VENTILATING THE SKATE EGG CAPSULE: THE TRANSITORY TAIL PUMP OF EMBRYONIC LITTLE SKATES (*RAJA ERINACEA*)

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Conspicuous morphological features of nearly all skate egg capsules are the curved hollow horns emanating from the four corners. In the distal half of each horn is a slit which at oviposition is plugged by a dense, gelatinous egg jelly. After approximately a third of development is completed, this gel disappears, admitting sea water into the body of the capsule. That the skate embryo actively moves water in and out of the capsule with its caudal tail appendage after the slits open was first discovered by Clark (J. Mar. Biol. Assoc. U.K. 12, 577-643, 1922). Although this observation has been independently made by many investigators since then, anatomical and empirical investigations have to our knowledge never been attempted. We therefore set out to examine the morphology and mechanics of the putative tail pump in little skate (*Raja erinacea*) embryos.

The transitory tail appendage: The two dorsal fins on adult little skates are located just proximal to the end of the tail. In contrast, when the dorsal fins first appear on the embryo, they are far proximal to the tip of the tail (Fig. 1). As development proceeds, the length of the tail distal to the second dorsal fin changes little. The dorsal fins of near-hatching embryos are well proximal to the tip of the tail, and a morphologically distinct tail appendage approximately 12 mm in length can readily be discriminated by gross examination. After the embryo hatches, the tail appendage disappears over the next several weeks, and the dorsal fins assume their permanent position relative to the tail tip.

Tail morphology: Alcian blue and H & E stained sagittal sections of the tail appendage from a formalin fixed, near-hatching embryo revealed that the appendage consists predominantly of notochord and muscle. The notochord extends to the tip of the tail. No traces of cartilage or developing vertebrae were detected throughout the length of the appendage, whereas they were well developed at the end of the future tail tip. X-rays of the tail confirmed the absence of calcified structures in the appendage.

Embryonic tail behavior: Videotapes of young embryos just after gel dissolution from the slits revealed that the tail appendage at this early stage is fully capable of producing the motions necessary to pump water through the capsule. The embryo inserted the tail appendage into the lumen of one horn and began movements which were essentially identical to those utilized by the near-hatching embryos to pump water through the horn. The embryo changed position freely utilizing all four horns for the tail pump. Videotapes of embryos at various intermediate stages showed that the tail pump functions throughout the last two thirds of development. The tail of near-hatching embryos, which fill most of capsule, is reflected 180^0 and thereby restricted to utilizing one of the two horns close to the rostrum. However, the embryo can turn horizontally so that the rostrum faces either the anterior or posterior (hatching) seam.

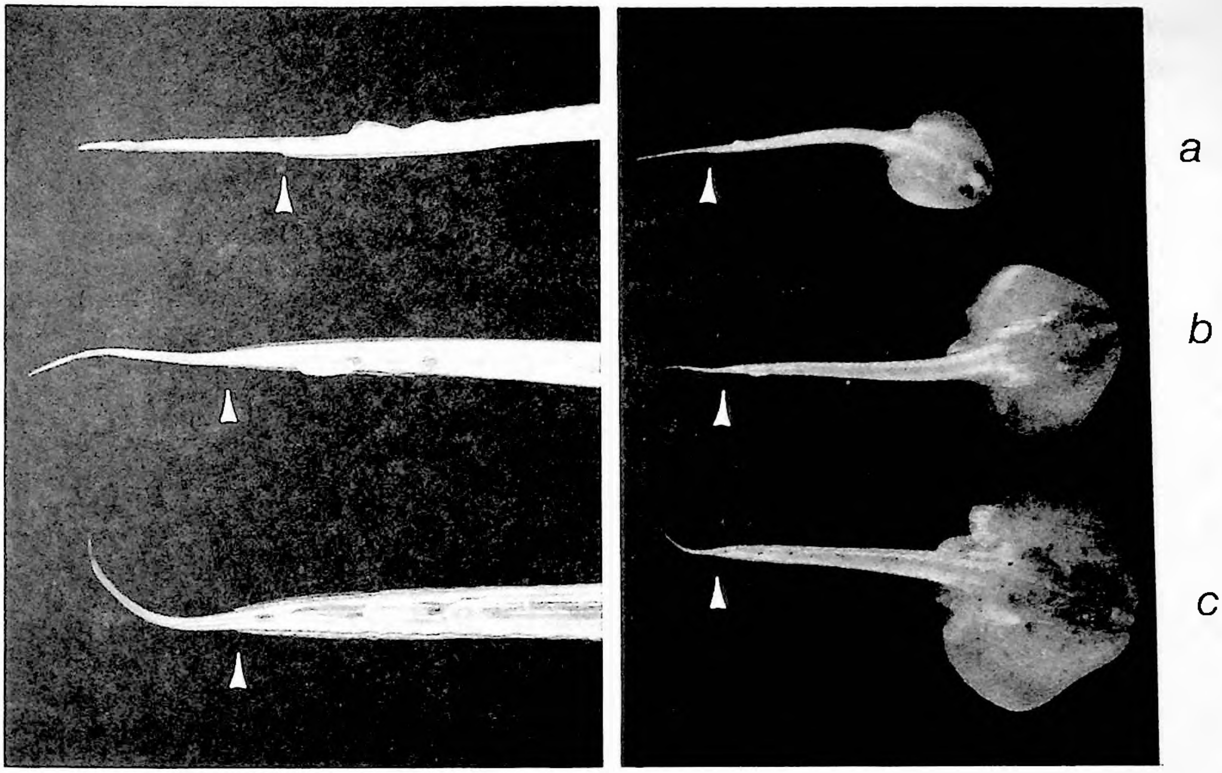

Figure 1. Little skate embryos at three developmental stages: a) after approximately 1/3 of development shortly after the slits on the capsule horns open; b) approximately 2/3 developed; c) near-hatching. The arrow heads indicate the future tail tip; the tail appendages extend to the left of the arrow heads.

Tail movement: The motion of the tail pump of near hatching embryos was measured using high-speed video (Kodak EktaPro 1000EM high-speed camera at 125 frames/sec). When in the lumen of one of the capsule's four horns, the tail appendage produced highly regular waves of bending that traveled proximo-distally. The traveling wave was frequently reversed. To assess tail pump motion independent of the capsule horn, one horn was cut off at the base and motion of the undulating appendage as it extended out of the capsule was recorded with high-speed video. Without the physical presence of the horn, the regular lateral amplitude and axial curvature disappeared.

Tail pump: To determine whether the tail appendage drives ventilation, air bubbles were injected through a canula into the capsule at the base of the horn occupied by the tail appendage. High-speed video of bubble movement showed that the undulating tail pumped the bubbles out of the horn when the waves traveled proximo-distally. Air bubbles injected into the horns not occupied by the pumping tail were drawn into the capsule. When the bending waves were reversed, bubbles moved into the capsule through the occupied horn.

Pumping pressure: A pressure transducer was inserted into the lumen of one horn distal to the tip of the tail appendage to record pressure changes during tail pumping (Strathman-Gould

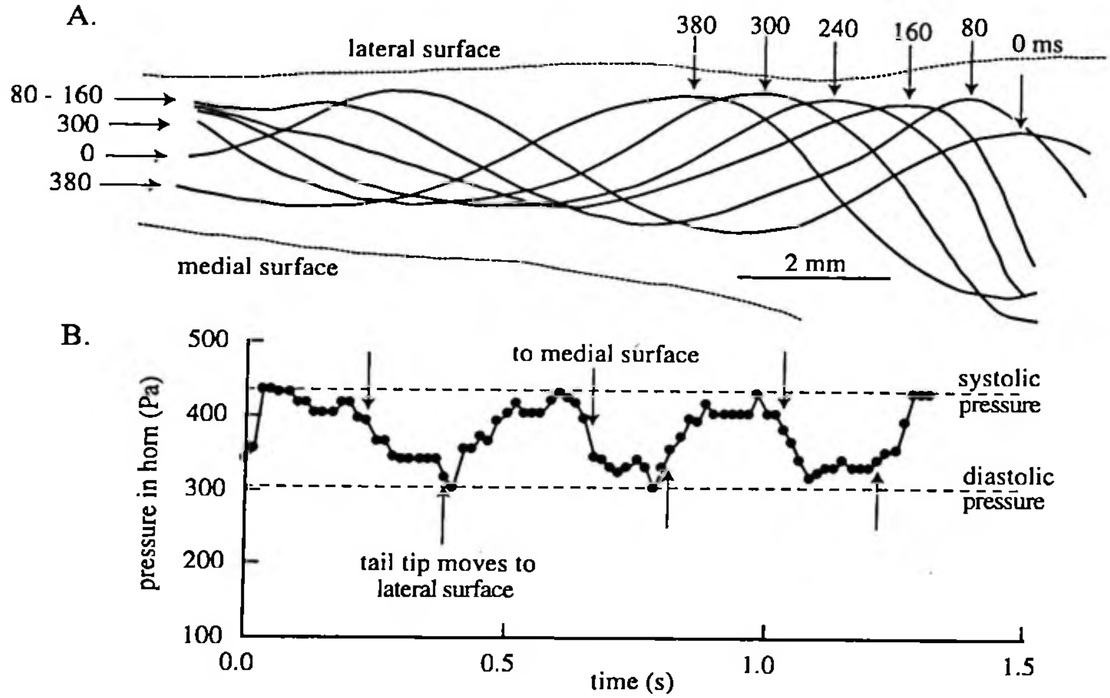

Figure 2. Mechanics of capsule ventilation. A. Ventral view of tail (lateral edge traced through time), showing waves of bending traveling proximal to distal (right to left). B. Pressure in the occupied horn is generated by the beating tail; when it stops, the pressure drops to 0 Pa.

P23ID transducer connected to the lumen of the horn with Intramedic PE50 polyethylene tubing; pressure calibrations were routinely performed immediately after each experiment). When the transduced horn was occupied by the active tail pump, a positive pulsatile pressure head was generated (Fig. 2). Nearly steady state negative pressures were generated if the tail pump was operating in one of the horns not occupied by the transducer.

Little skate embryos pump sea water into and out of the capsule during the last two thirds of development using a specialized tail appendage. This appendage attains the appropriate functional size and morphology early in embryonic development, and remains this size until hatching. The tail pump operates by producing bending waves of highly regular lateral amplitude and axial curvature which travel along the tail and are physically governed by the horn. Tail pumping generates a positive pressure in the occupied horn, causing a negative pressure in the three unoccupied horns, thus causing water to exit the pumped horn and enter the three empty horns. Thus, the tail pump/capsule horn system appears to be co-adapted for ventilating the capsule. We predict that the embryo ventilates the capsule in response to reductions in dissolved oxygen or increases in metabolic waste products. These observations suggest that, as the embryo develops, oxygen diffusion through the capsule walls is no longer sufficient to supply the continually increasing oxygen demand.

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