

Metabolic Rate of Embryonic Little Skate, *Raja erinacea* (Chondrichthyes: Batoidea): The Cost of Active Pumping

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ABSTRACT Near-hatching embryonic little skates, *Raja erinacea*, are highly active within their egg capsules, displaying a characteristic tail beating, which pumps water through the capsule. We measured the metabolic rate of late-stage embryos to determine whether oxygen sufficient for the embryo's needs will diffuse through the egg capsule, and to assess the energetic cost of tail beating. Metabolic rate was inferred from oxygen consumption rates while embryos were in the capsules, unencapsulated, and anesthetized and unencapsulated. Anesthesia inhibited voluntary movements, including tail wagging, allowing an estimate of the standard metabolic rate (SMR). Averaged over five embryos, the SMR was $0.032 \pm 0.004 \text{ ml O}_2 \text{ g}^{-1} \text{ hr}^{-1}$. There was no significant difference in metabolic rate between encapsulated ($0.058 \pm 0.009 \text{ ml O}_2 \text{ g}^{-1} \text{ hr}^{-1}$) and unencapsulated ($0.049 \pm 0.009 \text{ ml O}_2 \text{ g}^{-1} \text{ hr}^{-1}$) skates. Tail beating was found to be energetically expensive, requiring a 53%–81% increase over the SMR. From literature values for the oxygen permeability of the egg capsule we conclude that tail beating is required to supply sufficient oxygen to the embryonic skate. This observation is consistent with the proposal that actively pumping water through the capsule, by tail beating, has played an evolutionary role in the shape of the capsule. *J. Exp. Zool.* 283:13–18, 1999. © 1999 Wiley-Liss, Inc.

Skates are dorsoventrally flattened chondrichthyan fishes inhabiting shallow and deep water of all the oceans. The little skate (*Raja erinacea*) is a common member of the Northwestern Atlantic marine ecosystem. Like many elasmobranchs, populations of this fish have come under increasing fishing pressure off the coast of New England, primarily because of the decrease in groundfish in the area (Hutchings and Meyers, '94; Meyers et al., '96). There is a growing targeted fishery for elasmobranchs in the region, including skates. Reproduction in elasmobranchs is generally characterized by low fecundity and consequently they are at risk of over-exploitation (Cailliet, '90). Currently, there is little known about hatching success and the survival of skate offspring in the wild, particularly because the spawning grounds have not been identified for this species. An increased understanding of the reproduction and early development of elasmobranchs is crucial to devel-

oping responsible management regimes for these fishes.

All skates are oviparous, laying rectangular egg capsules that usually contain a single embryo (Fig. 1). A distinctive feature of most of these capsules is the horns that emanate from the corners, two pointing posteriorly and two anteriorly. Each horn has a slit that, although plugged with albumen early in development, allows sea water to enter the case during the latter two thirds of the embryonic period (Clark, '22; Luer and Gilbert, '85). Skate embryos remain in the egg capsule for several months (e.g., *Raja eglanteria*) to a year or longer (e.g., *Raja erinacea*) prior to hatching.

While in the egg case, skate embryos demonstrate a characteristic tail-beating behavior. This

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movement is rhythmic and rapid and has also been observed in several other oviparous elasmobranch species (e.g., *Raja eglanteria*, Luer and Gilbert, '85; *Scyliorhinus canicula*, Diez and Davenport, '87). Luer and Gilbert ('85) reported that the embryonic activity increased within the egg case after the albumen plugs were removed from the horns. The embryonic skate tail is equipped with a thin appendage which is visible during the last few weeks before hatching (Libby, '59; Long and Koob, '97) and which appears to be lost after hatching in most skate species (Clark, '23; Bigelow and Schroeder, '53). This appendage is frequently inserted into a horn of the egg case where it is rapidly oscillated (Fig. 1) (Clark, '22). This rhythmic action pumps water through the capsule (Long and Koob, '97). While the purpose of the pumping is not entirely clear, it has been observed that blocking the horns with sediment causes 100% mortality in the embryos (Richards et al., '63). The assumption is, that though the capsule is permeable to many metabolites (Foulley and Mellinger, '80; Evans, '81), as the embryo grows diffusion will not suffice, and water must be pumped through the capsule to obtain oxygen and/or remove metabolic waste.

In addition to this active pumping mechanism it has been shown experimentally that water flows through the capsule in the presence of an external current (Koob and Summers, '96). Such a method of ventilating the capsule comes with no metabolic cost to the embryo since it relies only on the shape of the capsule and the speed of the external current.

The purpose of this study was twofold: (1) to determine the standard metabolic rate of an embryonic batoid fish; and (2) to quantify the cost to the embryo of actively pumping water through the capsule.

METHODS

Fish

Egg capsules were laid by captive, adult *Raja erinacea* in the fall of 1995 at the Mount Desert Island Biological Laboratory in Salsbury Cove, Maine. The newly laid egg capsules were kept in the flow-through sea water system at ambient ocean temperatures in indoor tanks until October

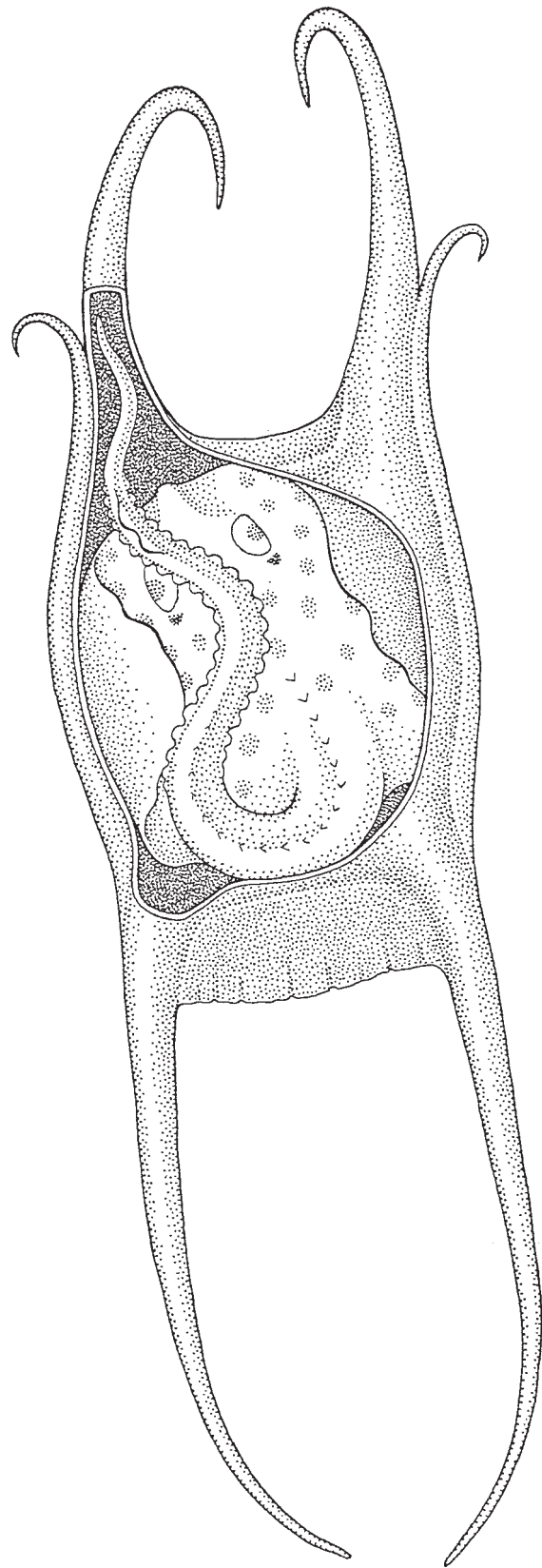


Fig. 1. Drawing of the dorsal view of an egg capsule of the little skate (*Raja erinacea*). The near-hatching embryo seems to fill the capsule and its wings are curled over its back. Part of the capsule has been cut away to reveal the embryo with its tail appendage inserted into one of the anterior horns.

1996. All the embryos were near hatching and had a 1–2 mm ball of yolk in an external sac. Fifteen embryonic skates were used for this study, ranging from 372 to 390 days old, and 5.15–6.93 g. Of these, ten were removed from their egg capsules 24–36 hr prior to experimentation. Five remained in their capsules. Encrusting bryozoans, algae and molluscs were removed from the capsules.

Equipment

Two identical box respirometers were built from 1-cm thick acrylic. Each was approximately 15 cm × 9 cm × 6 cm (inner dimension) with a total volume of 845 ml. Each respirometer was equipped with an acrylic lid that clamped in place over a rubber gasket to seal the box. Each lid had two 2-cm vents for oxygen probe insertion and removal of air bubbles. Oxygen was monitored by self-stirring oxygen probes and meters (YSI Inc. model 5905 and 5730 probes, and model 58 meters; Yellow Springs, OH). The probes were calibrated in water-saturated air prior to use. The oxygen meters sent calibrated voltage output signals to a LiCor datalogger (Lincoln, NE), which were downloaded to a personal computer. Measurements of dissolved oxygen within the box respirometers were recorded every minute. Data were recorded for at least 1.5 hr for each trial, and two trials were allowed to run for 12 hr. The probes were capable of detecting changes greater than 0.05–0.20 mg O₂ hr⁻¹ chamber⁻¹, depending upon the oxygen probe used.

The respirometer boxes were placed in a shallow sea water table with freely flowing sea water supplied to control temperature within the chambers. All experiments were therefore carried out at ambient ocean water temperature which ranged from 12.8 to 13.1°C during the course of the study.

For each run the respirometer chamber was filled with sea water and an embryo placed in the chamber. The embryos were always placed with their dorsal surface facing up. Since the egg capsule is translucent it was possible to make sure that even encapsulated embryos were right side up. The lid was then sealed, any air bubbles were removed through the lid vents, and the oxygen probe was inserted. For unencapsulated, unanesthetized animals, activity was recorded using a Hi-8 video camera to determine tailbeat frequency.

Metabolic rate

Standard metabolic rate (SMR) of the embryos was measured by anesthetizing them with tricaine methanesulfonate (MS-222) to stop voluntary

muscle activity. Benetti et al. ('95) used both MS-222 and neuromuscular blocking agents and demonstrated no effect of the anesthesia on metabolic rate, other than skeletal muscle immobilization. Five of the unencapsulated skates were anesthetized with 100 mg l⁻¹ MS-222 buffered to pH 7.0 with NaHCO₃ and NaOH prior to placement in the respirometers. This dosage was the minimum that would stop tail beating without affecting the rhythmic contraction of the gills. Sea water with MS-222 (100 mg l⁻¹) was also used in the respirometer for these fish. No tail movements were seen in these fish, though the gills continued to ventilate throughout the experiment. After the experiment each embryo was weighed. No attempt was made to remove the yolk sac from the animal. Animals in the different groups were randomly distributed between the two respirometers. As a control, the oxygen uptake of empty respirometer chambers (n = 2) was measured, as was the oxygen consumption by an empty egg capsule (n = 2).

Oxygen consumption was measured over five-minute periods starting when the chamber was closed. Post-hoc examination of the two 12-hr respirometry runs indicated that after 1 hr the stress response, often a sharp elevation in oxygen consumption caused by moving the embryo, had run its course. Though one hour may seem a short time for a stress response to disappear, it should be noted that in these experiments the embryo was hardly disturbed as it was moved from the sea-table into the chamber. The embryos had been equilibrated to the sea-table for weeks, and the unencapsulated embryos had more than 24 hr to adjust to the removal of the capsule. The subsequent analysis is of data collected for 30 min, starting 1 hr after the respirometer was closed. Data were analyzed using a two-way analysis of variance (ANOVA), with individual and treatment as factors, followed by Sheffé's post-hoc analysis.

RESULTS

All the fish in the three treatment groups (encapsulated, unencapsulated, unencapsulated and anesthetized) survived respirometry. The anesthetized embryos rapidly recovered from the MS-222 treatment when returned to clean sea water, as indicated by a resumption of tail beating activity. There was no significant difference in embryo mass between the groups, with the average mass being 5.9 g ± 0.6 S.E.

The tail beat intermittently in all of the embryos after removal from the capsule. The rate of tail beating averaged 47 ± 14 beats per minute

during the data-collection period (60–90 min) in unencapsulated, unanesthetized embryos. The amplitude and the number of undulatory waves on the tail were not quantifiable from the video, though both characteristics seemed to vary. Other than tail beating, there was minimal movement in the unencapsulated embryos, though occasionally the animal would curl its tail into a circle. This usually preceded a period with no tail beating activity. There was no tail beating in the anesthetized embryos.

Metabolic rates of the three experimental groups are shown in Figure 2. Initially the water was at 85–90% of oxygen saturation, and in no case did it drop by more than 16% (range 3–16%, median 10%) during the experiment. The respiration data were normal and homoscedastic. The mean mass specific metabolic rate of the anesthetized embryos, which we are calling the standard metabolic rate (SMR), was $0.032 \pm .004 \text{ ml O}_2 \text{ g}^{-1} \text{ hr}^{-1}$. Encapsulated, active embryos had an 81% higher rate ($0.058 \pm 0.009 \text{ ml O}_2 \text{ g}^{-1} \text{ hr}^{-1}$), while unencapsulated, active embryos were 53% higher, consuming $0.049 \pm .009 \text{ ml O}_2 \text{ g}^{-1} \text{ hr}^{-1}$. This represents a significant increase in oxygen consumption rate over the SMR, though the two unanesthetized groups were not significantly different.

There was no significant effect of individual on the oxygen consumption ($P = 0.88$) nor was there a significant interaction between individual and

treatment ($P = 0.10$). We detected no oxygen consumption by the empty egg case ($n = 2$) or the empty chambers. The metabolic rates of all three experimental groups are well above the detection limit of our probes.

DISCUSSION

The metabolic rate of *Raja erinacea* embryos is lower than that of the other chondrichthian embryo for which there is published data. Oxygen consumption of unencapsulated small-spotted catshark, *Scyliorhinus canicula*, embryos was approximately $260 \mu\text{l h}^{-1}$ for 3.2 g embryos (Diez and Davenport, '87). Oxygen consumption by unencapsulated skate embryos averaged $196 \mu\text{l h}^{-1}$ for 5.9 g embryos, or 24% lower for embryos nearly twice as large. Since the consumption of oxygen scales exponentially with body size (Withers, '92) we would expect a larger value for the skates. This difference seems too large to be due only to the higher temperature of their experiment (15°C vs. 13°C), it may reflect a fundamental difference between these species in metabolic rate during ontogeny.

The possibility that late-stage embryos of catsharks and skates have different metabolic rates is credible based on the different phylogenetic history, morphology, and behavior of near-hatching embryos. Oviparity in catsharks (*Scyliorhinidae*) was evolved independently from skates (Dulvy and Reynolds, '97). They are quite morphologically distinct as well. Catshark capsules do not have the long horns of *Raja* capsules, and shark embryos have no tail appendage. Shark capsules are typically attached to bottom structure above the substrate rather than being laid directly on the bottom. This would imply a very different hydrodynamic regime for the capsule. The behavioral contrast is also marked; skate embryos beat their tail vigorously until hatching (Luer and Gilbert, '85), while the shark embryos stop moving as they get near term (Diez and Davenport, '87).

Our results demonstrate the large cost (53–81% above SMR) of tail beating in *R. erinacea* embryos. These data, and the presence of a specialized embryonic organ, the tail appendage, suggest that embryo-generated currents within the egg capsule are important to the success of the embryo. Two possible functions have been proposed for this active pumping mechanism: (1) movement of oxygenated water into the capsule (Libby, '59; Luer and Gilbert, '85); and (2) removal of metabolic wastes (Luer and Gilbert, '85). Our metabolic rate data combined with the diffusion rate of oxygen

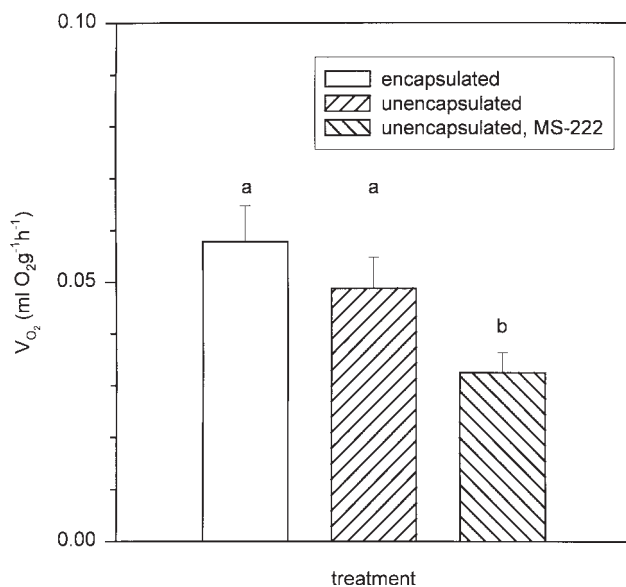


Fig. 2. Relationship between treatment and metabolic rate (VO_2) in embryonic little skate (*Raja erinacea*). $N = 5$ for each group. Bars with the same letter are not significantly different.

through the capsule indicate that the need for oxygen drives the behavior. The diffusion rate (J) per unit area for an arbitrary concentration gradient can be calculated from:

$$J = \frac{D\Delta C}{\Delta X}$$

where D is the diffusion coefficient, ΔC is the concentration gradient, and ΔX is the distance over which diffusion takes place. The diffusion coefficient for oxygen passing through capsule material is $2.95 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$ for shark (Diez and Davenport, '87), and $3.1 \times 10^{-8} \text{ cm}^2 \text{ s}^{-1}$ for skates (Gannon, '91). The large difference between these two values may reflect differences between the capsules or it may be due to differences in measuring techniques. We will use both values to establish a range of possible diffusion rates with the following calculations. For intact *Scyliorhinus* capsules the gradient was measured at 25–35 mmHg (Diez and Davenport, '87). Assuming a similar gradient is found in the skate egg capsule, the O_2 diffusing across 20 cm^2 of capsule wall is $0.1\text{--}7.9 \mu\text{l O}_2 \text{ hr}^{-1}$. The oxygen needed by an embryo not beating its tail is about $200 \mu\text{l O}_2 \text{ hr}^{-1}$, therefore simple diffusion can not provide enough oxygen for the embryo.

In addition to the active pumping mechanism, the skate egg capsule has a passive pumping mechanism. Water is induced to flow through the capsule by current passing around the capsule. The flow through empty capsules has been determined to be biologically significant at flow rates typical of the ocean floor (Koob and Summers, '96). The diameter of the slits in the horns is very small compared to the diameter of the body of the capsule; so even with an embryo occluding about half the cross sectional area, capsule flow through should not be decreased by the presence of the embryo. The development of an efficient passive pumping mechanism benefits the developing skate by reducing the need for active pumping. The relative contributions of these two methods to ventilation of the capsule is an area for future study.

The high metabolic cost of pumping water through the capsule means an egg capsule (through which it is marginally easier to pump water) is of energetic benefit to the embryo. Devoting less energy to tail beating means a larger hatching size, which has been correlated with higher survivorship in fishes (Hutchings, '91). We predict that the relative importance of active and passive

pumping would vary with bottom type and topography, oxygen content of the water, and current flow. Perhaps the trade-off between the two pumping mechanisms, active and passive, explains some of the variation in horn length, capsule shape, slit placement, and capsule size seen in skate egg capsules.

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