THE EGG CASE OF RAJA ERINACEA PLAYS ONLY A MINIMAL ROLE AS AN IONIC/OSMOTIC BARRIER

Gregg A. Kormanik
Department of Biology, UNCA, Asheville, NC 28814

The little skate, Raja erinacea, is fairly typical of the oviparous elasmobranchs (Wourms, Am. Zool. 21; 379-410, 1977). Embryos are encapsulated in egg cases, which act as mechanical protection during the time the egg incubates in sea water. The role of the egg case as an ionic or osmotic barrier is less clear. Smith (Biol. Rev. 11; 49-82, 1936) and Price and Daiber (Physiol. Zool. 40; 248-260, 1967) suggest that the egg case in oviparous species acts as an osmotic barrier, especially against urea loss, and protects the embryo until it may osmoregulate on its own. Evans (J. Exp. Biol. 92; 337-340, 1981) suggests that the egg case of Raja erinacea may osmoregulate, since it can maintain significant gradients between the embryo and the surrounding sea water. The ability of the embryo to iono- and osmoregulate may play an important role as a selective pressure in the evolution of maternal support. In order to further examine embryonic osmoregulation, the role of the egg case as an ionic and osmotic barrier was examined.

Egg cases of the skate (Raja erinacea) were collected to insure that the eggs were exposed to sea water for less than 24 hours after they were laid. The role of the egg case as an osmotic barrier was examined by placing egg cases in 80% sea water. The eggs were weighed at various intervals to determine the rate of water movement and the time necessary to reach osmotic equilibration (= constant weight). All values were determined as previously described (Kormanik & Evans, J. Exp. Biol. 125; 173-179, 1986). Pco_2 for both sea water and egg case fluid was calculated using constants for sea water (Boutilier et al., in Fish Physiology, XA, pp. 403-430, 1984).

Selected acid-base values for egg case fluids are presented in Table 1. The pH, Tco_2, Pco_2 and ammonia values are all somewhat lower than the values expected for normal elasmobranch blood (Kormanik & Evans, ibid.) and are intermediate between blood and sea water. Po_2 is lower than that of ambient sea water, but about the level expected for well-oxygenated blood.

After less than 24 hours exposure, the levels of Na^+, K^+ and osmotic pressure of the egg case fluid are not significantly different from ambient sea water (Table 2). Urea, while elevated over that of sea water, is far lower than blood levels (ca. 350 mM). Upon exposure to dilute (80%) sea

Table 1 Selected acid-base values for egg case fluid of Raja erinacea embryos determined less than 24 hours after laying (n = 8 to 9).

	pH	Tco_2 (mM)	Pco_2 (mm Hg)	Po_2 (mm Hg)	Ammonia (uM)
egg case fluid	7.44 $\pm$0.06	2.4 $\pm$0.5	1.3 $\pm$0.2	122 $\pm$ 1	107 $\pm$ 30
sea water	8.19 $\pm$0.02	2.04 $\pm$0.04	0.21 $\pm$0.01	155 $\pm$ 1	0

water, the egg cases came to equilibration after 96 hours and gained little weight (+3.15%), which indicated a substantial permeability to both water and osmolytes (see below). One of the seven eggs did not survive this transfer. After the 96 hours required for osmotic equilibration, all of the ion and osmolyte concentrations were reduced compared to the seawater controls (Table 2). Yet even after 96 hours, egg case fluid Na^+ and Cl^- concentrations were 4 to 6% lower than that of the ambient sea water, while K^+ was 13% higher. Egg case fluid was slightly but significantly hyperosmotic by 8 mOsm., or about 1% for these paired data.

Table 2 Ion and osmolyte values for egg case fluid of Raja erinacea embryos compared to sea water. Concentrations are expressed in mM, osmotic pressure (OP) is expressed in mOsm.

	Na^+	K^+	Cl^-	Urea	OP
-less than 24 hrs after laying (n = 3 to 11; unpaired data)					
egg case fluid	450^{ns} $\pm$ 11	10.8^{ns} $\pm$ 1.2	488** $\pm$ 8	8.7* $\pm$2.5	890^{ns} $\pm$ 5
sea water	460 $\pm$ 5	9.8 $\pm$ 0.1	536 $\pm$ 5	0	904 $\pm$ 5
-after 96 hrs exposure to 80% sea water (n = 6 to 7; paired data)					
egg case fluid	347** $\pm$ 2	8.8* $\pm$0.4	414** $\pm$ 4	5.5** $\pm$1.8	731** $\pm$ 1
sea water (80 %)	370	7.8	432	0	723

(ns -$p > 0.05$; * -$p < 0.05$; ** -$p < 0.01$)

The preliminary data presented above suggest that the ions, urea and water in the egg case fluids of R. erinacea rapidly approach the levels found in diluted ambient sea water. Upon exposure to dilute sea water, egg case fluids equilibrated after 4 days, and osmolyte differences were minimal. These data are in contrast to those of Evans (1981, ibid.) where gradients of 90 to 100 mM were established for Na^+ and Cl^-, respectively (egg case fluid < sea water), with 2 to 10 days exposure to full-strength sea water. However, the slightly longer exposure time to sea water may have been responsible. Nevertheless, the data presented here suggest that egg cases do not significantly regulate capsular fluid ion and osmolyte concentrations. Thus if the embryos maintain normal elasmobranch levels of osmolytes, then the embryonic epithelia must themselves osmoregulate at this earliest stage of embryonic development (supported by NSF DCB-850251).