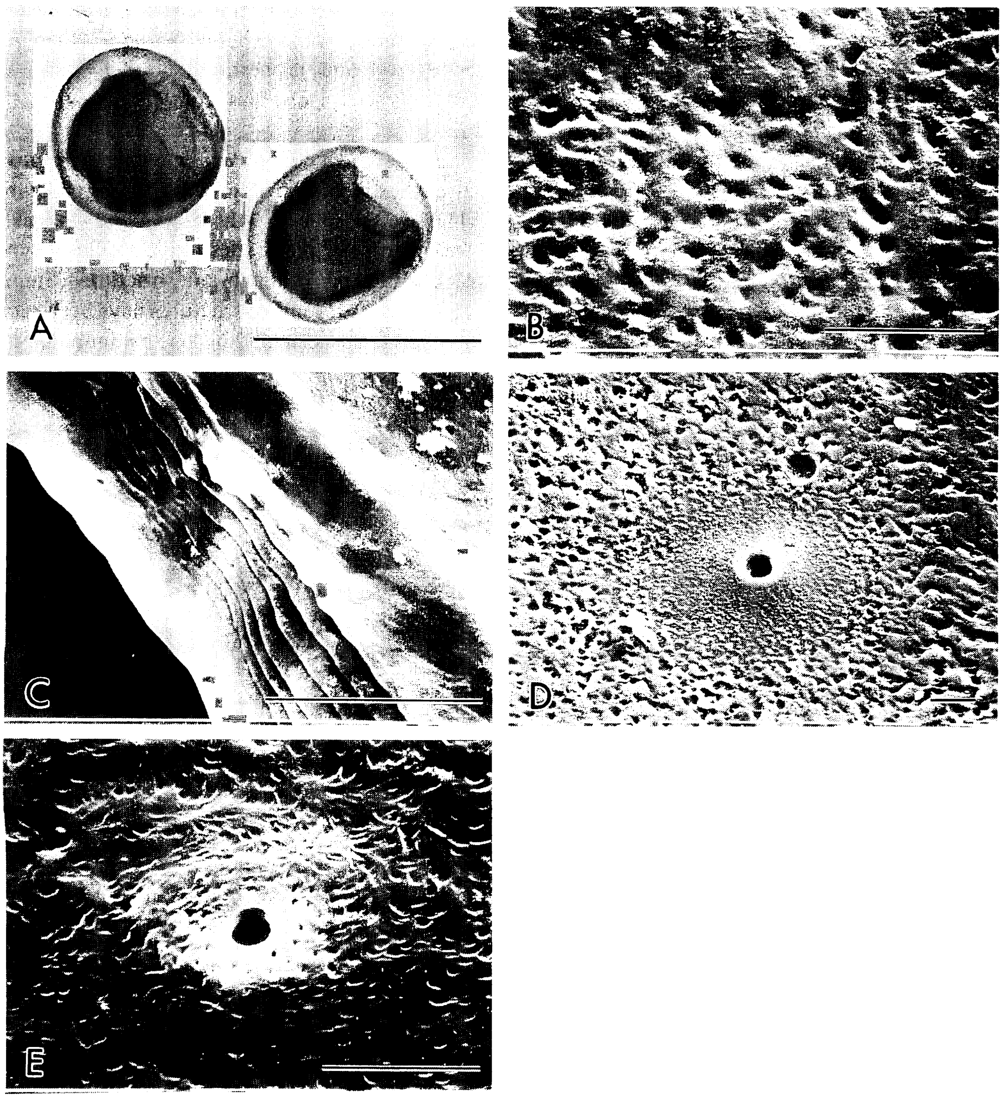

Fig. 4. *Pleuronectes yokohamae.* Fine structure of egg membrane. Bar scales indicate 1 mm (A) and 10 μm (B–E). A) Whole egg (light microscope); B) Outer surface of egg membrane (SEM); C) Cross section of egg membrane; D) Micropyle, exterior surface; E) Micropyle, interior surface.

brane could be clearly observed (Fig. 3B). Sculpturing of the egg membrane consisted of regular, hexagonally-arranged walls, about 3 μm in height and about 10 μm in length. Under higher magnification, these structures showed a further pattern of polygonal areas within each hexagonal unit. Each polygon was about 1.5 μm in diameter and possessed a pore canal opening in its center (Fig. 3C). Pore density was 27.7–35.3 per 100 μm^2. The egg membrane, about 3.2 μm thick, consisted of six, equally-thick lamellae (Fig. 3D). The opening of the micropylar canal was about 8.4 μm in diameter, the canal tapering toward the inner membrane surface. The region around the outer opening lack further structures (Fig. 3E). The internal surface also lacked any specialized apparatus except for the elevated region of the terminal aperture of the micropyle. This elevation was surrounded by small cavities and undulating structures (Fig. 3F).

Pleuronectes yokohamae

The eggs were spherical in shape, ranging between 0.8–0.9 mm in diameter, although somewhat modified by fixation. No specialized membrane structures were detected under light microscope (Fig. 4A).

Under SEM, that the egg membrane possessed a number of irregularly distributed pores or cavities (0.60–1.29 μm in diameter) at a density of 12.5 per 100 μm^2 (Fig. 4B). The egg membrane was 12.4 μm thick, consisting of about ten lamellae, of which the inner- and outermost were thickest. The outer lamella, about five times as thick as the middle lamellae, appeared to constitute an adhesive layer (Fig. 4C). The entrance of the micropyle was about 6 μm in diameter, the canal tapering toward the inner membrane surface. The region around the outer opening of the micropyle lacked an adhesive layer and pores (Fig. 4D). Cavities of various sizes were distributed irregularly on the internal surface. The terminus of the micropyle, measuring about 2.6 μm in diameter, was surrounded by a protuberance with about 30 pores (Fig. 4E).

Discussion

A rich diversity in both spawning ecology and egg morphology exists in pleuronectine fishes (Pertseva-Ostroumova, 1961; Minami, 1984). The eggs of each of the four pleuronectine species examined here, having their own morphologies under light microscope level and properties (Table 1), also, under SEM, showed a distinctive fine architecture (Table 2).

Among them, only *Pleuronichthys cornutus* possessed hexagonal structures on the egg membrane, which were visible by light microscope. At least four other pleuronectine species were have been reported to have hexagonal membrane structures (Sumida et al., 1979), with similar structures have been reported in other teleosts, including one or more species of Synodontidae, Callionymidae and Uranoscopidae (Mito, 1960; Ikeda and Mito, 1988). As for the ecological significance of such hexagonal structures, various functions including protection, resiliency and buoyancy have been proposed (Robertson, 1981; Stehr and Hawkes, 1983). However, there is little direct evidence relating fish behavior to a hexagonally-patterned egg membrane, although it is notable that all of the fishes so far known to have such a structure, have a creeping mode of behavior, irrespective of their phylogeny. This is suggestive of a certain functional significance.

The egg membrane of *Hippoglossoides dubius* was the thinnest of the four species examined here. In addition, *H. dubius* had a wide perivitelline space. Having the thinnest egg membrane seems to be related to the formation of a wide perivitelline space, the function of which was reviewed by Laale (1980).

Table 1. Comparison of morphological characters (light microscope level) and properties of pleuronectine eggs

Species	Egg diameter (mm)	Oil globule (mm)	Character of egg membrane*	Property of egg	Perivitelline space
Hippoglossoides dubius	1.5–1.7	Many** (0.01–0.001)	Smooth	Pelagic	Wide
Eopsetta grigorjewi	1.0–1.1	None	Smooth	Pelagic	Narrow
Pleuronichthys cornutus	1.1–1.2	Single (0.17–0.18)	Sculptured	Pelagic	Narrow
Pleuronectes yokohamae	0.8–0.9	None	Smooth	Demersal	Narrow

*Observations by light microscope; **Kuragami (1917).

Table 2. Comparison of fine structures of the egg membranes

Species	Egg surface with	Diameter of micropylar canal (μm)	Thickness of egg membrane (μm)	Number of lamelae
Hippoglossoides dubius	pores	4–6	2.7	5–6
Eopsetta grigorjewi	pores	8	3.3	7
Pleuronichthys cornutus	polygons and pores	8	3.2	6
Pleuronectes yokohamae	pores	6	12.4	10

Any increase in the egg size with a wide perivitelline space influences the ascent rate (Robertson, 1981). It is notable that the eggs of *H. dubius*, having a wide perivitelline space, and *P. cornutus*, having a hexagonal structure, possess one or many oil globules which confer buoyancy, whereas the eggs of many pleuronectine species lack oil globule.

On the other hand, the demersal eggs of *Pleuronectes yokohamae* have much more complex lamellae and a thicker membrane than pelagic pleuronectine eggs. Comparing the eggs of *Platichthys stellatus* and *Oncorhynchus gorbuscha*, Stehr and Hawkes (1979) mentioned that pelagic eggs tended to have a thin membrane, while demersal eggs tended to develop a much thicker membrane in relation to the egg diameter. They proposed that this difference might be related to a protective function. Pearcy (1962) considered that demersal eggs belonging to the order Pleuronectiformes might be less subject to dispersion by offshore currents or freezing than more buoyant eggs. Minami (1984) reported that *P. yokohamae* spawned demersal eggs on inshore, rocky sites, exposed to strong currents, whereas *Kareius bicoloratus*, with almost the same spawning season as the former, spawned pelagic eggs at offshore muddy sites. The thick membrane in association with the complex lamellar structure of *P. yokohamae* may have conferred advantage from the greater mechanical stresses.

Teleostean eggs are known to possess a number of pores or knobs uniformly distributed on the surface of the egg membrane (Hagström and Lönning, 1968; Lönning, 1972; Lönning and Hagström, 1975; Hosokawa et al., 1981; Stehr and Hawkes, 1979; Ohta et al., 1983; Groot and Alderdice, 1985; Hirai and Yamamoto, 1986; Hirai, 1988; Riehl and Kock, 1989). In the ovary, the pore canals distributed throughout the egg membrane contribute to the transportation of nutrients from the follicle cell to the developing egg body (Hurley and Fisher, 1966; Stehr and Hawkes, 1983) and it has been suggested that the pores of fertilized eggs are the remnants of previous canals (Hagström and Lönning, 1968; Lönning, 1972; Groot and Alderdice, 1985). As the cross-sectional observations of the egg membrane of *Eopsetta grigorjewi* revealed, the pore canals may be blocked or disappear completely by the process of oogenesis, in order to protect the embryo against exposure to the natural environment. The pore diameters of pleuronectine eggs are much larger and their densities lower than in other fishes (Table 3). Figure 5 clearly shows the difference in egg membrane be-

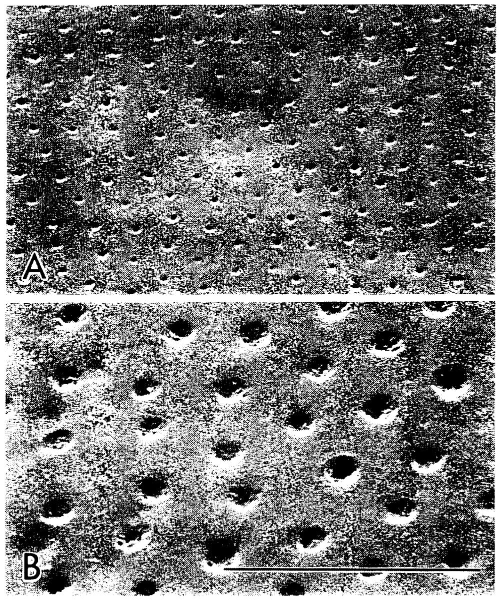

Fig. 5. Comparison of egg membrane pores of *Pagrus major* (A) and *Eopsetta grigorjewi* (B) at the same magnification (Bar: 10μm).

Table 3. Comparison of egg membranes pores between pleuronectine fishes and other teleosts

Family	Species	Pore diameter (μm)	Pore density (Pore/100μm^2)	Source
Pleuronectinae	*Hippoglossoides dubius*	0.29–0.40	8.4–8.6	This study
	Eopsetta grigorjewi	0.90–1.13	9.6–13.2	This study
	Pleuronichthys cornutus	0.28–0.33	27.7–35.3	This study
	Pleuronectes yokohamae	0.60–1.29	12.5	This study
Clupeidae	*Sardinops melanosticutus*	—	56–60	Kuroda et al., 1983
Salmonidae	*Oncorhynchus keta*	—	40.8±4.0	Groot and Alderdice, 1985
Sparidae	*Pagrus major*	0.16–0.24	47.7–53.8	Hirai, unpublished
Scombridae	*Scomber japonicus*	0.14–0.24	40–41	Kuroda et al., 1982

tween *E. grigorjewi* and *Pagrus major*, (Sparidae). Thus a large pore size and lower density may be a characteristic of pleuronectine fish.

Based on an examination of the membranes of pelagic eggs from nine marine species, Hirai (1988) reported that the micropylar region was characterized by a circular elevation in which the pores were larger than those elsewhere on the egg membrane. However, the eggs of *Kareius bicoloratus* (Pleuronectinae) had uniform pores around the micropylar canal, without any elevation. Similarly, the four pleuronectine species examined here lacked any characteristic structure around the micropylar canal. Such a lack may be a common feature of the Pleuronectinae.

Lönning and Hagström (1975) considered that the differences in teleost egg membrane structure were species specific. Ivankov and Kurdyayeva (1973) hypothesized that the morphological character of the primary membrane (zona radiata) indicated adaptation to spawning and egg development. The present observations indicate that the thickness, lamellar structure and surface sculpturing of the egg membrane are closely related to environmental factors, for example, giving protection from mechanical stresses or determining of ascent rate, whereas the pore density and diameter reflect systematic relationships.

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Literature Cited

Groot, E. P. and D. F. Alderdice. 1985. Fine structure of the external egg membranes of five species of Pacific salmon and steelhead trout. Can. J. Zool., 63: 552–566.

Hagström, B. E. and S. Lönning. 1968. Electron microscopic studies of unfertilized and fertilized eggs from marine teleosts. Sarsia, 33: 73–80.

Hirai, A. 1988. Fine structures of the micropyles of pelagic eggs of some marine fishes. Japan. J. Ichthyol., 35: 351–357.

Hirai, A. and T. S. Yamamoto. 1986. Micropyle in the developing eggs of the anchovy, *Engraulis japonica*. Japan. J. Ichthyol., 33: 62–66.

Hosokawa, K., T. Fushimi and T. Matsusato. 1981. Electron microscopic observation of the chorion and micropyle apparatus of the porgy, *Pagrus major*. Japan. J. Ichthyol., 27: 339–343.

Hurley, D. A. and K. C. Fisher. 1966. The structure and development of the external membrane in young eggs of the brook trout, *Salvelinus fontinaris* (Mitchill). Can. J. Zool., 44: 173–190.

Ikeda, T. and S. Mito. 1988. Keys of fish eggs and hatched larvae. Pages 999–1083 *in* M. Okiyama ed. An atlas of the early stage fishes in Japan. (In Japanese.)

Ivankov, V. N. and V. P. Kurdyayeva. 1973. Systematic differences and the ecological importance of the membranes in fish eggs. J. Ichthyol., 13: 864–873.

Kuragami, M. 1917. Eggs and larvae of two flatfishes (Pleuronectidae) in Hokkaido. Hokusuishi Chosa Houkoku, (6): 307–313. (In Japanese.)

Kuroda, K., T. Yamamoto and Y. Hirano. 1982. Development and identification of the egg of the Pacific mackerel (*Scomber japonicus* Houttuyn) and a similar egg. Bull. Tokai Reg. Fish. Res. Lab., (107): 33–52. (In Japanese with English abstract.)

Kuroda, K., T. Yamamoto and Y. Hirano. 1983. Identification of the eggs of the Japanese sardine, *Sardinops melanosticta* (T. & S.), the gizzard shad, *Konosirus punctatus* (T. & S.) and the Japanese shad, *Harengula zunasi* B. Bull. Tokai Reg. Fish. Res. Lab., (110): 81–91. (In Japanese with English abstract.)

Laale, H. W. 1980. The perivitelline space and egg envelopes of bony fishes: A review. Copeia, 1980: 210–226.

Lönning, S. 1972. Comparative electron microscope studies of teleostean eggs with special reference to the chorion. Sarsia, 49: 41–48.

Lönning, S. 1981. Comparative electron microscope studies of chorion of the fish egg. Rapp. P.-v. Reun. Cons. int. Explor. Mer, 178: 560–564.

Lönning, S. and B. E. Hagström. 1975. Scanning electron microscope studies of the surface of the fish egg. Astarte, 8: 17–22.

Minami, T. 1984. Early life history of flatfishes—III. Characteristics of eggs. Aquabiol., 6: 46–49. (In Japanese with English abstract.)

Mito, S. 1963. Pelagic fish eggs from Japanese water—IX Echeneida and Pleuronectida. Japan. J. Ichthyol., 11: 81–102. (In Japanese with English abstract.)

Ohta, H., K. Takano, T. Izawa and K. Yamauchi. 1983. Ultrastructure of the chorion and the micropyle of the Japanese eel, *Anguilla japonica.* Bull. Japan. Soc. Sci. Fish., 49: 501.

Pearcy, W. G. 1962. Distribution and origin of demersal eggs within the order Pleuronectiformes. J. Cons. Perm. Int. Explor. Mer, 27: 232–235.

Pertseva-Ostroumova, T. A. 1961. The reproduction and development of far eastern flounders. Izdatel'stvo Akademie Nauk, SSSR., Moskva. 484pp. (In Russian; Japanese transl. avail., Japanese-Soviet Fish. Scien. Techni. Coop. Transl., Fisheries Agency. 1–690)

Riehl, R. and K. H. Kock. 1989. The surface structure of antarctic fish eggs and its use in identifying fish eggs from the Southern Ocean. Polar Biol., 9: 197–203.

Robertson, D. A. 1981. Possible functions of surface structure and size in some planktonic eggs of marine fishes. New Zealand J. Mar. Freshw. Res., 15: 147–153.

Stehr, C. M. and J. W. Hawkes. 1979. The comparative ultrastructure of the membrane and associated pore structures in the starry flounder, *Platichthys stellatus* (Pallas), and pink salmon, *Oncorhynchus gorbuscha* (Walbaum). Cell Tissue Res., 202: 347–356.

Stehr, C. M. and J. W. Hawkes. 1983. The development of the hexagonally structured egg envelope of the C-D sole (*Pleuronichthys coenosus*). J. Morphol., 178: 267–284.

Sumida, B. Y., E. H. Ahlstrom and H. G. Moser. 1978. Early development of seven flatfishes of the eastern North Pacific with heavily pigmented larvae (Pisces, Pleuronectiformes). Fish. Bull., 77: 105–145.

カレイ亜科魚類4種の魚卵の卵膜微細構造

平井明夫

カレイ亜科魚類4種の魚卵の卵膜微細構造を走査型電子顕微鏡を用いて観察した．アカガレイ（*Hippoglossoides dubius*）は囲卵腔の広い浮性卵を産み，最も薄い卵膜を持ち，層構造も単純であった．一方，マコガレイ（*Pleuronectes yokohamae*）は囲卵腔の狭い沈性卵を産み，その卵膜は他のカレイ亜科魚類のものと比べて厚く，より複雑な層構造を呈していた．ムシガレイ（*Eopsetta grigorjewi*）とメイタガレイ（*Pleuronichthys cornutus*）の卵は囲卵腔が狭く浮性であり，卵膜が前2者の中間の厚さと層構造を示した．メイタガレイでは卵膜表面に，直立した平板で形成された亀甲構造が観察された．今回調べたカレイ亜科魚類の卵は，これまで報告された他の魚種に比べ，卵膜全体に分布する微孔の分布密度が小さく，その直径も大きいという共通の特徴を持っていた．卵膜の厚さ，その層構造および膜表面上の構造物は，外圧からの保護，浮上速度の調整といった機能により卵の生育環境と密接な関連を持ち，一方，微孔の密度や大きさは，これら魚種の属する分類群の特徴を示していると考えられる．

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