ON THE ATTACHMENT FIBERS OF LITTLE SKATE (*RAJA ERINACEA*) EGG CAPSULES

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Elasmobranch egg capsules at oviposition are provisioned with structures that are used as anchoring devices. Catshark (*Scyliorhinus* spp.) capsules have long tendrils arising from each of the four corners which the female winds around submerged objects like sea weed and corals. Hornshark (*Heterodontus* spp.) capsules have a spiral flange that secures the capsule in rock and coral crevices. Skate egg capsules have various forms of fibrous masses. Attached along the lateral seams of freshly oviposited little skate (*Raja erinacea*) egg capsules are loose masses of sticky fibers. While it seems clear that these fibers moor the capsule in some manner, nothing is known about how they accomplish attachment. The present study examined their structure, composition and physicochemical properties and evaluated their potential anchoring ability.

For biochemical analyses, attachment fibers were obtained from untanned regions of partially formed capsules removed from the uterus and solubilized in gel electrophoresis sample buffer containing 3% SDS and dithiothreitol. The solubilized proteins from the untanned attachment fibers were compared to those solubilized from the other regions of the capsule by electrophoresis on 4-20% linear gradient SDS/PAGE gels (Fig. 1). The fibers contained all of the proteins previously characterized from the body walls (see Koob & Cox, Environ. Biol. Fishes 38; 151-157, 1993), However, the relative proportion of capsule proteins comprising the attachment fibers differed from that in all other regions of the capsule. The predominant protein in the fibers was the 23 kDa protein (which is a minor component of the body walls, but is enriched in the seams). The fibers also contain significant amounts of the 20 kDa protein (which is in relatively low abundance in the other regions of the capsule). The fibers contain relatively small amounts of the major proteins found in the seams and body walls.

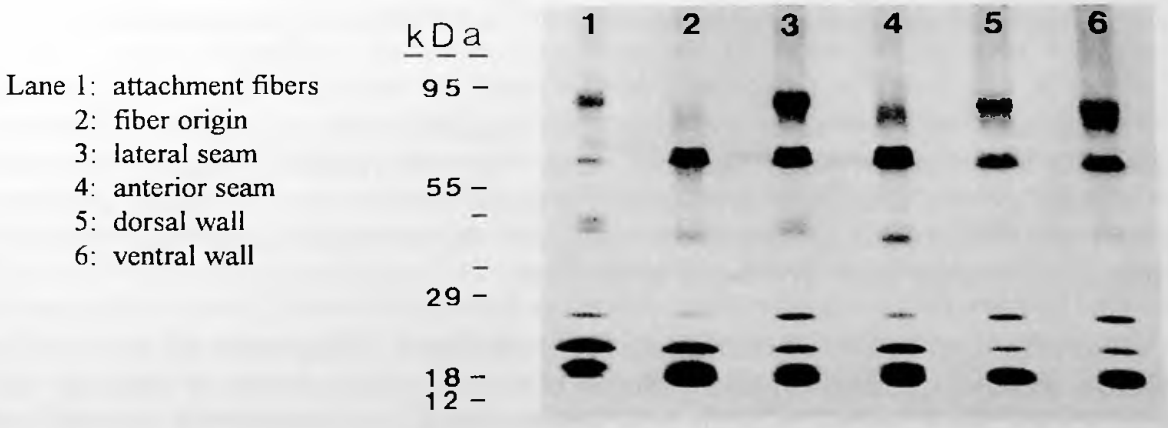

Figure 1. 4-20% linear gradient SDS/PAGE of capsule proteins solubilized from structurally diverse sites of an untanned, partially formed capsule.

After oviposition, attachment fibers separate from the body of the capsule to form a loose, diffuse mass of numerous individual fibers (Fig. 2). Each fiber mass remains joined to the body of the capsule at two structurally distinct sites. At the short horned or anterior end, fibers arise from a solid, cylindrical bar which extends outward from the keeled lateral seam. Near the base of the posterior horns, fibers insert separately along the lateral edge of the capsule. In the expanded form attained soon after oviposition, fiber masses appear light gold in marked contrast to the dark greenish-brown capsule body. Fibers feel sticky in sea water when freshly oviposited and quickly pick up various small particles present in the tanks (Fig. 2). After several days in the tanks, the fibers lose their adhesive properties and darken, whether from chemical changes or saturation is not presently known.

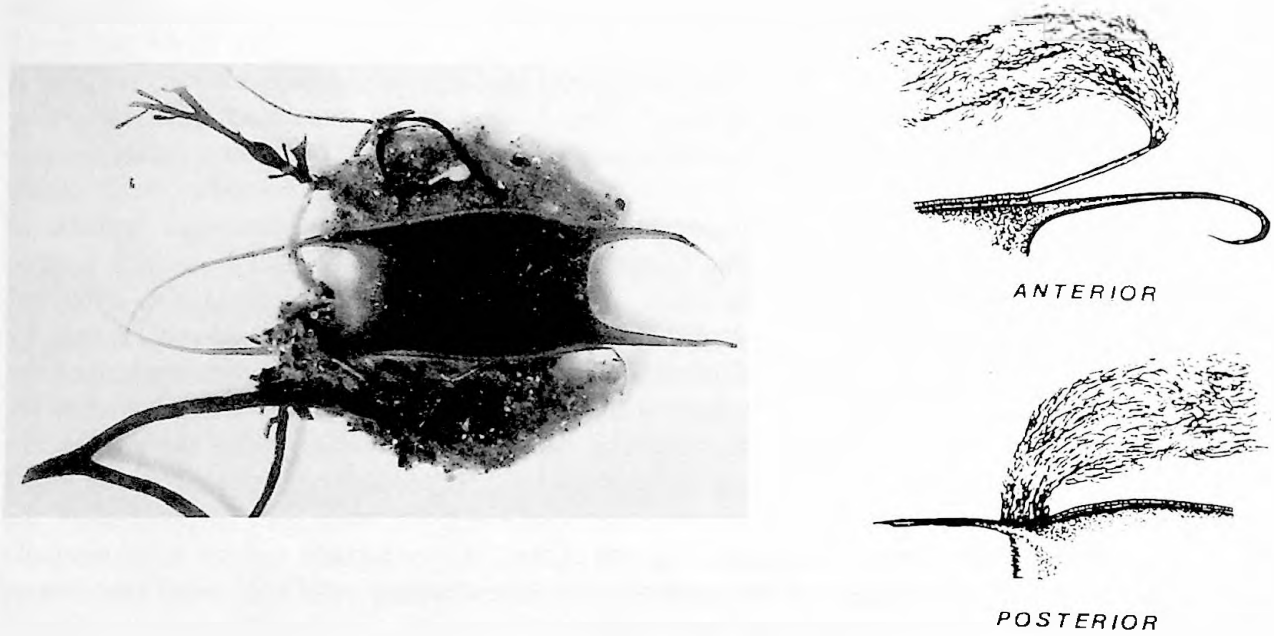

Figure 2. Little skate egg capsule with bound particulates. The illustration shows the distinct morphology of the anterior and posterior connections of the attachment fibers to the capsule.

Attachment fibers from freshly oviposited capsules were exposed to a variety of particulates in sea water and the amount bound after 1 hour determined by weight. Fibers avidly bound crushed mollusk shells with as much as 8 g bound per fiber mass. Crushed chicken egg shells were also avidly bound by these fibers. Silica sand was not bound by the fibers. Both anion and cation ion exchange resins were bound by the fibers.

The rate at which fibers experimentally bound particles was determined by presenting fiber masses from freshly oviposited capsules with one of two polystyrene based ion exchange resins, Dowex AG1-X4 200-400 mesh (strongly basic anion exchanger) and AG50W-X4 200-400 mesh (strongly acidic cation exchanger). Chromatographic resins were selected because of their uniform size and chemistry. The fibers were stirred with the resins in sea water and the weight of the fiber mass plus bound resins after blotting was determined at regular intervals. Fibers rapidly

bound these resins reaching saturation levels at about 1 hour, after which the amount bound remained constant for five days.

To determine the effect of bound particles on egg capsule weight, capsules were removed from the urogenital sinus and immediately placed in 500 ml of sea water containing crushed mollusk shells. The capsules were then gently mixed with the shells for 5 min to allow the fibers to bind shells. The matching capsules were collected and kept in particle free sea water. The capsules with and without attached shells were first weighed on a balance. They were then weighed in sea water by hanging them with monofilament from a pre-calibrated 100 g force transducer (Schaevitz) and recording the force due to gravity. The weights of eight capsules with bound shells were compared to those of the matching capsules which were not exposed to the shells. The average weight of eight capsules lacking bound shells was 13.0 $\pm$ 1.0 g compared to 21.4 g $\pm$ 6.2 g for capsules with bound shells. The average weight in sea water of capsules without bound shells was 0.66 $\pm$ 0.13 g (5% of their weight out of water), indicating that egg capsules at oviposition are very buoyant. The average weight in sea water of the matching capsules with bound shells was 4.82 $\pm$ 2.82 g in sea water. The latter represents a 7-fold increase in weight in sea water when shells are bound to the fibers compared the matching capsules lacking bound shells.

These capsules were next exposed to currents in a laminar flow tank (described in Koob & Summers, Bull. MDIBL, 35, 108-111, 1996) in order to determine whether the additional weight resulting from the bound shells would significantly influence their response to currents. The capsules were placed with the short horns forward in the tank with no flow, then the current speed was increased in increments from 6 to 50 cm/sec. The current speed which caused the capsule to move through a distance of 1 m was determined. Displacement of capsules without bound shells occurred at an average current speed of 10.8 $\pm$ 0.5 cm/sec, whereas, capsules with bound shells were displaced at an average speed of 40.9 $\pm$ 14.2 cm/sec.

At oviposition little skate egg capsules are remarkably buoyant and weigh surprisingly little for benthic eggs of their size. To prevent untoward movement following oviposition, egg capsules come equipped with attachment fibers which will bind a variety of particulates, but have a certain affinity for calcium carbonate based materials. Binding can be rapid with complete saturation occurring in less than an hour. Whether the female selects a site at which these materials are abundant, or, alternatively, she oviposits the egg at a location where the capsule will be moored to a solid object is unknown since natural oviposition sites of skates have never been discovered. For either scenario, the attachment fibers provide an effective means of anchoring the capsule. The basis for this adhesion appears to reside in part with two of the capsule proteins which make up the preponderance of the fiber mass. The shell gland apparently produces mixtures of the capsule proteins that result in specific site-related properties adapted to particular functions. In the case of the attachment fibers, this particular mixture, together with post-secretory chemical events, results in fibers with adhesive properties. Whether the proteins themselves are adhesive or the adhesive properties of the fibers rely on post-secretory events correlated with quinone tanning is unknown. We are currently exploring the chemical basis for adhesion.

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