section of the hind-gut epithelium. Scale bar = 10  $\mu$ m; E) immunostaining section of the mid-gut epithelium. Scale bar = 10  $\mu$ m; F) immunostaining section of the hind-gut epithelium. Scale bar = 10  $\mu$ m.

embryos were 39 mm TL, 170 mg and 20 mg in wet and dry weights, respectively, and had a yolk-sac. Embryos completed yolk absorption in late November, having attained 50 mm TL, and 300 mg and 35 mg in wet and dry weights, respectively. In February, the embryos grew further (Fig. 1A, inset *b*), reaching 57 mm TL, and 540 mg in wet weight (65 mg dry weight).

*Absorption of external protein.*—Immunohisto-

chemical observations of BSA uptake was performed on the sagittal sections that extended from the head to the anus. Figure 2A shows a photomicrograph of a H-E stained section of a yolk-sac embryo, 39 mm in total length, in October. The abdominal cavity of the embryo was occupied by a well-developed intestine, recognizable as two parts owing to their morphological differences. The front part of the intestine (mid-gut) was a simple winding tube, whereas the

posterior part (hind-gut) was thickened having numerous, intricately-arrayed folds (Fig. 2A). Positive immune localization of BSA was detected in both the mid- and hind-guts (Fig. 2B). In the mid-gut, the cytoplasm of the epithelial cell was stainable by H-E, with the nucleus being localized in the center of the cell (Fig. 2C). On the other hand, the epithelial cells of the hind-gut were occupied by a vacuole-like structure which was not stained H-E, and had the nucleus localized in the basal part of the cell (Fig. 2D). Positive immuno-reaction in the mid-gut occurred in the cytoplasm of most of the epithelial cells (Fig. 2E), although that in the hind-gut epithelium was restricted to the cortical and thin lateral parts of the cytoplasm (Fig. 2F). In the control sections, no positive reactions were observed. Similar results were obtained from embryos that had completed yolk absorption by late November.

### Discussion

The present study demonstrated that embryos of *Zoarces elongatus* increased from 7 mg to 65 mg in dry weight during gestation. Such an increase indicates that *Z. elongatus* is a matrotrophic species in which embryos depend on a continual supply of maternal nutrients. Various grades of maternal dependence occur in matrotrophy, involving both the initial yolk reserves as well as the amount of nutrients provided during gestation. For example, yolk reserves are lacking in the eggs of *Embiotoca lateralis* (Wourms, 1981), whereas abundant yolk reserves occur in the eggs of *Zoarces viviparus* (Bretschneider and DeWit, 1947; Korsgaard, 1986). *Z. elongatus* forms very large eggs (4.4 mm in diameter) which have abundant reserves of yolk (Koya et al., 1993). Thus, they are similar to *Z. viviparus* in terms of their initial provision of nutrients.

In *Z. elongatus*, the dry weight increased from 7 mg ripe eggs to 20 mg for embryos that still had a yolk-sac one month after hatching. The results of tracer experiments demonstrated that the intestine of embryos having a yolk-sac had the ability to absorb protein. The embryos of *Z. elongatus* thus begin to receive maternal nutrients from their intestine during the period of yolk absorption. Therefore, the embryos of this species utilize their own yolk and maternal nutrients during early gestation, thereafter depending solely on additional maternal nutrients for more than three months.

Several types of external trophic adaptations, such

as "trophotaeniae" or "follicular pseudoplacenta," in the embryos of viviparous teleosts are known (see Wourms, 1981). In *Z. viviparus*, Kristoffersson et al. (1973) suggested that an enlarged hind-gut with a hypertrophied intestinal epithelium was the main site of nutrient absorption. The present study indicated that the embryos of *Z. elongatus* absorb maternal nutrients both from the simple mid-gut as well as from the enlarged hind-gut. Based on apparent differences in the absorptive area, the hind-gut surpassing the mid-gut, it was concluded that the hind-gut is the main site of nutrient absorption in *Z. elongatus*. The difference in the morphology of mid- and hind-gut epithelial cells may reflect differences in function.

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#### 胎生魚ナガガジの妊娠期における胎仔の成長

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胎生魚のナガガジ (*Zoarces elongatus*) が、卵黄栄養依存 (lecithotrophy) および母体栄養依存 (matrotrophy) のいずれの胎生型に属するのかを明らかにするために、1992年9月から2月までの妊娠期中の胎仔の体長、湿重量、および乾燥重量の変化を調べた。本種の未受精卵は直径4.4 mm、乾燥重量7 mgで、胎生魚の卵としては極めて大きかった。9月に受精した卵は10月下旬に

は孵化していた。孵化後1か月以内の卵黄嚢を持つ胎仔は体長39 mmで、乾燥重量は20 mgに増加していた。その後の妊娠期を通じて体長、湿重量、および乾燥重量とも直線的に増加し、2月には体長57 mm、乾燥重量65 mgに達した。このような乾燥重量の増加は、本種が母体栄養依存型の胎生魚であることを示している。また、孵化後間もない胎仔の乾燥重量が未受精卵のそれよりも大きかったことは、この時期からすでに胎仔が母体由来の栄養を摂取していることを示唆している。牛血清アルブミン (MW 67,000) をトレーサーに用いて胎仔の栄養吸収能を調べた結果、卵黄嚢を持つ時期には既に腸で外因性の栄養を吸収できることが示された。従って、本種の胎仔は孵化後自身の卵黄嚢を吸収しつつ母体からも何らかの栄養を受け、卵黄嚢吸収後は母体からの栄養供給のみに依存して成長するものと思われる。

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