WHAT DEGRADES SKATE EGG CAPSULES?

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Egg capsules of oviparous elasmobranchs protect the eggs and developing embryos for protracted incubation periods in the harsh and unpredictable marine environment. Little skate (*Leucoraja erinacea*) eggs require at least one year to develop to hatching when incubated at ambient sea water temperatures in the northern Gulf of Maine. On one occasion we found that capsules incubated in the waters off the Laboratory had not hatched after two years yet they contained viable, near-hatching embryos. The capsule material, composed almost entirely of protein, must be exceptionally durable in order to withstand such protracted periods exposed in sea water. The capsule protects the developing embryo against bacterial assault, predatory attacks (Koob and Cox, Environ. Biol. Fishes 38; 117-125, 1993), and the caustic action of sea water. But after the embryo hatches, what happens to the capsule? How long does it survive? Is there any biological consequence of capsules remaining intact beyond hatching?

In 1992, a wire cage containing 96 *Leucoraja erinacea* egg capsules oviposited in the Laboratory tanks was set in approximately 40 feet of water off the Laboratory point. The cage was not located the following year and subsequent attempts to find the cage in later years were unsuccessful. However, in the summer of 2000, Davin O'Connell, while diving for specimens for the Laboratory, by chance discovered this cage. Further inspection revealed that, while the majority of the capsules had disappeared (due to degradation since they were tethered with monofilament and the cage mesh was too small to allow the capsules to escape), 14 hatched capsules were relatively intact. One of these capsules is shown below. While this particular capsule was remarkably intact and differed little from a capsule immediately after hatching, other capsules remaining in the cage had degraded to various extents. This finding raises questions concerning the average time hatched capsules survive in the marine environment, why some capsules degrade and others are more refractory to degradation, and what is responsible for their natural degradation.

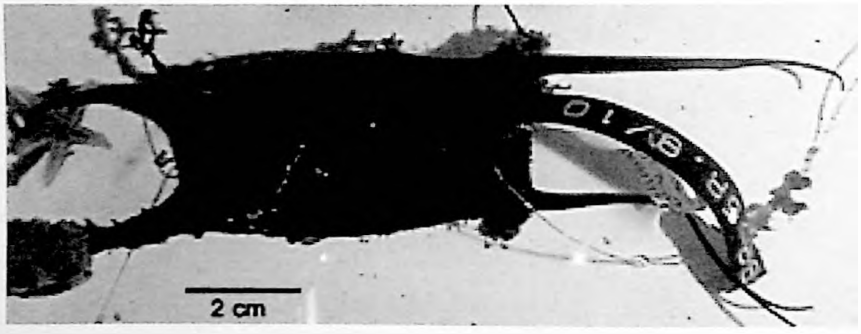

Figure 1. A little skate egg capsule that had incubated in Frenchman Bay for eight years.

To address the latter question, an initial experimental approach examining the stability of capsule material to a variety of agents was employed. One possibility for degradation enlists the action of proteolytic enzymes derived from animal sources. To examine the susceptibility of the

capsule material to proteolytic attack, capsules were collected at oviposition and uniform specimens 5 x 1 mm were prepared by dissection. Specimens were then subjected to a variety of commercially available and pure proteinases, including papain, porcine pepsin, bovine elastase, and a variety of bacterial proteinases, protease (*Staph. grisues*), dispase (*Bacillus polymyxa*), V8 protease (*Staph. aureus*), proteinase K (*Tritarachium album*), clostripain (*Clostridium histolyticum*), and collagenase (*Clostridium histolyticum*). Since all but one of the structural proteins that comprise the capsule contain hydroxyproline (Koob and Cox, Environ. Biol. Fishes 38; 151-157, 1993), measurements of the amount of hydroxyproline in the incubation buffer and capsule residue ware performed colorimetrically on dried acid hydrolyzates in order to estimate the extent of degradation.

Of the enzymes examined, papain was the only one that significantly degraded the capsule material (Fig. 2). Pepsin, protease, and dispase appeared to solubilize small amounts of the capsule. The values for the other enzymes were within the error of the assay and indicate that they had little affect on the specimens.

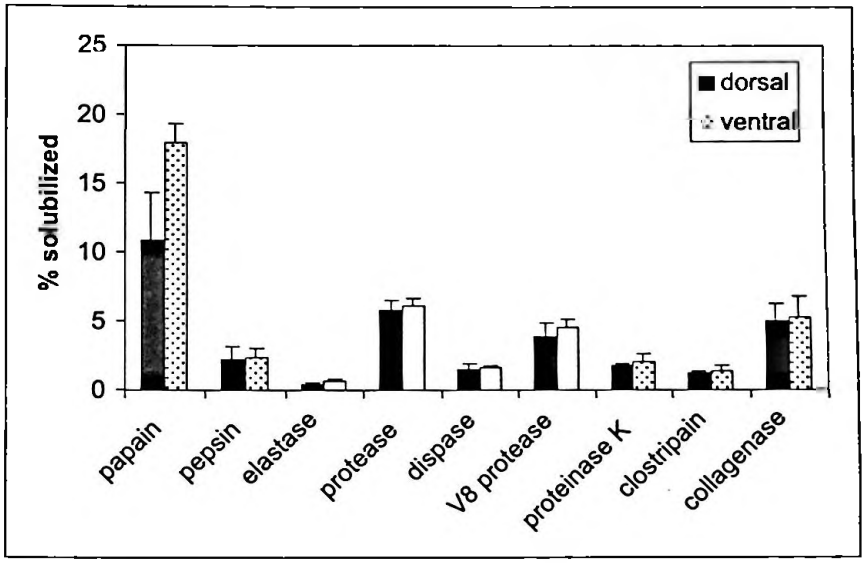

Figure 2. Extent of degradation of egg capsule material by proteinases after 24 hr incubation as expressed as the percentage of hydroxyproline released into the incubation medium. The bacterial enzymes were dissolved at 1 mg/ml in sea water. Pepsin was in acetic acid at pH 2.5, and papain was in sodium acetate/cysteine/EDTA buffer. Incubations were at room temperature except for papain treatment which was done at 60°C. N = 5 for each bar; error bars show the S.D.

The outer and inner surfaces of the capsule are coated with a dense, quinone tanned layer (Fig. 3), which could serve to protect the capsule from proteolytic attack. To examine whether the degradation by papain was primarily through the cut surfaces, identically sized specimens were further sectioned to expose more area of the central layers. They were then subjected to

papain treatment as above. The results indicate that exposure of the central capsule layers allowed more degradation by papain (Fig. 3).

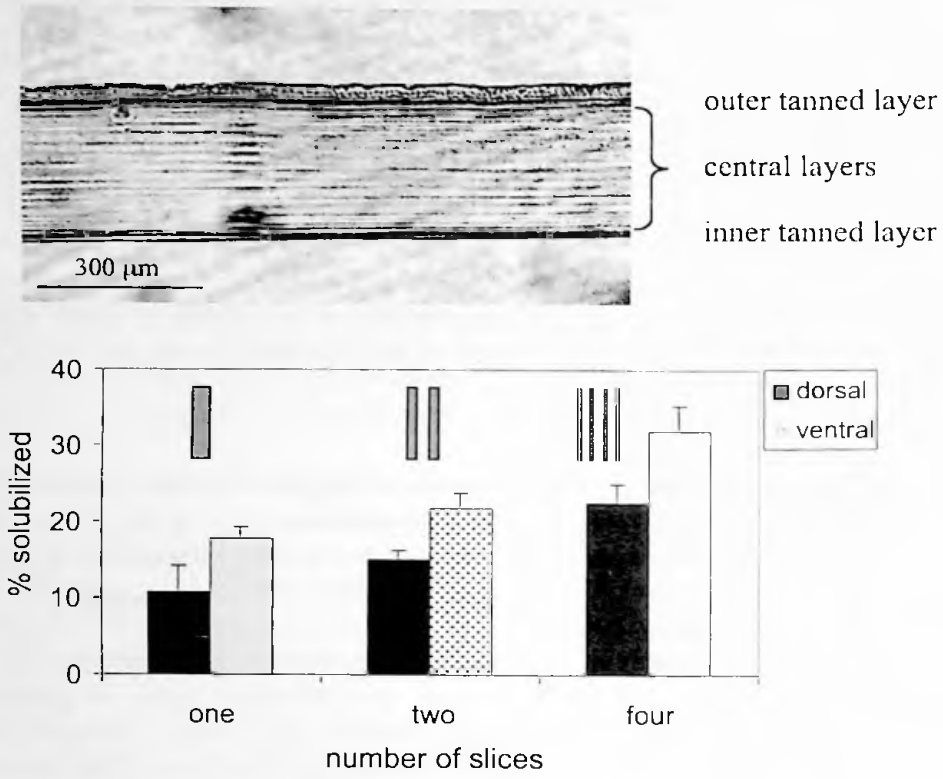

Figure 3. Effect of exposing the central layers of capsule on the extent of degradation by papain. The upper panel shows an unstained section of the dorsal wall of the little skate egg capsule to illustrate the two tanned layers that bound the central layers. The bottom graph shows the percentage of hydroxyproline containing material solubilized by papain. N = 5 for each bar; error bars show S.D.

What degrades skate egg capsules? These experiments clearly can not answer the question. However, the results do provide a provisional but solid basis for speculations that bear directly on the question. While papain is not a biologically relevant enzyme, especially given the 60°C incubation and reducing conditions, it did reveal that the central layers are more susceptible to degradation than the outer and inner tanned layers. This may be why the surfaces exposed to sea water, both on the outer and inner surfaces, are varnished with this tanned material. Structural integrity of the specialized layers may be crucial for fending off enzymatic attack. Can the other proteinases degrade the capsule? They have little effect over 24 hr; however, longer incubations will be necessary to unequivocally determine how intractable the capsule is to these and other enzymes. Protracted incubations are currently underway, as are experiments with caged capsules in Frenchman Bay addressing potential biological consequences of the capsule's remarkable durability.

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