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The Comparative Ultrastructure of the Egg Membrane and Associated Pore Structures in the Starry Flounder, *Platichthys stellatus* (Pallas), and Pink Salmon, *Oncorhynchus gorbuscha* (Walbaum)*

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Summary. Eggs of the starry flounder (*Platichthys stellatus*) and pink salmon (*Oncorhynchus gorbuscha*) were examined by scanning and transmission electron microscopy to determine differences in egg membrane structure with reference to contrasting ecological conditions in which the eggs normally develop. The egg membrane of the starry flounder constitutes 0.22–0.50% of the egg's diameter. The zona radiata is composed of 6 continuous horizontal lamellae, covered by a thin triple layered border, and pierced by numerous regularly spaced pore canals. The micropyle canal measures 8 μm at the opening and tapers to 3.6 μm as it penetrates the membrane. In contrast, the thicker membrane of the pink salmon egg forms 0.80–1.0% of the egg's diameter, is composed of numerous short discontinuous lamellae which are pierced by pore canals, and is covered by a coating of irregular thickness. The 15–16 μm micropyle opening is surrounded by an area of protrusions, and the funnel-shaped canal tapers to 2 μm at its terminal aperture. Contrasting environmental conditions during embryogenesis of these two species may be reflected by the thin membrane and simple lamellar structure in the pelagic egg of the starry flounder, and the thick membrane and complex lamellar structure in the demersal egg of the pink salmon.

Key words: Egg membrane – Teleosts – Micropyle – Zona radiata – Pore canals.

An intricate membrane covers and protects the teleost egg and developing embryo. Several authors (for review, see: Ginzburg 1968, Anderson 1974) have de-

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monstrated differences in membrane structures among eggs of several different teleost species. Lönning and Hagström (1975) consider these differences as species specific, and Ivankov and Kurdyayeva (1973) hypothesize that the morphology of the primary membrane (zona radiata) is an indication of ecological conditions for spawning and egg development.

An understanding of the role of specific membrane structures in protecting the egg and embryo is important in determining the effects of natural and manmade environmental stresses on development. Starry flounder (*Platichthys stellatus*) eggs, which are pelagic, and pink salmon (*Oncorhynchus gorbuscha*) eggs, which are demersal, develop under different environmental conditions and reveal substantial differences in their membrane structure. In the present study, egg membrane structures are described for these two species, and differences that may be related to their protective function are discussed.

Materials and Methods

Ripe starry flounders were stripped and the eggs fertilized and incubated at the National Marine Fisheries Service (NMFS) Laboratory, Mukilteo, Washington. Pink salmon eggs were fertilized and incubated at the Auke Bay Laboratory of NMFS, Auke Bay, Alaska. Eggs were fixed two to three minutes after fertilization unless otherwise noted.

Flounder eggs were fixed for 24 h and pink salmon eggs for one week in a solution of 0.75% glutaraldehyde, 3% formalin, 0.5% acrolein in 0.1 M sodium cacodylate buffer with 0.02 $CaCl_2 \times H_2O$ and 5.5% sucrose (Hawkes 1974). Prior to dehydration, portions of the pink salmon egg membranes were dissected or punctured to allow complete passage of fluids. For scanning electron microscopy (SEM), eggs were dehydrated in a graded series of ethanol and Freon, critically point-dryed in Freon, coated with gold-palladium and examined with an AMR-1000 microscope.[1] For transmission electron microscopy (TEM), dissected portions of pink salmon egg membranes and whole starry flounder eggs were post-fixed $1^1/_2$ h in 1% OsO_4 in buffer, and embedded in plastic (Spurr 1969). The sections were stained with lead citrate, uranyl acetate, and again with lead citrate, and examined with a Philips EM-301 microscope.

It should be noted that the membrane and egg diameter measurements listed in Table 1 are from eggs treated in different laboratories by several techniques, some of which may involve shrinkage artifacts. For example, in our laboratory, about 10% shrinkage occurred during tissue preparation for SEM. Comparison of TEM and SEM micrographs demonstrated that structural integrity was not compromised.

Results

The membranes of the eggs examined are composed of a primary membrane known as the zona radiata (nomenclature follows Hurley and Fisher 1966), and lack a secondary membrane or zona pellucida.

Platichthys stellatus

The membrane (zona radiata) of the starry flounder egg consists of six continuous horizontal lamellae of approximately equal thickness (Figs. 1–3). Numerous pore

[1] Mention of commercial products is for information only and does not constitute endorsement by the U.S. Department of Commerce.

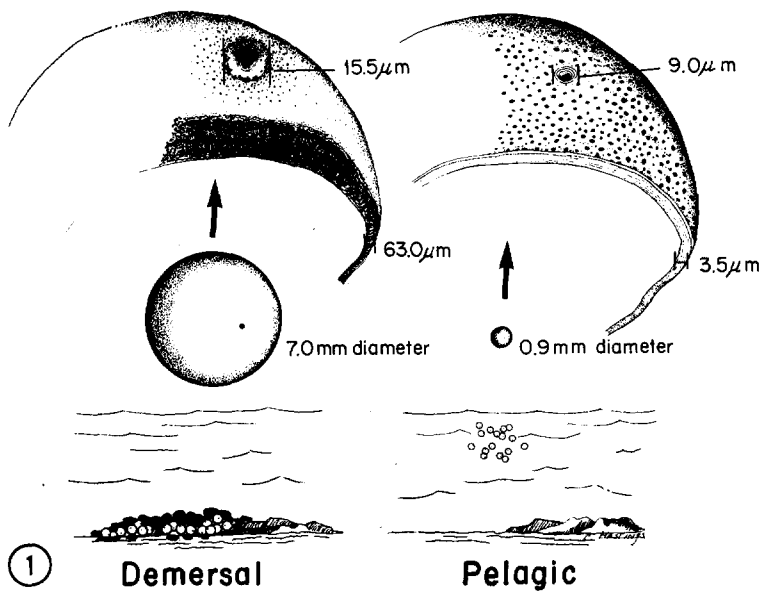

Fig. 1. Comparison of the habitat, size, membrane and micropyle of pink salmon and starry flounder eggs

canals penetrate the 2.0–5.0 μm thick zona radiata (Figs. 2, 3). A 0.1 μm triple-layered border forms the outer surface of the zona radiata and shapes a slightly depressed lip as it circumvents the openings to pore canals. An outer 0.02 μm electron-opaque layer, a center 0.04 μm electron-transparent layer, and an inner 0.04 μm electron-opaque layer compose the border. Beneath the pore openings the canals are wider than the surface aperture but narrow again (Fig. 2) and a shelf is apparent beneath the outer opening when several aspects of the upper 0.3 μm of the pore canals are compared (Figs. 2–4). The micropyle is funnel-shaped, is 8–10 μm in diameter at the outer surface (Fig. 4), and tapers to about 4 μm. Surrounding the outer opening of the micropyle is a distinct edge of the thin surface border. Radiating 8–10 μm from the edge is a region in which some of the pore openings are noticeably smaller, ranging from 0.3 to 0.7 μm in diameter. Beyond this area, pore openings are more uniform in diameter, 0.6–0.7 μm, and are distributed over the entire surface of the egg. At the terminus of the micropyle there is a protuberance, 23 μm in diameter, with a center depression, 9.5 μm across (Fig. 5). Numerous 0.5 μm pores spaced about 1.6 μm apart border the outside edge of the depression. A centrally-located protrusion 3.6 μm across occurs within the depression and in the area where the terminal opening to the micropyle would be expected.

Oncorhynchus gorbuscha

The pink salmon egg membrane is composed of numerous short, discontinuous lamellae (Figs. 1, 6, 9). Pore canals penetrate the 55–70 μm thick zona radiata,

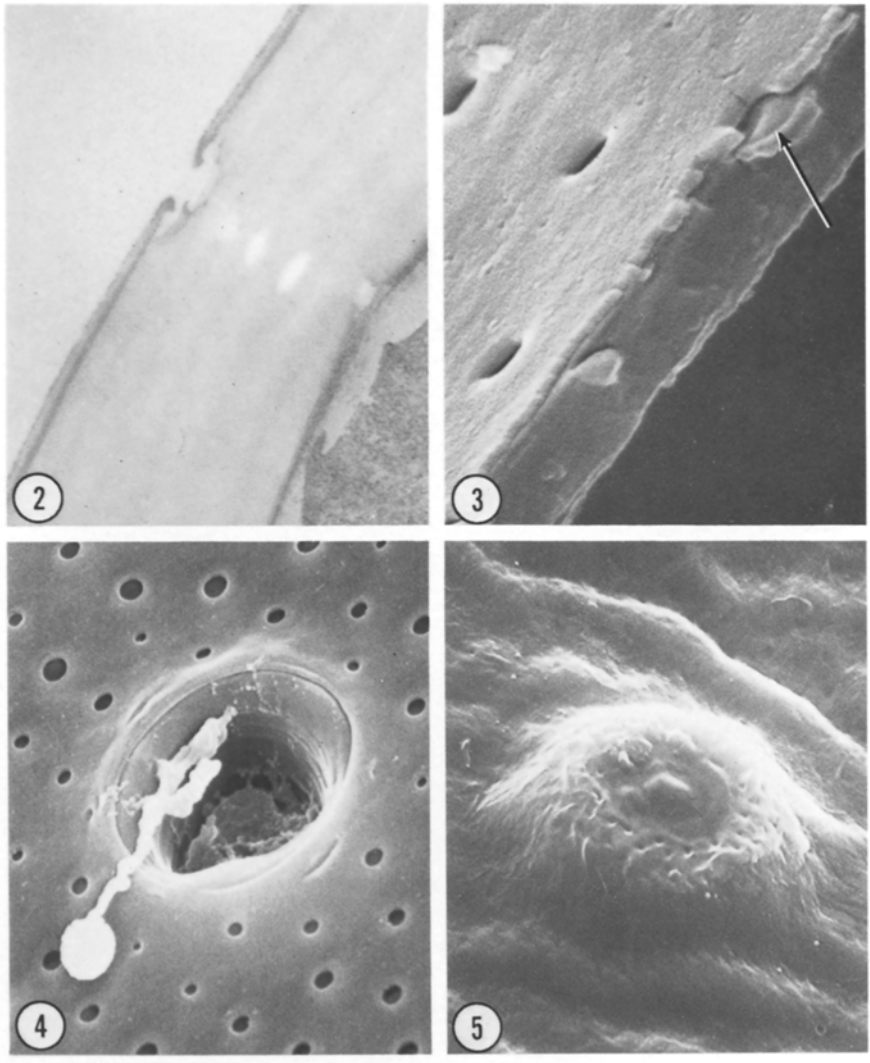

Fig. 2. Transmission electron micrograph (TEM) of starry flounder egg membrane. Pore openings and portions of a canal penetrate six lamellae. ×22,000

Fig. 3. Scanning electron micrograph (SEM) of broken edge of starry flounder egg membrane, pore canal shelf (*arrow*). ×11,000

Fig. 4. Micropyle of starry flounder egg (SEM) 2 min after sperm was added. Note sperm beside micropyle, diameter of head, 2.0 μm. ×3900

Fig. 5. Internal face of terminal aperture of starry flounder micropyle (SEM). In contrast with pink salmon (Fig. 11), internal surface is smooth and continuous. ×2000

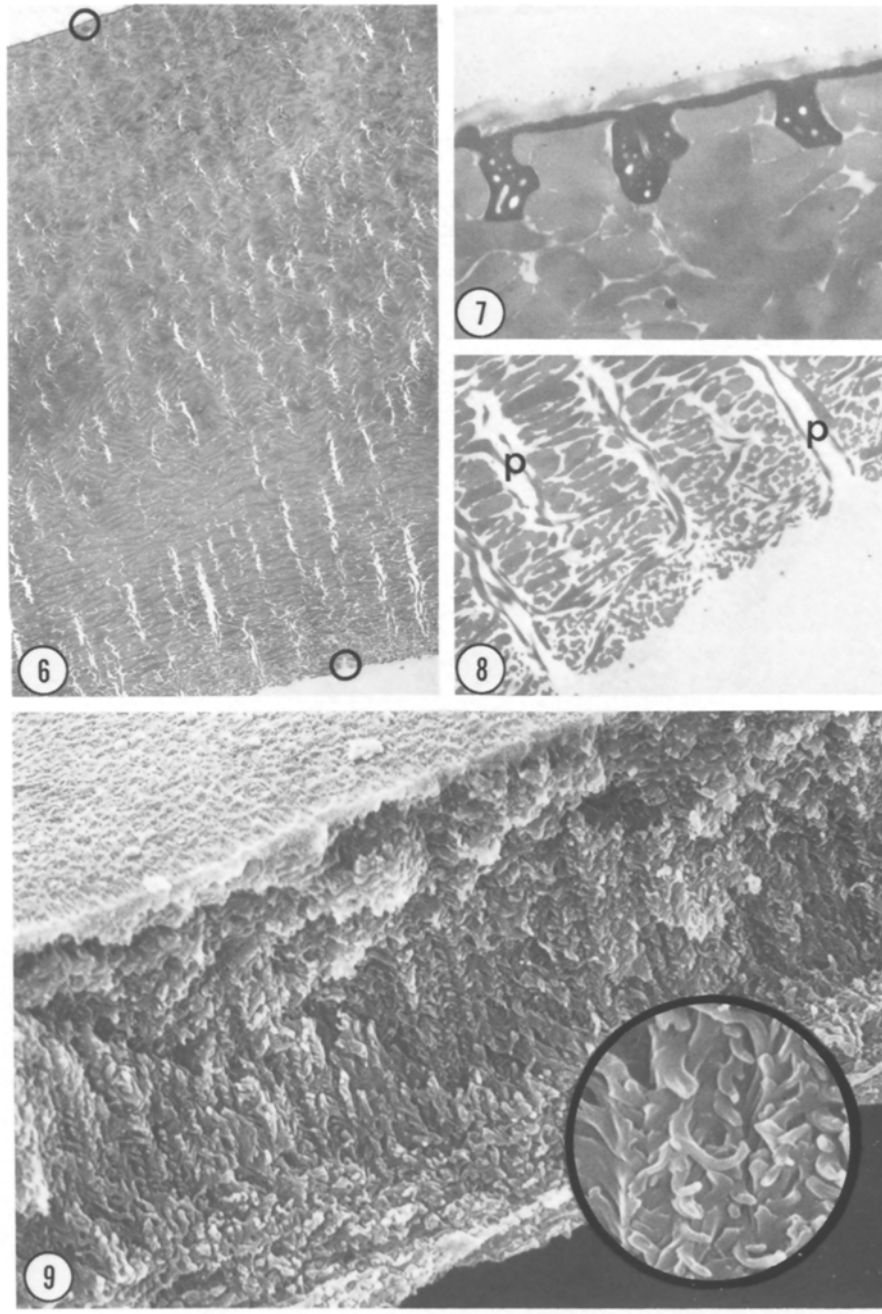

Fig. 6. Pink salmon egg membrane (TEM). ×1300

Fig. 7. Pink salmon membrane surface, enlarged area of upper circle in Fig. 6 (TEM). Note electron dense material or "plugs" at opening of pore canals. ×12,000

Fig. 8. Lamellar structure and pore canals (*P*) at internal face of membrane, enlarged area of lower circle in Fig. 6 (TEM). ×4700

Fig. 9. Cross section of pink salmon membrane (SEM). Note columnar arrangement of short, discontinuous lamellae penetrated by pore canals. ×1000. Inset, ×4200

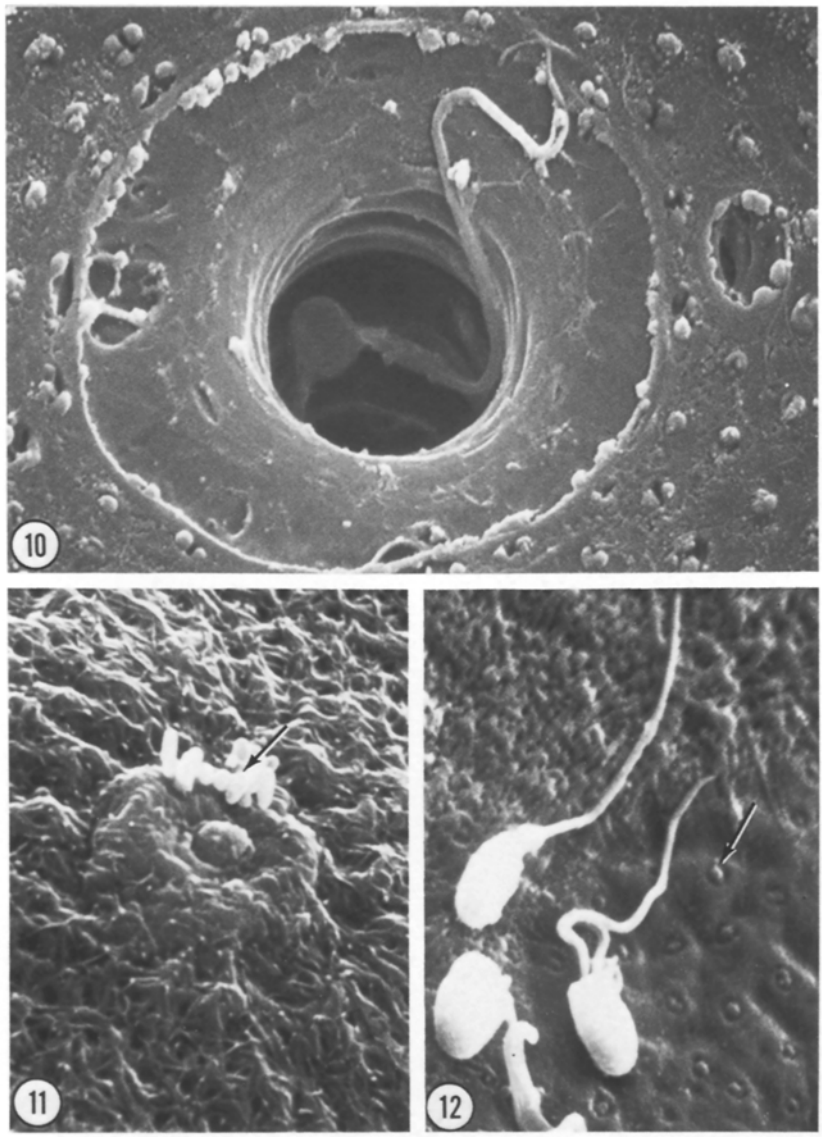

Fig. 10. Pink salmon micropyle (SEM) 45 seconds after sperm was added to eggs. Single sperm in micropyle canal. Protrusions surround micropyle; secondary opening on right. ×4700

Fig. 11. Terminal aperture of pink salmon micropyle (SEM). Note irregular and porous appearance of internal membrane face. Extraneous bacteria (*arrow*). ×3800

Fig. 12. Pink salmon egg surface (SEM). Gap in coating reveals pore canal openings containing plug-like structure (*arrow*). Sperm on surface average 2 μm in diameter. ×4000

which produce the columnar-like appearance of the lamellae when viewed in cross-section (Figs. 6, 8, 9). Most of the pore canal openings and the surface of the zona radiata are covered with an irregular coating, 0.2–1.5 μm thick. Occasional gaps in the coating reveal pore canal openings 0.7–0.8 μm in diameter (Fig. 12) some of which are slightly raised with a centrally located protrusion. Cross-sections of the egg surface examined with TEM show an osmiophilic substance that forms plug-like structures at the opening to the pore canals. This material also seems to be part of a continuous 1.0 μm thick outer border of the zona radiata (Fig. 7).

The micropyle is 15–16 μm in diameter, and surrounded by an irregular edge formed by a thin outer coating that includes a population of protrusions 0.6–0.8 μm in diameter (Fig. 10). These protrusions extend over a 30–50 μm area adjacent to the micropyle and are unique to that region of the egg. Occasionally, small secondary openings of varying sizes are observed within the area of protrusions (Fig. 10). These openings pass through the outer coating into the zona radiata; however, the extent of penetration is unknown. The inner terminus of the micropyle appears as a 10 μm protuberance, with a 5–6 μm crater-like depression and a central 2 μm terminal aperture (Fig. 11). A plug-like structure occludes the terminal aperture in ferilized pink salmon eggs (Fig. 11).

Discussion

Starry flounder eggs are pelagic, float at various depths throughout the water column in the marine environment, and hatch after about 14 days in 2.0°–5.4° C water (Yusa 1957). Pink salmon eggs, on the other hand, are demersal, incubate within gravel nests in estuary or stream environments, and require 5–6 months to develop under natural conditions (Hart 1973). The size of the egg, and the thickness and structure of the membrane may reflect adaptations to these different ecological conditions.

In general, marine teleost eggs that are pelagic tend to have thin membranes in relation to egg diameter (Table 1). A simple lamellar structure has also been noted in several pelagic species (Hagström and Lönning 1968, Lönning 1972). The structures of the starry flounder egg membrane are typical of eggs that maintain buoyancy during development. Demersal eggs, on the other hand, tend to develop much thicker membranes in relation to their egg diameter (Table 1). The thick membrane of the pink salmon in combination with the complex lamellar structure may provide strength for protection from mechanical stresses during a long demersal development. In addition, pink salmon eggs develop in fresh water and probably maintain osmotic properties that are different from those of marine eggs, and may affect membrane requirements and structure. The egg membranes of the marine rock sole, *Lepidopsetta bilineata,* which has demersal eggs, do not have the simple lamellar structure normally found in flounder eggs that are pelagic, but instead are composed of complex discontinuous lamellae and a thick membrane similar to that of the pink salmon (Stehr, unpublished data), supporting the hypothesis that this structure may be a general characteristic of demersal eggs.

In the ovary, teleost eggs are known to be pierced by numerous pore canals containing microvilli which transport nutrients from the follicle cell to the

Table 1. Comparison of egg membrane thickness to egg diameter in several species of pelagic and demersal teleost eggs

Species	Egg diameter (mm)	Zona radiata thickness (μm)	Membrane thickness to egg diameter (%)	References
Pelagic eggs				
Pleuronectidae				
Platichthys stellatus	0.9	2–5	0.22–0.50	Stehr and Hawkes
Platichthys flesus	0.8–1.4[a]	2.5[b]	0.18–0.31	(a) Muus 1962, (b) Lönning 1972
Pleuronectes platessa	1.6[a]	15.5[b]	0.96	(a) Muus 1962, (b) Lönning 1972
Limanda limanda	0.7–1.0[a]	2.5[b]	0.25–0.35	(a) Muus 1962, (b) Lönning 1972
Hippoglossoides platessoides	1.3–3.2[a]	2.5[b]	0.19–0.78	(a) Muus 1962, (b) Lönning 1972
Labridae				
Centolabrus rupestris	1.0[a]	2.0[b]	0.20	(a) Muus 1962, (b) Lönning 1972
Gadidae				
Gadus morhua	1.5[a]	4.5[b]	0.30	(a) Muus 1962, (b) Lönning 1972
Theragra chalcogramma	1.4–1.6	5–10	0.36–0.63	Stehr (unpubl.)
Demersal eggs				
Pleuronectidae				
Lepidopsetta bilineata	0.92	40–50	4.3–5.4	Stehr (unpubl.)
Labridae				
Crenilabrus melops	0.80–0.85[c]	7.0–8.0[d]	0.87–0.94	(c) Quignard 1967, (d) Hagström and Lönning 1968
Osmeridae				
Osmerus eperlanus	1.3[e]	13.32[f]	1.0	(e) Cunningham 1887, (f) Sadov 1963*
Clupeidae				
Clupea harengus harengus	1.2–1.5[g]	32.5[h]	2.1–2.7	(g) Brook 1886, (h) Hoffmann 1881
Salmonidae				
Oncorhynchus gorbuscha	7.0	55–70	0.80–1.0 5.0[i]	Stehr and Hawkes, (i) Ivankov and Kurdyayeva 1973
Salmo gairdneri	5.5–6.0	37–45	0.67–0.75	Stehr (unpubl.)
Salmo salar	5.0–6.0[j]	46–57[k]	0.92–0.95	(j) Berg et al. 1949, (k) His 1873

* In: Ginzburg 1968.

developing oocyte (Ginzburg 1968, Hurley and Fisher 1966). However, it is not known if and to what extent these pores remain open after fertilization. Hurley and Fisher (1966) report that in the rainbow trout the number of pore canals decreases due to "filling in" as the egg matures. However, Flügel (1967) attributes the striated appearance of the *Salvelinus fontinalis* egg membrane to portions of canals that remain open after oogenesis. Only the outer openings of the canals are plugged with an osmiophilic material that appears shortly before ovulation. In some fertilized eggs of other teleosts, Hagström and Lönning (1968) and Lönning (1972) describe "crypts," blind openings and sections of canals which they suggest to be remnants of previous canals. In the present study, pore canals of both the starry flounder and pink salmon (Figs. 2, 6, 8) were observed to penetrate the membrane, and their linear arrangement suggests continuous penetration, although structures similar to the "plug" described by Flügel (1967) were seen in the pore canal openings of the pink salmon (Fig. 7). The original function of pore canals in transporting nutrients

during oogenesis occurs within the fish before exposure to the external environment. However, if these pores remain open after spawning, they may be important in influencing the extent of exposure of the embryo to the natural environment or contaminants. Further research is underway to determine if pore canals partially or fully penetrate the egg membrane after fertilization.

Surface structures unique to the micropyle area occur in both species. In the pink salmon egg, the protrusions surrounding the micropyle are of the same diameter as the pore canal openings and are, perhaps, related to or extruded by the canals. These protrusions and/or secondary openings in the pink salmon, and the small pores surrounding the micropyle of the starry flounder conceivably may be specialized structures for production of phermones to attract sperm to the micropyle. It should be noted that attraction of sperm to the micropyle area has been shown to occur in herring, *Clupea pallasii* (Yanagimachi 1957), and three species of bitterling, *Acheilognathus lanceolata, Acheilognathus tabirua,* and *Rhodeus ocellatus* (Suzuki 1958, 1961a). A sperm-attracting component has been isolated from these bitterlings (Suzuki 1961b), but the mechanism of its production and dispersal is not well known.

The micropyle in the pink salmon egg is smaller, but otherwise structurally similar to that in the rainbow trout. Both Szöllösi and Billard (1974) and Stehr and Hawkes (unpublished data) have noted the occurrence of concentric ribs in the sides of the micropyle canal in rainbow trout. The current study also shows rib-like structures in the micropyle of the pink salmon egg. The bordering edges of the lamellae may form these structures.

The opening of the pink salmon egg micropyle is twice the size of that of the starry flounder; however, micropyle canals in both species taper to 2–4 μm at their terminal aperture and, similarly, the sperm of both species are the same diameter. In addition, the terminal apertures are centered within a protuberance which appears closed by a membrane in the starry flounder and is filled by a plug-like structure in the pink salmon. Szöllösi and Billard (1974) report a delicate membrane occurring across the aperture in response to fresh water exposure of the rainbow trout egg. This may correspond to the membrane across the terminal aperture of the starry flounder and may have been destroyed during processing of the pink salmon egg. The plug-like structure may also be a fertilization response or the actual head of a sperm. Kuchnow and Scott (1977) and Ginzburg (1968) suggest that in teleost eggs the terminal aperture of the micropyle is only large enough to allow entry of a single sperm. In support of this hypothesis, the terminal aperture diameter is similar to the sperm diameter in the rainbow trout (Szöllösi and Billard 1974), and in the present study of starry flounder and pink salmon. The funnel-shaped micropyle, therefore, appears to be implicated in both attracting sperm and restricting entry to one individual.

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