MORPHOLOGICAL CHANGES OF THE EMBRYO OF A VIVIPAROUS TELEOST, *NEODITREMA RANSONNETI* STEINDACHNER DURING GESTATION

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Introduction

Concerning the development of embiotocids, a group of viviparous teleosts, several valuable contributions have been made by Girard (1854), Ryder (1885), Eigenmann (1892), Turner (1938), Triplett (1960), etc. with the American species, *Embiotoca jacksoni* AGASSIZ, *Cymatogaster aggregatus* GIBBONS and *Amphistichus argenteus* AGASSIZ, Uchida (1938), Ishii (1960) and Mizue (1961) have also referred briefly to the development of *Ditrema temmincki* BLEEKER, one of two embiotocids in Japan, in their reports on ecological studies of the species.

In the preceding paper (1961), the present writer reported on the histological and cytological changes of the ovary during gestation with the other species in Japan, *Neoditrema ransonneti* STEINDACHNER. In the present paper the writer intends to describe observations made on the morphological changes of the embryo during gestation with the same species, in comparison with the results obtained by the above mentioned workers.

Before going further, the writer wishes to express his hearty thanks to the late Prof. Shin-ichi Sato and Prof. Shun Okada of the Faculty of Fisheries, Hokkaido University for their various sorts of advice and kind encouragement in the prosecution of the work. His thanks are offered also to Messrs. Kiyu Kobayashi and Isao Hirano for their valuable help in the collecting of the specimens.

Materials and Methods

The materials, *Neoditrema ransonneti*, utilized in the study were collected during the months April to August, 1960 on the shores in the vicinity of Hakodate. The ovaries were dissected out as soon as possible from the females after the materials were captured; they were preserved in Bouin's or Zenker's fixative fluids or in formalin. Sufficient samples of various stages of embryos during gestation were obtained. Most of them were observed under low magnification, while some cases *in toto*, being stained with borax carmine. Longitudinal serial sections especially in the portion of the hind gut were also made in some embryos which measured 3~45 mm in length. A double staining method with Delafield's haematoxylin and eosin was used for the staining.

Observations

The ovary of *Neoditrema ransonneti* shows a remarkable difference in external features from the ovaries of oviparous or ovoviviparous teleosts. Namely, the part corresponding to the oviduct in ordinary teleosts expands and is transformed into a single and unpaired ovary which is bifurcated for a short distance only at the anterior end.

The ovary of a female just after birth is small in volume and remains without showing any increase in relative volume to the growth of the body to January. It grows gradually from February to March and the volume increases rapidly after April. The peak of growth appears in June and then the ovary decreases rapidly in volume after July. The increase in ovary volume is not due to maturation and addition of oocytes as in ordinary teleosts, but to the growth of embryos in the ovary. There is not found any embryo in the ovary after the middle of July. In the vicinity of Hakodate the discharge of embryos from the maternal body seems to occur during two weeks or so from late July to early August. The testis of a male can be clearly identified from the ovary of a female just after birth.

Mating is perhaps carried out between the sexes in late September after the birth. Spermatozoa are found in the ovarian cavity of females collected after late September, but are never found before that time. Fertilized eggs are discharged from an ovigerous fold into an ovarian cavity and continue to develop there. The ovarian cavity of *Neoditrema ransonneti* is divided into 6 longitudinal folds which are free ventrally and attached at the dorsal mid-line. The folds are thin and highly pleated, increasing the surface area in a small space. The embryos are dicharged late in July or early in August from the maternal body after gestation during more than ten months.

The embryo at the earliest stage is slender transparent needle-like, measuring about 3 mm total length (Plate I. Fig. 1). The tail is formed to a certain extent and terminates in a blunt lobe. A yolk sack lies at the posterior part of a pericardium, containing a mass of minute yolk at the anterior end. The heart is yet simple in the form of a slender tube extending from the posterior wall of the pericardium upward and forward. The alimentary canal is straight without looping. The hypertrophy of the hind gut has begun at this stage (Plate II. Fig. 1).

There appear several changes of features in the embryo a little more than 5 mm in length (Plate I. Fig. 2). The notochord extends to the tip of the tail while a caudal fin fold is not yet developed. The dorsal and anal fin folds are both developed, with a thickening at the posterior part of the latter. Later the posterior parts of the dorsal and anal folds extend to the tail and consequently the caudal fin fold is finally formed. The pectoral fin is present also as a small flap on the shoulder. The liver is well developed and fills the most part of the space formerly occupied by the yolk sack. The intestine becomes much larger while, as a whole, it is still a simple tube. The hind gut lined with many folds which later transform to villi protrudes distinctly outside from the surface of the body (Plate II. Fig. 2).

In the embryo of about 8 mm length, the yolk has been absorbed completely and the mouth is now opened (Plate I. Fig. 3). The simple tube of the heart has been transformed into an auricle, a ventricle and arteries. The intestine bends down and forms a sigmoid curve. The hind gut is very hypertrophic and protrudes outside, the wall being thin and highly folded in its inner side (Plate II. Fig. 3). It is presumable that most of the absorption of nutrients brought into the mouth and through the gill-cleft takes place here.

The embryo beyond about 8 mm long exhibits the peristaltic movement of the hind gut. At the same time the eyes are pigmented. Scales are formed principally along the lateral line and around the eye when the embryo is about 11 mm long. The definite fins appear at this stage.

When the embryo grows to 15 mm length in the ovary at late May, the dorsal, anal and caudal fins suddenly get large. All the margin of the fin membrane of each fin is enlarged and projects, especially in the embryos soon before birth (Plate I. Figs. 4, 5). This suggests that the respiration and the absorption of nutrition may be performed by the spatulated extensions of these fins. The rectum lined with many villi protrudes outside at the highest degree in this stage (Plate II. Figs. 4, 5).

The embryos vary in length at the time of birth, ranging from 40 to 45 mm. The body is comparatively thick, the flesh firm, the vertical fins comparatively short and thick. The spatulated fin extensions have disappeared (Plate I. Fig. 6). The scales develop with many chromatophores and are arranged as imbricated. The body is very much paler in color even in the largest embryos than in the young soon after birth among which the male is darker than the female. The protruding rectum becomes small and is normal in appearance, while the hind gut in the abdominal cavity still remains swollen (Plate II. Fig. 6).

Soon after birth, most of the young leave the shallow waters in which they were born. Only a few of them remain there in company with the breeding adults, probably for a very short time.

Discussion

It is well known that adaptations to various degrees develop morphologically and physiologically between an embryo and the maternal body in viviparous animals. Of course, identical facts are seen in viviparous teleosts, too.

The family Goodeidae in the order Cyprinodontes, a group of viviparous fresh-water fishes, includes species which show distinctly such adaptive changes between the mother and her embryos during gestation. Turner ('33, '37, '40a, '40b) and Mendoza ('37, '39, '40, '41, '43, '56, '58) studied the adaptations in the family from the structural, histological and physiological points of view. After performing extensive studies on the goodeids, Turner concluded that the respiration and absorption of nutrients by the embryos might occur in the following ways: from the entire body surface, through the vascular trophotaeniae, through the vascular gills which are protruded markedly outside the body in some species, by the specially differentiated rectum, etc.

The respiration and nutrition of the embryos of the family Embiotocidae were described by Eigenmann (1892) and Turner ('38, '52) with *Cymatogaster aggregatus* Gibbons, by Triplett ('60) with *Amphistichus argenteus* Agassiz, by Mizue ('61) with *Ditrema temmincki* Bleeker. According to them, the ovary is not only the place producing eggs but also the home of developing embryos until the time of birth. Neither placenta nor conspicuous placenta-like structure is developed on either embryos or maternal body during gestation. Accordingly several interesting changes or adaptations occur on the morphological characters of the embryos and maternal body so that the former may obtain oxygen and nutrients.

An accessory organ of absorption, the trophotaeniae, develops in all species of goodeids (Turner, '37, '40a, '40b; Mendoza, '56) from an early stage to the end of gestation, while such an organ does not develop in the embryos of embiotocids during gastation. In the latter embryos, doubtless, the part where the absorption chiefly takes place is the hypertrophic hind gut, as mentioned by Eigenmann (1892), Triplett ('60) and Mizue ('61). In the embryos of *Neoditrema ransonneti* exceeding about 8 mm length the rectum expands and protrudes outside, the wall being very thin and lined with long, vascular villi. This agrees with the conditions found by Mizue ('61) in the embryos of the same stages of *Ditrema temmincki*. It is surely one of the adaptations for the absorption of nutrition in the embryo of *Neoditrema ransonneti* as the trophotaeniae are so in the goodeids.

When the embryo of *Neoditrema ransonneti* grows to about 15 mm long in the ovary in late May, the vertical fins enlarge suddenly and spatulated extensions appear at the margin of these fins. Various studies have been undertaken to determine whether the peculiar structure is concerned with respiration or nutrition or both functions. Blake (1867) assumed many years ago that the embryos of *Cymatogaster* might obtain nutritive substances through the extensions. Eigenmann (1892) ascertained with the embryos of *Cymatogaster* that the extensions were concerned with not only nutrition but also with the respiratory function. Mendoza ('58) recently reported that the enlarged fin folds in the embryos of *Goodea luitpoldii*, one of the species of Goodeidae, might be concerned with respiratory, excretory and nutritive functions. The spatulated extensions in the embiotocids are very similar to the fin folds in goodeids in regard to the vascular structure. It seems certain that the hypertrophic fins in *Neoditrema ransonneti* may be concerned with both respiration and the absorption of nutrients.

Summary

The morphological changes in the embryos of a viviparous teleost, *Neoditrema ransonneti* STEINDACHNER during gestation may be summarized as follows:

1. In the embryo which is larger than 5 mm long, the yolk has been absorbed entirely. Subsequently, it seems that the embryo absorbs the fluid in the ovary directly from the surface of the body and continues to grow.
2. The rectum lined with many folds and villi protrudes outside the body until the embryo grows to about 35 mm long. This seem also in the embryo to be one of the organs for the absorption of nutritional matter.
3. When the embryo grows to about 15 mm long in ovary, the dorsal, anal and caudal fins increase rapidly in size; the margin of each fin is enlarged and projects. The extensions develop in the highest degree specially in the embryos soon before birth. This suggests that the respiration and nutrition are performed by these fins.

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Explanation of Plates

PLATE I

External appearance of various stages of embryos. All embryos were stained with borax carmine except one embryo of 42.3 mm length.

Fig. 1. An embryo of 3.2 mm total length (collected on 10th April)

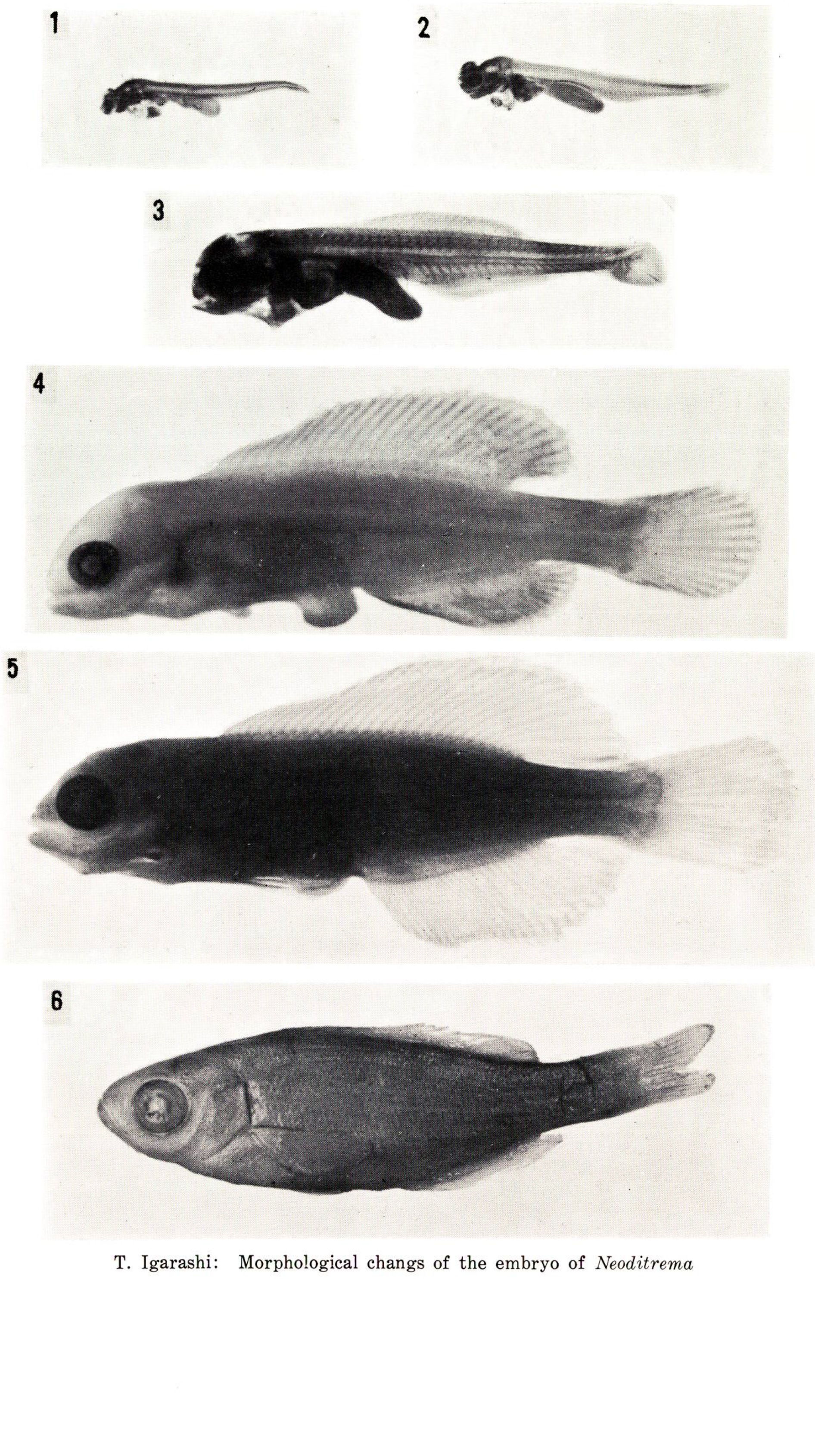
Fig. 2. An embryo of 5.4 mm total length (collected on 10th April)

Fig. 3. An embryo of 8.5 mm total length (collected on 16th April)

Fig. 4. An embryo of 15.5 mm total length (collected on 22th May)

Fig. 5. An embryo of 28.6 mm total length (collected on 18th June)

Fig. 6. An embryo of 42.3 mm total length (collected on 28th July)



T. Igarashi: Morphological changs of the embryo of *Neoditrema*

PLATE II

Longitudinal sections of the hind guts in various stages of embryos.

Fig. 1. An embryo of 3.2 mm total length (collected on 10th April)

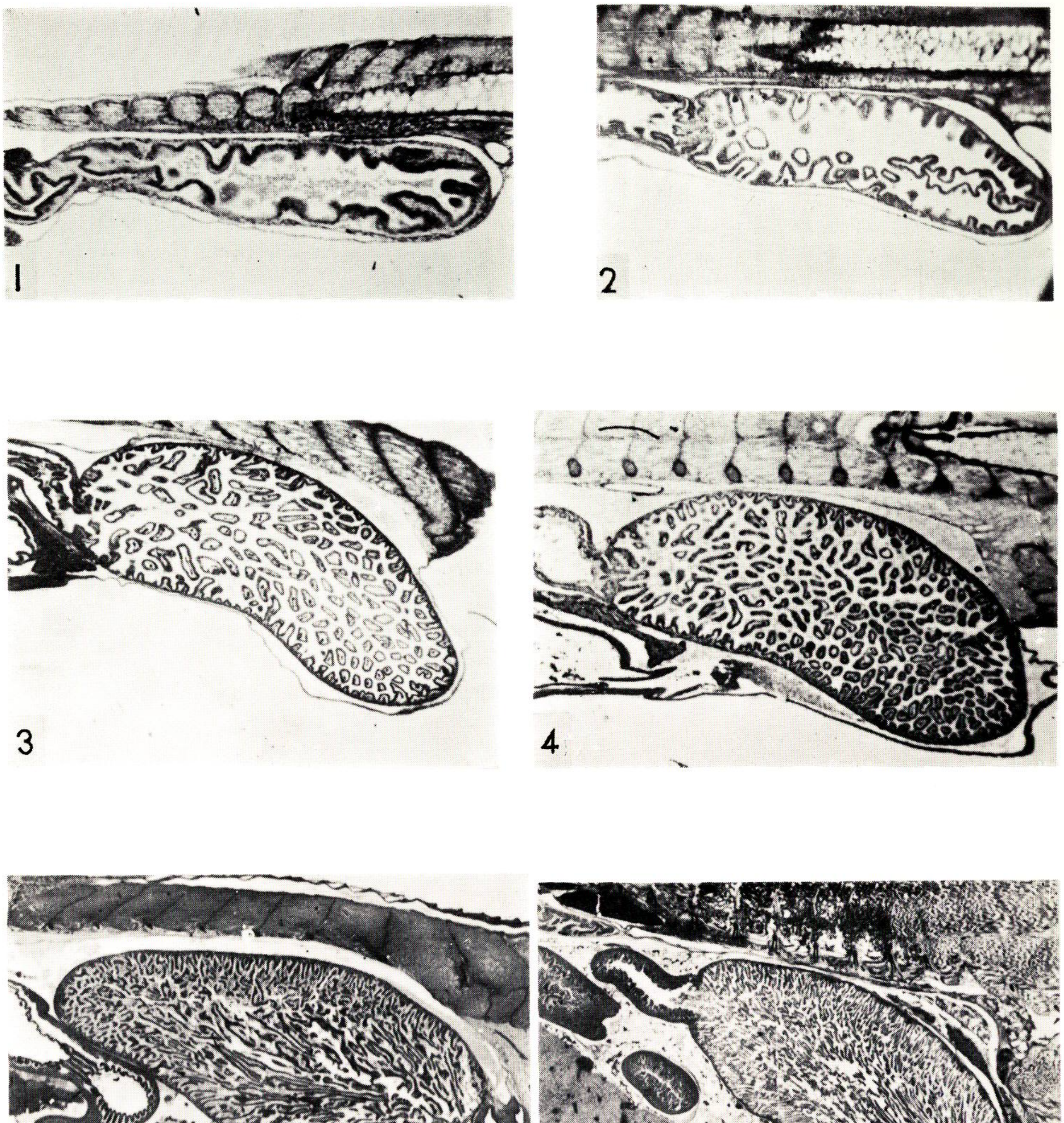
Fig. 2. An embryo of 5.4 mm total length (collected on 10th April)

Fig. 3. An embryo of 8.5 mm total length (collected on 16th April)

Fig. 4. An embryo of 15.5 mm total length (collected on 22th May)

Fig. 5. An embryo of 28.6 mm total length (collected on 18th June)

Fig. 6. An embryo of 42.3 mm total length (collected on 28th July)



T. Igarashi: Morphological changes of the embryo of *Neoditrema*