

Fine Structures of the Micropyles of Pelagic Eggs of Some Marine Fishes

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Abstract The micropyles and their surrounding structures of pelagic fish eggs of nine species were examined and compared using a scanning electron microscope (SEM). The surface of the egg membrane possesses uniformly distributed pores. However, *Lateolabrax japonicus* possesses a number of small knobs. The region of the micropyle of fish eggs examined is characterized by a circular elevation in which the pores are larger than those on the other parts of the egg membrane. In contrast, *Kareius bicoloratus* egg has no elevation and possesses uniform pores to the edge of the micropylar canal. The funnel-like structure is seen in the opening of the micropyle in *Sardinops melanostictus*, *Saurida elongata*, *Sillago japonica*, *Parapristipoma trilineatum*, *Hypodytes rubripinnis* and *K. bicoloratus*. These specific features of the micropyle may be used for the identification of pelagic eggs.

The identification of pelagic eggs of fishes, especially of those preserved in formalin, is often difficult under an optical microscope when their egg membranes exhibit no special structures.

The recent studies with an electron microscope (Riehl and Schulte, 1977, 1978; Riehl, 1980) revealed that the fine structure of the egg membrane and micropyle could be a criterion for the identification of fresh water teleostean eggs. Similarly, electron microscopy was introduced to investigate the possibility that the micropylar structure of the pelagic eggs of marine fishes can be used for identification of the parental species. The present paper compares the pelagic eggs of nine marine fishes with regard to their fine structure of the egg membranes and micropyles by SEM.

Materials and methods

As shown in Table 1, the eggs of nine marine fishes were examined. These eggs were fixed and preserved in 10% formalin in sea water.

In preparation for SEM, the egg membrane was separated from the embryonic body and rinsed with distilled water to remove formalin. After locating the position of the micropyle under an optical microscope, the egg membrane was cut into a small piece containing the micropyle with a sharp needle. As the small transparent pieces were unmanageable on the opaque mount, they were placed on a glass disk with attention to face the exterior. The sample was dried naturally on

a disk. The glass disk with a sample was set on an aluminum mount with silver paste and was coated with gold-palladium. The samples were then examined under an SEM (Hitachi-Akasi MSM-2).

Observations

The eggs of nine species were spherical in shape and their diameters ranged from 0.7 mm of

Table 1. List of marine fishes of which fine structure of the micropyles was observed, and the origins of eggs. c, eggs spawned by parent fish in captivity; w, wild eggs collected with a plankton sampler. ¹⁾ Collected off the northern coast of Kyushu near Genkai-cho, Saga Prefecture; ²⁾ collected in the Seto Inland Sea near Takehara, Hiroshima Prefecture; ³⁾ collected in eastern Wakasa Bay, the Japan Sea.

Family	Species	Origin of eggs
Clupeidae	<i>Sardinops melanostictus</i>	w ¹⁾
Synodontidae	<i>Saurida elongata</i>	w ²⁾
Percichthyidae	<i>Lateolabrax japonicus</i>	c
Sillaginidae	<i>Sillago japonica</i>	c
Pomadasyidae	<i>Parapristipoma trilineatum</i>	c
Oplegnathidae	<i>Oplegnathus fasciatus</i>	c
Synanceiidae	<i>Inimicus japonicus</i>	c
Congiopodidae	<i>Hypodytes rubripinnis</i>	w ³⁾
Pleuronectidae	<i>Kareius bicoloratus</i>	w ¹⁾

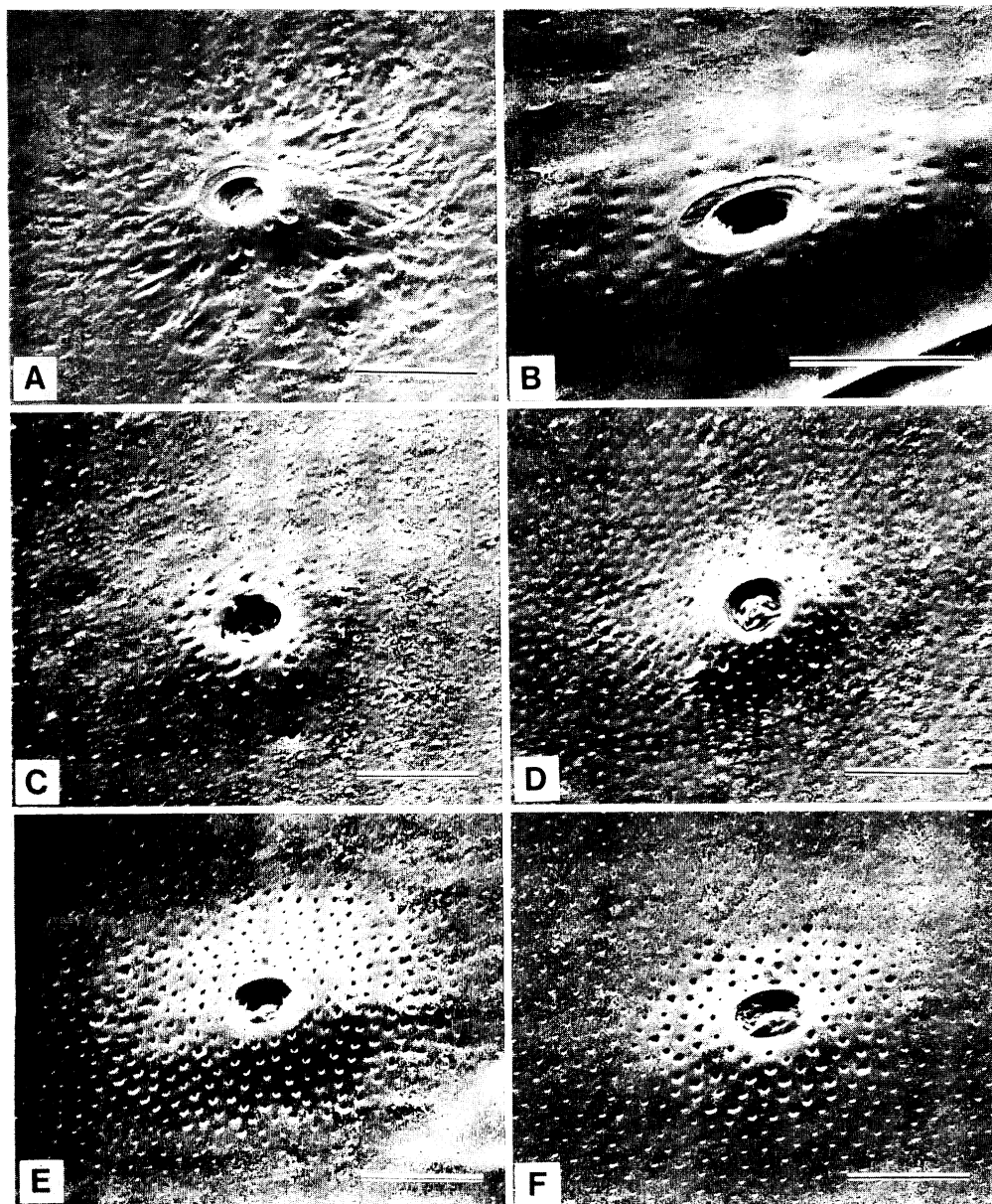


Fig. 1. Micropyle and its surrounding structure. Each scale indicates 10 μm . A, *Sardinops melanostictus*; B, *Saurida elongata*; C, *Lateolabrax japonicus*; D, *Sillago japonica*; E, *Parapristipoma trilineatum*; F, *Oplegnathus fasciatus*.

Sillago japonica to 1.3 mm of *Sardinops melanostictus*. The egg membranes were almost transparent and no specialized apparatus was detected under the optical microscope. However, observations by a SEM showed various appearances on the surface of egg membranes and micropyles.

Sardinops melanostictus (Fig. 1A): The egg

membrane had a number of pores uniformly distributed on the surface. In the micropylar region, the surface of the egg membrane showed a circular elevation measuring about 20.0 μm in diameter in which distinct cavities of various sizes were observed. The micropylar canal opened outward like a funnel of about 6.9 μm in diameter and its

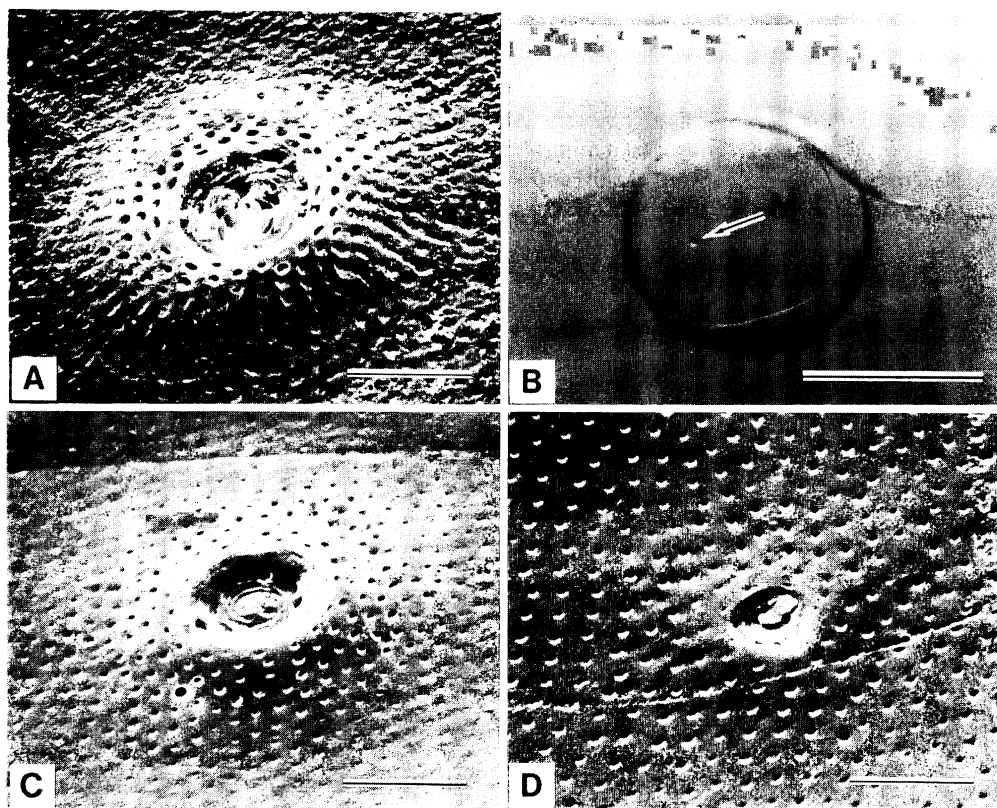


Fig. 2. Micropyle and its surrounding structure. Each scale indicates $10\text{ }\mu\text{m}$, except one in B which indicates 1 mm . A, *Inimicus japonicus*; B, *Inimicus japonicus* egg separated from an embryonic body (M, micropyle); C, *Hypodytes rubripinnis*; D, *Kareius bicoloratus*.

slope was gentle. The outer opening of the micropylar canal was observed in the center of the funnel measuring about $4.2\text{ }\mu\text{m}$ in diameter.

Saurida elongata (Fig. 1B): The outer opening of the micropyle showed a funnel-like structure with a gentle slope measuring about $8.3\text{ }\mu\text{m}$ in diameter. The funnel was surrounded with about 70 shallow cavities, although they were indistinct throughout the egg membrane. No circular elevation was observed in the micropylar region. The diameter of the micropylar canal in the center of the region was $3.7\text{ }\mu\text{m}$ approximately in which the annular lamellae were observed on the inside surface of the canal.

Lateolabrax japonicus (Fig. 1C): The outer surface of the egg membrane possessed a number of small knobs, which showed a uniform distribution throughout the egg membrane with the exception of the micropylar region. The micropylar region had a flat surface in which about 60

pores were counted. The diameter of the region was about $18\text{ }\mu\text{m}$. The outer opening of the micropylar canal in the center of the pores was about $4.8\text{ }\mu\text{m}$ in diameter. The micropyle had no funnel structure and the canal was leading directly into the interior. A convoluted structure was seen inside of the canal where several pores were observed on the side of its entrance.

Sillago japonica (Fig. 1D), *Parapristipoma trilineatum* (Fig. 1E) and *Oplegnathus fasciatus* (Fig. 1F): These three species had similar structures of micropyles having uniformly distributed pores on the egg membrane. The micropylar region showed a circular elevation in which the size of pores was larger than those found throughout the egg membrane. *P. trilineatum* had the largest elevation of about $29.6\text{ }\mu\text{m}$ in diameter among the three species, whereas those of *S. japonica* and *O. fasciatus* were about $15.8\text{ }\mu\text{m}$ and $21.2\text{ }\mu\text{m}$, respectively. Also *P. trilineatum* showed the great-

est number of large pores of about 280 around the micropylar canal while those of the other two species were about 110.

S. japonica and *P. trilineatum* had the funnel-like opening of about 6.0 μm and 5.3 μm in diameter, respectively. The micropylar canal of *O. fasciatus* was leading directly into the interior.

Inimicus japonicus (Fig. 2A, B) and *Hypodytes rubripinnis* (Fig. 2C): The micropylar structures of *I. japonicus* and *H. rubripinnis* were basically similar to those of the former three species. Their eggs possessed a large number of pores uniformly distributed over the egg membrane and had a circular elevation with larger pores in the micropylar region. However, the micropyles of these two species were much larger, that is, a circular elevation of *I. japonicus* and *H. rubripinnis* was about 27.0 μm and 28.0 μm in diameter, respectively. In addition, the diameters of their micropylar canal were about 12.0 μm and 5.7 μm , respectively. Therefore, these micropyles were easy to find out under an optical microscope (Fig. 2B). The micropylar opening of *H. rubripinnis* was stretched outward like a funnel of about 11.9 μm in diameter and several pores were observed on the side of the funnel. *I. japonicus* had no funnel-like structure. *H. rubripinnis* showed a distinct convoluted structure.

Kareius bicoloratus (Fig. 2D): *K. bicoloratus* had a different structure of the micropyle from those of the other species. The pores distributed throughout the egg membrane were uniform to the edge of the micropylar opening. The pore density of *K. bicoloratus* was much lower than those of the other species and its diameter was much larger. The neighborhood of the micropyle was nearly flat. The outer opening of the micropyle had a funnel-like structure measuring about 6.7 μm in diameter whose canal locating in the center of the funnel was about 2.6 μm in diameter.

Discussion

In this study, SEM observation was conducted by placing an object onto a sample plate without dehydration and critical point drying. The sizes of canal and elevation measured by this method accord with those by a light microscope (interference and phase-contrast microscope) which were measured before the treatment. This method makes good samples certainly and rapidly

even if the sample is small.

The examined egg membranes were almost transparent and no specific characters were recognized under an optical microscope. However, observation by SEM revealed the pores or knobs distributed on the egg membranes and the various types of the micropyles (Table 2).

The examined eggs possessed the pores on their membranes except for *Lateolabrax japonicus* which had a number of small knobs. Similar structures of pores and knobs are observed in *Pagrus major* (Hosokawa et al., 1981) and *Engraulis japonica* (Hirai and Yamamoto, 1986), respectively. Groot and Alderdice (1985) reported that the externus of the salmonid egg membrane was usually pierced by hexagonally arranged pores that were often closed by plugs. These plugs of some species are shaped like knobs. Observation by SEM, however, could not reveal the detailed structure of the knobs of *L. japonicus*.

The diameter of the micropylar canal varied among the species, ranging from about 2.6 μm in *Kareius bicoloratus* to about 12.0 μm in *Inimicus japonicus*. The micropylar canals of *L. japonicus* and *Hypodytes rubripinnis* had a distinct convoluted structure. This structure may be formed through swelling of the interna of the egg membrane (Hirai and Yamamoto, 1986). The annular lamellates were observed on the side of the canal of *Saurida elongata*.

The micropylar regions of the eggs examined were divided into two types: one type showed a circular elevation around the micropyle as seen in *Sardinops melanostictus*, *Sillago japonica*, *Parapristipoma trilineatum*, *Oplegnathus fasciatus*, *Inimicus japonicus* and *Hypodytes rubripinnis*, and the other type showed a flat structure as seen in *Saurida elongata*, *Lateolabrax japonicus* and *Kareius bicoloratus*. Except for *K. bicoloratus*, the pores in the micropylar region were larger than those on the egg membrane. *L. japonicus* and *H. rubripinnis* had some pores inside of the micropylar canal. It is reported that *Engraulis japonica* egg has a circular elevation in the micropylar region (Hirai and Yamamoto, 1986) and *Pagrus major* has larger pores in the neighborhood of the micropylar canal (Hosokawa et al., 1981). The micropylar canals of *S. melanostictus*, *S. elongata*, *S. japonica*, *P. trilineatum*, *H. rubripinnis* and *K. bicoloratus* opened outward like funnels as observed in some salmonoid fish eggs by Riehl (1980).

Table 2. Measurements of the studied eggs. mp, micropylar.

	<i>Sardinops melanostictus</i>	<i>Saurida elongata</i>	<i>Lateolabrax japonicus</i>	<i>Sillago japonica</i>	<i>Parapristipoma trilineatum</i>
Egg surface with	pores	—	knobs	pores	pores
Diameter of mp canal	4.2 μm	3.7 μm	4.8 μm	3.5 μm	3.2 μm
Funnel structure in mp region (diameter)	yes (6.9 μm)	yes (8.3 μm)	no	yes (6.0 μm)	yes (5.3 μm)
Circular elevation in mp region (diameter)	yes (20.0 μm)	no	no	yes (15.8 μm)	yes (29.6 μm)
Number of pores in mp region	62	72	58	104	282
Pores of inside of mp canal	no	no	yes	no	no
	<i>Oplegnathus fasciatus</i>	<i>Inimicus japonicus</i>	<i>Hypodytes rubripinnis</i>	<i>Kareius bicoloratus</i>	
Egg surface with	pores	pores	pores	pores	
Diameter of mp canal	6.2 μm	12.0 μm	5.7 μm	2.6 μm	
Funnel structure in mp region (diameter)	no	no	yes (11.9 μm)	yes (6.7 μm)	
Circular elevation in mp region (diameter)	yes (21.2 μm)	yes (27.0 μm)	yes (28.2 μm)	no	
Number of pores in mp region	108	113	253	—	
Pores of inside of mp canal	no	no	yes	no	

Regarding the eggs of *S. melanostictus* and *S. elongata*, the slope from the edge of the funnel to the micropylar canal was gentle compared to the others.

In some pelagic fish eggs, unique micropyles are observed. For example, the micropyle of the ostraciid fish is surrounded by hollow bumps (Leis and Moyer, 1985). The regions around the micropyles of *Stolephourus* and of some paralicthiid fishes rise extremely like warts (Delsman, 1931; Mito, 1963; Hirai, 1987). As described above, the region of the micropyle may present a remarkable structure such as an elevation and large pores which may be a species-specific character.

Lönning (1972) proposed that the structure of the chorion is determined more by the biology of the reproduction of the species than by their taxonomic affinities. Similarly, in the micropyle, the funnel-like structure and elevation showed distinct similarities even among the distantly related species. In contrast, it is interesting that *I. japonicus* and *H. rubripinnis* with close relationship have larger micropyles than those of the others. Further, Lönning (1972) described that geographic differences in the thickness and ultrastructure of the chorion were found among eggs of the same species. The variation of the micropyle may also exist in different geographic localities.

Riehl and Götting (1974) classified the micropyles of fish eggs into three types. Judging from the images under SEM, the micropyles in the present study belong to the type III of Riehl and Götting which has no clear micropylar depression (vestibule) but the micropylar canal may be wider at the upper end like a funnel. The micropyles of pelagic fish eggs may be of the type III.

The usefulness of the micropyle as a character for the identification of the freshwater fish eggs, which are almost demersal, is suggested by Riehl and Schulte (1977, 1978). This study suggests that the micropylar structure of pelagic eggs of marine fishes presents their own characteristics as well. For example, in the early stage of *S. elongata* and *I. japonicus* eggs are difficult to distinguish under an optical microscope due to the similar morphology. However, their micropyles showed remarkably different conformations (Figs. 1B, 2A). It is quite possible that eggs with similar morphologies can be identified by comparing their micropyles and surface patterns.

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海産浮遊性魚卵数種における卵門の微細構造

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海産浮遊性魚卵 9 種の卵門及びその周辺構造を、走査型電子顕微鏡により観察した。浮遊性魚卵の卵膜全体に

は、多数の小孔あるいは小瘤が均一に分布する。マイワシ、キス、イサキ、イシダイ、オニオコゼ、ハオコゼの卵では、卵門周辺の表面が隆起し、この隆起した部分に点在する小孔は、卵膜全体に分布する小孔より、やや大きく、明瞭である。卵膜上の小孔が不明瞭なトカゲエソ卵は、卵門周辺はほとんど隆起せず、卵門のまわりにはいくつかの窪みが見られる。小瘤が卵膜全体に分布するスズキ卵も、卵門周辺は平坦で、卵門トンネル部の回りは小孔でかこまれる。イシガレイでは、卵門周辺は平坦で、卵膜上の小孔はトンネル部まで均一に分布する。マイワシ、トカゲエソ、キス、イサキ、ハオコゼ、イシガレイでは、卵門は表面で漏斗状に開口する。卵門及びその周辺部の形態から、海産浮遊性魚卵の魚種の同定が可能であると考えられる。

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