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Freshwater Stingray Embryos Store Yolk in Their Intestinal Epithelial Cells

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Abstract：Intestines of freshwater stingray embryos sampled in South America were examined histologically. During embryonic development, yolk, which is transported through the yolk stalk from the external yolk sac, appeared to be accumulating in the intestinal epithelial cells of larger embryos. The yolk (or yolk derived/originated substances) that accumulated in the intestinal epithelial cells may provide nutrients that enable the embryos to survive the vulnerable period immediately after birth.

Key words：freshwater stingray, yolk, intestinal epithelial cells, *Disceus thayeri, Potamotrygon magdalenae, P. motoro*

Introduction

To investigate reproduction in freshwater stingrays (elasmobranches) in rivers and lakes in South America (Fig. 1) the senior author (KT) participated in a research trip conducted during August through September 1980[1)]. During this trip, 117 individuals of the freshwater stingray, *Potamotrygon magdalenae*, were collected in lakes near San Cristobal, Colombia and we reported on its reproductive biology[2)]. In the process of studying the reproductive biology, we also examined various visceral organs including the intestine. These observations uncovered a quite unique phenomenon that has not yet been reported. The reproductive mode of the species examined here is non-placental viviparity as defined by Teshima[2, 3)]. That is, the animal has two uteri (right and left) and generally a single uterus has several embryos, but the placentae are not developed in the uteri. Therefore, the yolk is very important for the development of embryos. Although observed materials were rather limited, this report describes an unusual phenomenon with respect to the yolk absorption in specimens of *Disceus thayeri, P. magdalenae*, and *P. motoro* sampled in the Amazon, Magdalena, and Parana Rivers.

Materials and Methods

Materials for this study included three embryos of *Disceus thayeri* with disk widths of 20, 75 and 123 mm and a single embryo each of *Potamotrygon magdalenae* of 65 mm in disk width and *P. motoro* of 80 mm in disk width respectively (Fig. 1, Table 1).

Disceus thayeri specimens were captured by Japanese scientists in cooperation with local people in Rio Amazonas near Manaus, Brazil during a period from September 4th through 10th, 1980. A small-sized purse seine was employed in capturing the stingrays at night in the shallow areas of Rio Solimones near Manaus[1)].

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Embryos found in the uterus of the stingrays were fixed in 10% formalin solution. With the aid of local fishermen 117 individuals of *Potamotrygon magdalenae* were taken by gillnet in a lake constituting Rio Magdalena system near San Cristobal in Colombia during a period between August 13th and 16th, 1980[1]. Only one embryo with 65 mm disk width was taken from the uterus and fixed in 10% formalin. A single *P. motoro* embryo preserved in formalin was kindly provided from the Santa Fe Museum. The *P. motoro* specimen preserved in the museum was taken from Rio Parana[1].

All samples preserved in 10% formalin solution at the sampling sites were brought to Japan. After the external characters had been observed, the intestines were removed and paraffin sections of about 6 μm thick were made and stained with haematoxylin and eosin.

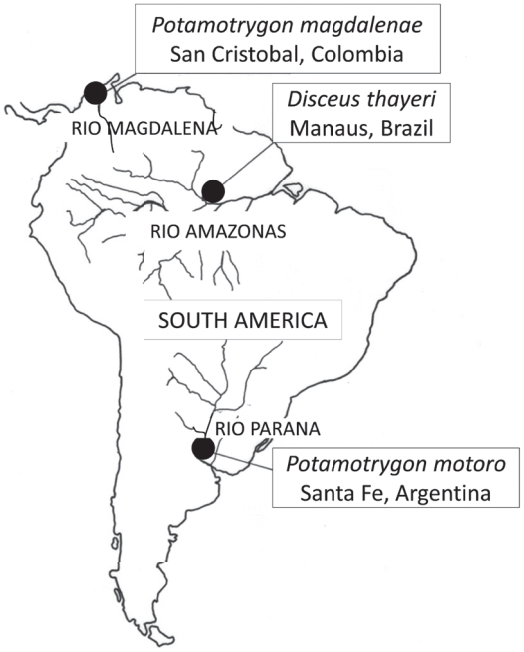

Fig. 1. Sampling site of the stingrays used in the present study.

Results

Macroscopic features

The central area of the abdominal skin of a *Disceus thayeri* embryo of 123 mm disk width was removed and the distal end of the yolk stalk was found to be linked to the anterior end of the intestine (Figs. 2A, 2B). The intestine removed from the body was transversely cut into the seven serial blocks to show the inner conditions as shown with the number 1 to 7 in Fig. 2B. Transverse sections of the fourth to the seventh block, i.e., the posterior portion of the intestine appeared to be mostly solid and filled with dark substances while those of the first to third block, i.e., the anterior portion seemed to be rather soft and had some empty spaces (Fig. 2B).

The dorsal side and ventral side having the external yolk sac of a *Potamotrygon magdalenae* embryo of 65 mm disk width are shown in Figs. 3A and 3B respectively. The conditions we observed in the embryo of this species (Fig. 3C and Fig. 3D) were almost identical to those described above in the *Disceus thayeri* embryo. Among the seven tissue blocks, the fourth to seventh one, i.e., posterior portion appeared to be extremely

Table 1. Embryos of freshwater stingrays sampled in three South American rivers

Animal examined		Size of yolk sac	Presence of intraepithelial	Sampling Mo/Yr
Species	Disk width (mm)	Length × width (mm)	yolk-like substances in the intestine	Location*
Disceus thayeri	20	a	None	September, 1980
	75	b	Large amount	Rio Amazonas
	123	50 × 10	Large amount	Manaus, Brazil
Potamotrygon magdalenae	65	30 × 7	Large amount	August, 1980
				Rio Magdalena
				San Cristobal, Colombia
Potamotrygon motoro	80	25 × 6	Large amount	September, 1980
				Rio Parana
				Santa Fe, Argentina

*See the map shown in Fig. 1

a, b; Yolk sac was broken when removed from the uterus.

a; Yolk sac was estimated to be several times larger than the embryo because in the non-placental viviparous species the smaller embryo possesses the larger yolk sac.

b; smaller than a, but larger than a 123 disk width embryo.

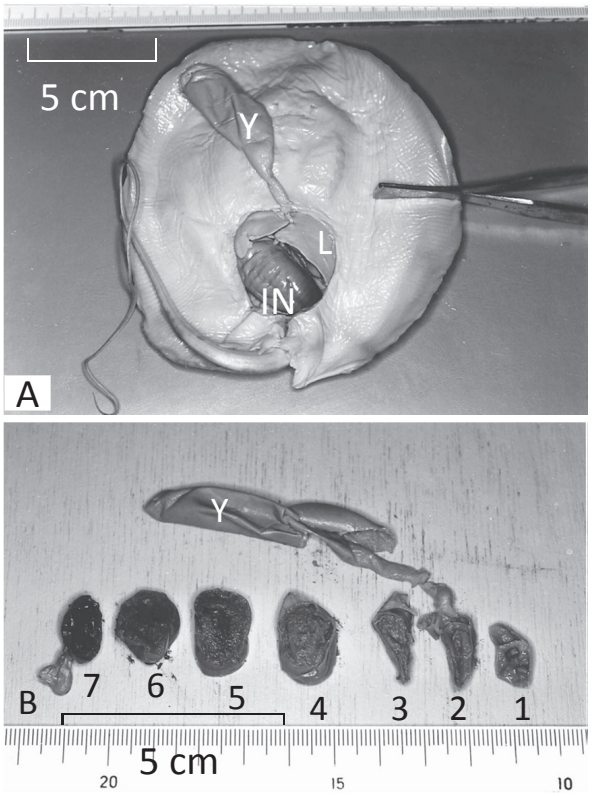

Fig. 2. **A,**Ventral view of *Disceus thayeri* embryo of 123 mm disk width. The central area of the abdominal skin was removed to show the intestine (IN), the liver (L), and the external yolk sac (Y); **B**, Seven serial blocks (1 to 7) of the intestine to show the inner conditions in the 123 mm disk width of *D. thayeri* embryo.

solid while the first to third one, i.e., the anterior portion seemed to be rather soft with some empty spaces being formed inside (Fig. 3E).

The macroscopic features of the embryo of *Potamotrygon motoro* were similar to those of the *P. magdalenae* embryo.

Histological features

The valvular intestine and neural tube were observed in a transverse section of the whole body from a *Disceus thayeri* embryo with a 20 mm disk width (Fig. 4A). At this developmental stage no yolk-like substances were recognized in epithelial cells that compose spiral folds of the intestine (Fig. 4B). However, in a larger more developed embryo with a 123 mm disk width, a large numbers of yolk-like substances (arrows) were observed to be accumulated in epithelial cells composing the spiral folds (Fig. 4D and F). When the anterior portion was compared with the posterior portion of the intestine, some luminal spaces were seen between intestinal spiral folds in the anterior portion (Fig. 4C from the second tissue block in Fig.

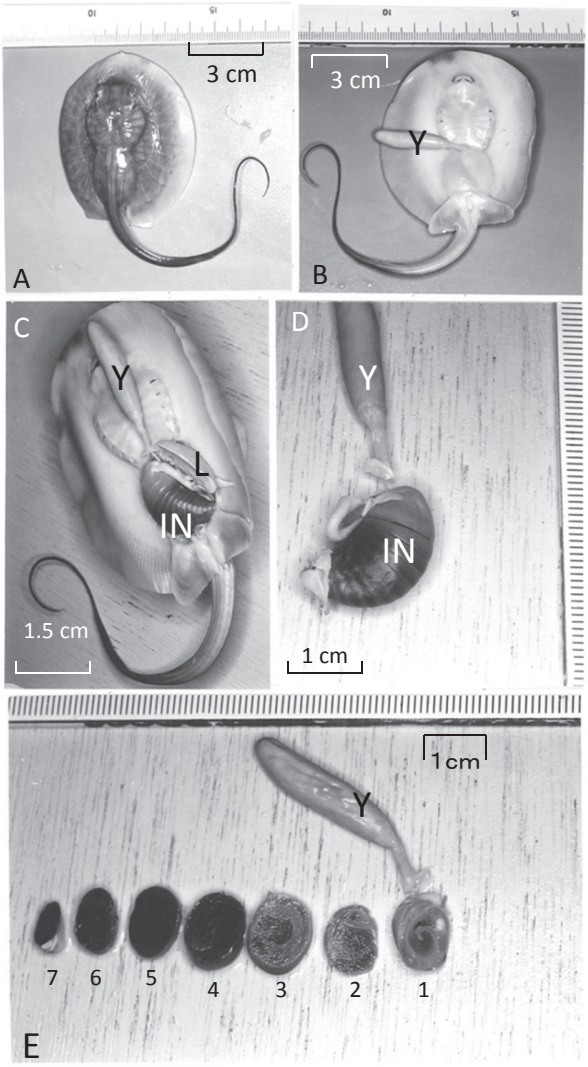

Fig. 3. **A**, Dorsal view of *Potamotrygon magdalenae* embryo of 65 mm disk width; **B**, Ventral view of *P. magdalenae* embryo of 65 mm disk width with the external yolk sac (Y); **C**, Ventolateral view of Fig. 3B. The central area of the abdominal skin was removed to show the intestine (IN), the liver (L), and the external yolk sac (Y); **D**, External yolk sac (Y) and the intestine (IN) removed. *P. magdalenae* embryo of 65 mm disk width; **E**, Seven serial blocks (1 to 7) of the intestine to show the inner conditions in the 65 mm disk width of *P. magdalenae* embryo.

2B) while the intestine was observed to be filled with a numerous cells similar to parenchymatous organ in the posterior portion (Fig. 4E from the fourth tissue block in Fig. 2B).

Histological features, similar to those in the posterior portion of the intestine in a *Disceus thayeri* embryo with 123 mm disk width, were observed in a *Potamotrygon magdalenae* embryo of 65 mm disk width and in a *P. motoro* embryo of 80 mm disk width. They all had numerous cells similar to parenchymatous organ that appeared to be filled abundantly in the intestine (Fig.

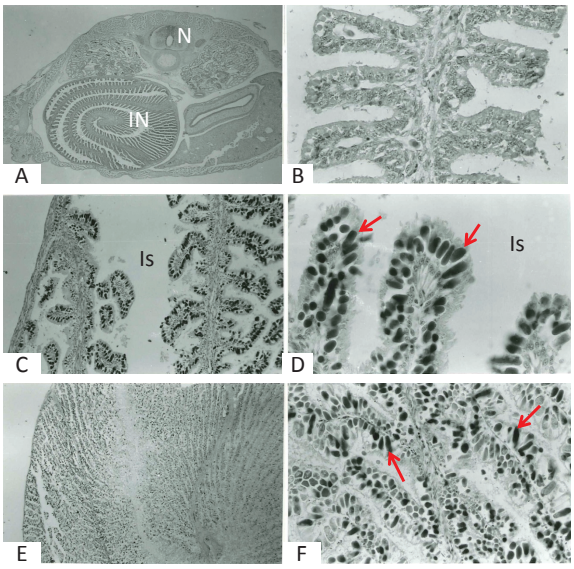

Fig. 4. **A**, Cross section of the whole body of *Disceus thayeri* embryo of 20 mm disk width. IN: intestine, N: neural tube. x10; **B**, Enlarged view of the intestine of Fig. 4A. Note, no yolk-like substances are observed in the epithelial cells. x200; **C**, Histology of the second block tissue in Fig. 2B. Some luminal spaces (ls) were seen between the intestinal spiral folds. x50. *D. thayeri* embryo of 123 mm disk width; **D**, Enlarged view of Fig. 4C. Note, the large numbers of yolk-like substances (arrows) in the epithelial cell. x200; **E**, Histology of the fourth block tissue in Fig. 2B. The intestine was filled with numerous cells similar to parenchymatous organ. x20. *D. thayeri* embryo of 123 mm disk width; **F**, Enlarged view of Fig. 4E. Each of the epithelial cells was filled with yolk granules (arrows). x100.

5A and C) and a large number of yolk-like substances (arrows) that appeared to be stored in each of the intestinal epithelial cells (Fig. 5B and D).

Discussion

It is clear and natural that the smaller embryos have larger yolk sacs, and the larger embryos have smaller ones, indicating that the yolk has been used as nutrients through embryonic development (Table1). The surprising phenomenon uncovered in this investigation was the very presence of an enormous amount of yolk (or yolk derived/originated substances) in the intestinal epithelial cells in the later stage of embryos (Table1). Most of the intestine, especially posterior parts, was quite solid, similar macroscopically to that found in parenchymatous organ such as the liver. It is not clear whether or not the chemical composition of intraepithelial yolk (or yolk derived/originated substances) is exactly identical to yolk in the yolk sac, because the occurrence of intracellular chemical modification cannot be ruled out.

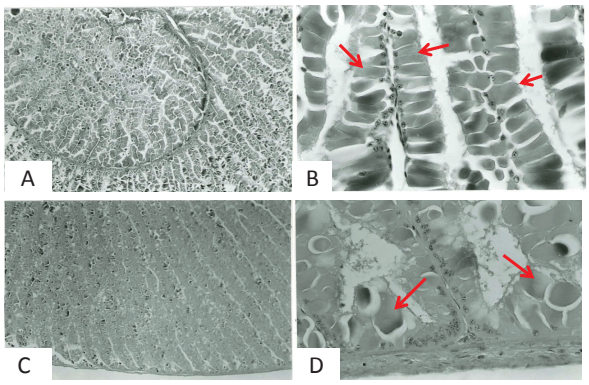

Fig. 5. **A**, Histology of the fourth block tissue in Fig. 3E. The intestine was filled with numerous cells similar to parenchymatous organ. x20. *Potamotrygon magdalenae* embryo of 65 mm disk width; **B**, Enlarged view of Fig. 5A. Note, each of epithelial cells was fully filled with yolk-like substances (arrows). x200; **C**, Histology of the posterior portion of the intestine. The intestine was filled with numerous cells similar to parenchymatous organ. x20. *P. motoro* embryo of 80 mm disk width; **D**, Enlarged view of Fig. 5C. Note, yolk granules (arrows) filled in the epithelial cells. x200.

Therefore, here we describe the accumulated intraepithelial substances as "yolk-like substances".

Among elasmobranchs, embryos of non-placental species have been reported to be nourished mainly by yolk in the yolk sac[3, 4]. Yolk absorption in elasmobranch embryos may be accomplished by endodermal epithelial cells of the yolk sac during the early stage of embryonic development and by intestinal epithelial cells during the late stage of embryonic development[5, 6]. Thus, it is reasonable to believe that the intestine of elasmobranchs plays a certain role in yolk absorption. However, the discovery of accumulated yolk (or yolk derived substances) in the intestine in this study, expands the functional concept of the intestine. It strongly suggests that the intestine of freshwater stingray is not only absorbing the yolk, but also storing the yolk. We speculate that the biological significance of yolk being stored in the intestine might be as a nutritional source after birth (Fig. 6), providing a possible adaptation to threatening environmental conditions in the evolution of this group of animals.

Since some of the freshwater stingrays were listed as one of the IUCN Red List of Threatened Species[7, 8], further investigations of the ecology and reproductive biology of the freshwater stingray may be important for the conservation of these species.

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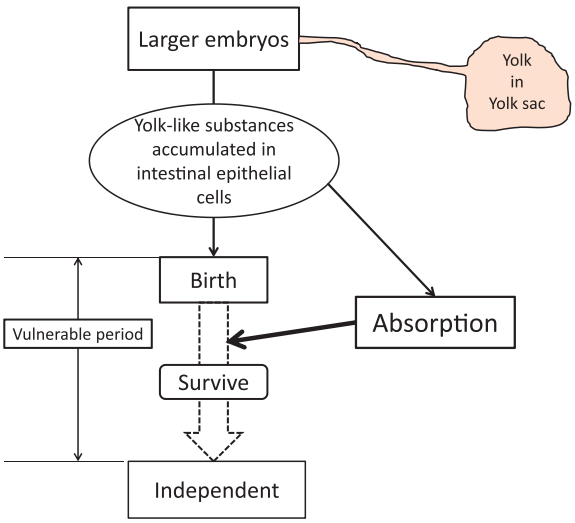

Fig. 6. The yolk-like substances that has been accumulated in the intestinal epithelial cells of the embryos suggests its role may be as a source of nutrients for the embryos after birth enabling them to survive its vulnerable period.

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References

1) Mizue K: The purpose and an Outline of the Research Investigation. *In*: Report on the Oversea's Scientific Research Investigation Entitled "Studies on Adaptability and Evolution of Freshwater Elasmobranchs" by University of Tokyo, 1-5 (1981) (in Japanese)

2) Teshima K, Takeshita K: Reproduction of the Freshwater Stingray, *Potamotrygon magdalenae* Taken from the Magdalena River System in Colombia, South America. *Bull Seikai Natl Fish Res Inst*, **70**, 11-27 (1992)

3) Teshima K: Studies on the Reproduction of Japanese Smooth Dogfishes, *Mustelus manazo* and M. *griseus*. *J Shimonoseki Univ Fish*, **29**, 113-199 (1981)

4) Wourms J P: Reproduction and Development in Chondrichthyan Fishes. *Amer Zool*, **17**, 379-410 (1977)

5) TeWinkel L E: Observations on Later Phases of Embryonic Nutrition in *Squalus acanthias*. *J Morph*, **73**, 117-205 (1943)

6) Jollie W P, Jollie L G: Electron Microscopic Observations on the Yolk Sac of the Spiny Dogfish, S*qualus acanthias*. *J Ultrastruct Res*, **18**, 102-126 (1967)

7) Charvet-Almeida P, Pinto de Almeida M: *Potamotrygon magdalenae*. *The IUCN Red List of Threatened Species*, **Version 2015. 1**, <www. iucnredlist. org>, Downloaded on 13 June 2015 (2009)

8) Drioli M, Chiaramonte G: *Potamotrygon motoro*. *The IUCN Red List of Threatened Species*. **Version 2015. 1**, <www. iucnredlist. org>. Downloaded on 13 June 2015 (2005)

淡水産エイ類の胚は腸管上皮中に卵黄を貯める

手島和之・友永　進

南米で採集された淡水産エイ類胚の腸管上皮細胞を組織学的に観察した。その結果、卵黄嚢から卵黄腸管を通して腸に運ばれる卵黄物質は、成長の途中から胚の腸上皮細胞中に蓄積されることが明らかとなった。卵黄様物質は、出産後も腸上皮細胞中に留まり、個体が vulnerable period（母体から産み出された直後からの受難期）を生き抜くための栄養として寄与していると考えられた。