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Fine Structure of the Egg Membranes in Four Species of Pleuronectinae

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Abstract The eggs of four species of closely related pleuronectine fishes were examined by scanning electron microscope to elucidate the significance of their different membrane structures. *Hippoglossoides dubius* lays pelagic egg with a wide perivitelline space. The egg membrane of this species was thinnest among the four species examined, consisting of a simple lamellar structure. In contrast, *Pleuronectes yokohamae* lays demersal eggs with a narrow perivitelline space. The egg membrane of this species was thicker and composed of a much more complex lamellar structure than those of the other species. *Eopsetta grigorjewi* and *Pleuronichthys cornutus* eggs, with the membranes of medium thicknesses and lamellar structures between the former two species, are pelagic with a narrow perivitelline spaces, *P. cornutus* showed ornamentation consisted regular hexagonally-arranged walls on the egg membrane surface. With respect to pores on the egg membrane, all the pleuronectine eggs examined in this study had larger pore diameters and lower pore densities than in other teleost species reported thus far. It is likely that the membrane thickness, lamellar structure and surface sculpturing of the eggs are closely related to environmental factors, whereas the pore diameter and pore density reflect systematic relationships.

Light microscope studies of the egg membranes of most teleostean fishes reveal few special structures, with the exception of a few species, which possess variable sculpturing on the membrane surface. However, electron microscope studies have revealed characteristic structures on the egg membranes of several species (Lönning, 1972, 1981; Lönning and Hagström, 1975), the significance of such having been discussed by Ivankov and Kurdyayeva (1973), Lönning and Hagström (1975) and Stehr and Hawkes (1979). However, the species compared in these investigations occupied widely divergent systematic positions.

The eggs of pleuronectine fishes show considerable variety, for example, the membrane may be smooth or with hexagonal structures on its surface, the perivitelline space may be narrow or wide, and an oil globule may be present or absent (Pertseva-Ostroumova, 1961; Minami, 1984). Furthermore, some species lay demersal eggs, although most have pelagic eggs (Pearcy, 1962).

This paper describes the fine structures on the eggs of four species in the Pleuronectinae and discusses the significance of such to the systematic position and spawning ecology of these species.

Materials and Methods

The eggs of four pleuronectine fishes, *Hippoglossoides dubius*, *Eopsetta grigorjewi*, *Pleuronectes yokohamae* and *Pleuronichthys cornutus*, were used, those of the first three species being obtained by artificial fertilization in April 1989, March 1988, Jan. 1987 respectively, and from the last-mentioned, by collection with a plankton sampler in the sea, off the northern coast of Kyushu near Genkai-cho, Saga Prefecture in Feb. 1990. The samples were fixed and preserved in 10% sea water-buffered formalin.

For scanning electron microscopy, the egg membranes were separated from the embryonic body, rinsed with distilled water to remove formalin, cut into small pieces and air-dried on glass disks. Each sample-bearing disk was then set onto an aluminum mount with silver paste. For examination of *P. cornutus* membranes, the samples were dehydrated in a graded series of acetone, and dried to critical point in CO_2. The dried samples were coated with gold-palladium and observed in ALPHA-30A and JEOL JSM-T20 scanning electron microscopes.

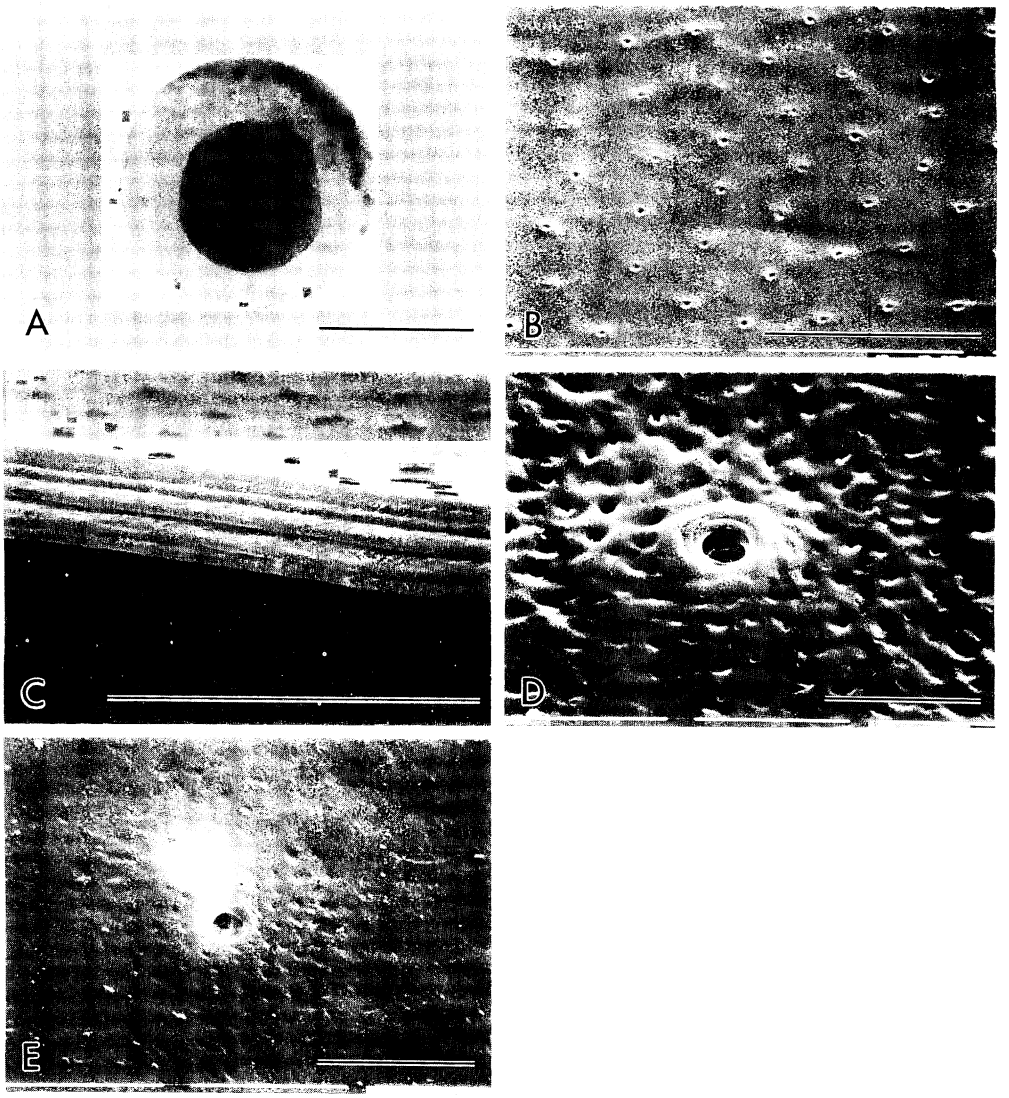

Fig. 1. *Hippoglossoides dubius.* Fine structure of the egg membrane. Bar scales indicate 1 mm (A) and 10 μm (B–E). A) Whole egg (light microscope); B) Outer surface of egg membrane (SEM); C) Cross section of egg membrane; D) Micropyle, exterior surface; E) Micropyle, interior surface.

Observations

Hippoglossoides dubius

The eggs were pelagic, spherical in shape and 1.5–1.7 mm in diameter. The yolk was about 1 mm in diameter, with a corresponding wide perivitelline space, and possessed many oil globules ranging from 0.001–0.01 mm in diameter. The egg membrane was almost transparent, with no specialized structures being detected under light microscope (Fig. 1A).

SEM, however, showed a number of pores, 0.29–0.40 μm in diameter and uniformly distributed on the surface of the egg membranes at a density of 8.4–8.6 pores/100 μm^2 (Fig. 1B). The egg membrane, 2.7 μm thick, consisted of five to six, more or less equally-thick lamellae (Fig. 1C). The micropyle was 4–6 μm in diameter at the outer surface, tapering to about 2 μm at the inner surface (Fig. 1D, E). Annular lamellae were observed on the inside surface of the

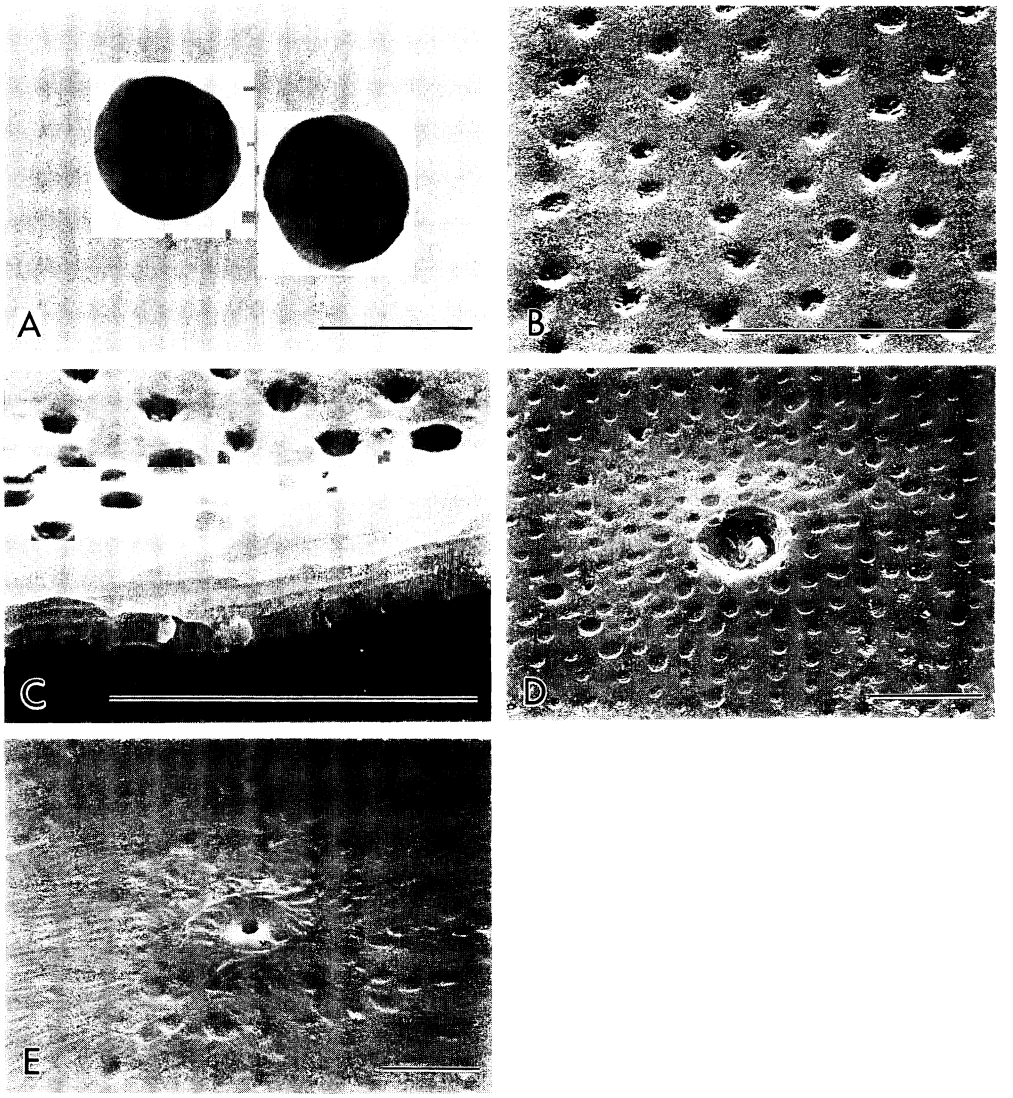

Fig. 2. *Eopsetta grigorjewi.* Fine structure of the egg membrane. Bar scales indicate 1 mm (A) and 10μm (B–E). A) Whole egg (light microscope); B) Outer surface of egg membrane (SEM); C) Cross section of egg membrane; D) Micropyle, exterior surface; E) Micropyle, interior surface.

canal. The outer opening of the micropyle was surrounded by pores and shallow cavities of various sizes, and the inner opening by about 50 small cavities.

Eopsetta grigorjewi

The eggs were pelagic, spherical in shape and 1.0–1.1 mm in diameter. The perivitelline space was very narrow and the yolk lacked oil globules. The egg membrane was smooth and almost transparent under light microscope (Fig. 2A).

Under SEM, the external surface of the egg membrane possessed a number of uniformly distributed pores, 0.90–1.13μm in diameter, at a density of 9.6–13.2 pores per 100μm^2 (Fig. 2B). The egg membrane, some 3.3μm thick, consisted of about seven lamellae, of which the outer most was thickest. A cross-section of the egg membrane showed the pores to be indented some two-thirds of the thickness of

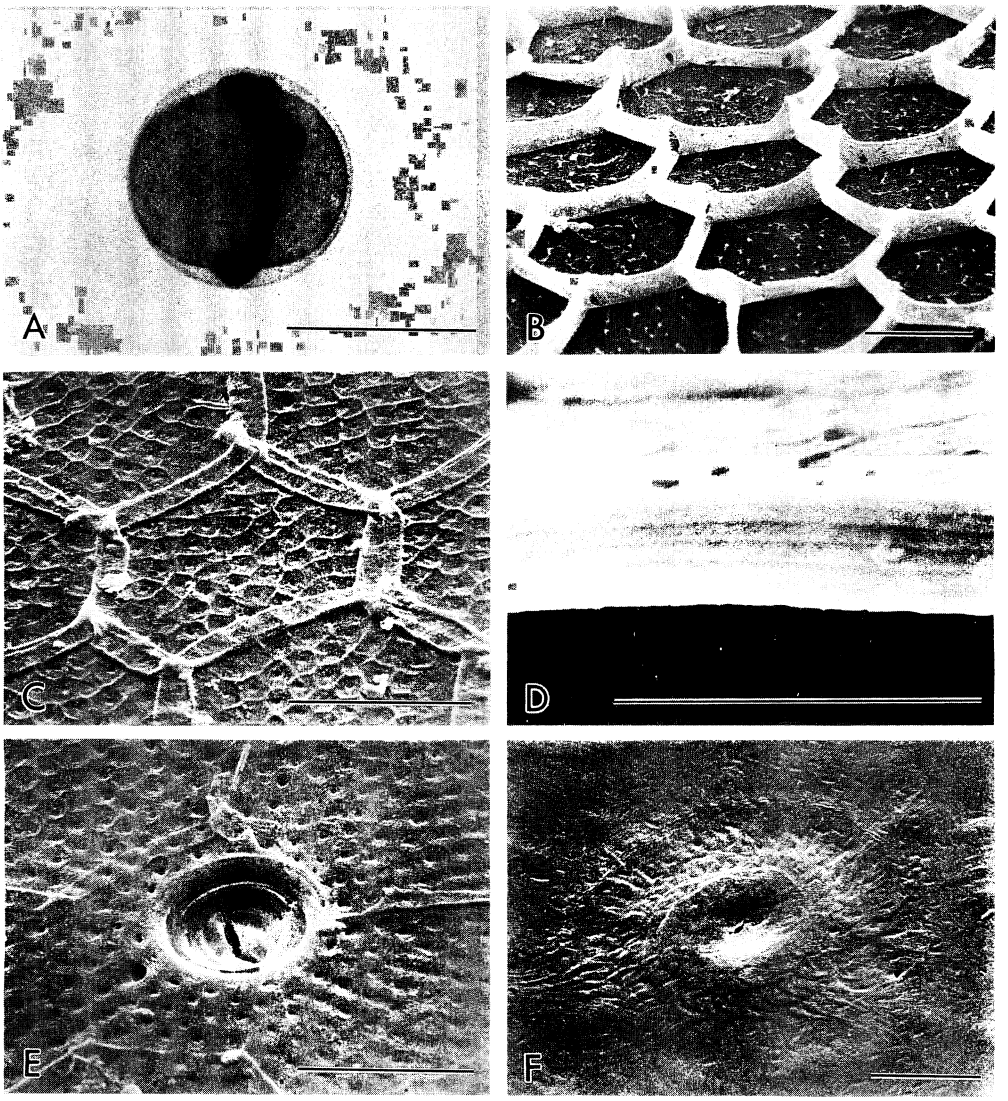

Fig. 3. *Pleuronichthys cornutus.* Fine structure of egg membrane. Bar scales indicate 1 mm (A) and 10μm (B–F). A) Whole egg (light microscope); B) Outer surface of egg membrane (SEM); C) High magnification of outer surface of egg membrane; D) Cross section of egg membrane; E) Micropyle, exterior surface; F) Micropyle, interior surface.

the first lamella (Fig. 2C). The outer opening of the micropyle (Fig. 2D) was about 8μm in diameter, with the adjacent area lacking further structures. On the inner surface of the micropyle, a protuberance, about 10μm in diameter, contained a central micropylar canal, 1.6μm in diameter. The protuberance was surrounded by irregularly distributed cavities, 2–3μm in diameter (Fig. 2E).

Pleuronichthys cornutus

The eggs were pelagic and spherical in shape (1.1–1.2 mm in diameter), with a single oil globule about 0.2 mm in diameter. The perivitelline space was narrow. Hexagonal meshes were apparent on the egg membrane surface under light microscope (Fig. 3A).

Under SEM, the ornamentation of the egg mem-