TOTAL NITROGEN, TRIMETHYLAMINE N-OXIDE AND UREA IN DEVELOPING EMBRYOS OF THE LITTLE SKATE, RAJA ERINACEA

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We have been examining nitrogen budgets in oviparous and viviparous elasmobranchs to determine the extent of maternal contribution and the manner by which nitrogen and other constituents are transferred to the developing embryos (Kormanik, J. Exp. Biol. 144:583-587, 1989; Kormanik et al., Bull. MDIBL 31:44-46, 1992). We are technically unable to monitor changes in total nitrogen within individuals through a period of development that may last nearly two years. Also, comparison of total nitrogen values in eggs and embryos or hatchlings of different origins is hampered by the variability of embryo and egg size within species. To minimize this variability, we took advantage of the fact that an individual female skate lays eggs that are fairly uniform in size. We analyzed eggs for several constituents, raised siblings to the time of hatch and then analyzed them as well, to determine the maternal contribution of nitrogen as well as several other constituents. The values we measured in oviparous species, where maternal contribution stops at oviposition, will help us to assess maternal contribution in more advanced viviparous species.

Female skates (Raja erinacea) were collected from Frenchman Bay over several seasons by commercial fisherman, and kept in running sea water. Eggs, usually laid in pairs, were collected daily. Half of the eggs collected were analyzed immediately, the other half were incubated in sea water at varying temperatures (4 to 25° C.) until hatching, ca. 7 to 12 months. Tissues were prepared, total nitrogen and urea were determined as previously described (Kormanik et al., 1992). Phosphate was determined by phosphomolybdate assay (Sigma Chem. Co.) and trimethylamine n-oxide (TMAO) using the method of Wekell and Barnett (J. Food Sci. 56:1;132-138, 1991).

The results of the analyses are presented in Table 1. Values for egg cells (="yolk") are presented since the capsular fluid/jelly content of these constituents is minimal. Total phosphorus was chosen as a benchmark for a constituent that was not likely to be lost by the embryo due to the bound and highly charged forms in which it is found. Nor is it likely to be extracted from surrounding sea water by the embryo, although we cannot rule out that possibility. Very little phosphorus is lost by the embryo. TMAO, total nitrogen and phosphorus content per gram of tissue declined, while urea increased. Hatchling weight increased due to increased water content (not shown). Urea and TMAO represent 10 and 2% respectively, of total nitrogen in the egg cell, and increase to 25 and 3% of total nitrogen at the time of hatch. Of greater interest are the ranges of total contents seen in egg and hatchling. If we assume that the smallest eggs develop into the smallest and the largest eggs into the largest of hatchlings, hatchlings contained 83 to 100% of TMAO, 171 to 187% of the urea, 60 to 68% of the total nitrogen and 96 to 114% of the total phosphorus with which they were endowed by the mother. Clearly the only constituent to increase was urea.

These data are instructive on several points. First, TMAO declines slightly through development. Unlike Raja binoculata (Read, Biol. Bull. 135:537-547, 1968), R. erinacea seems unable to synthesize TMAO to an amount

Table 1. Constituents of egg cells and hatchlings in siblings of Raja erinacea. Data are expressed as mean ± S.E.M., and as the range of total content per animal, n = 6 - 10.

	Wet wt. (g)	TMAO (μmol g^{-1})	Urea (μmol g^{-1})	N_{tot} (mg g^{-1})	P_{tot} (mg g^{-1})
Egg cell	4.32±0.29	96.4±2.7	230±10	66.0±1.6	5.70±0.12
Hatchlings	6.54±0.25	64.0±2.0	282± 6	32.1±2.1	3.96±0.12
Total content	(g)	(mmol)	(mmol)	(mg)	(mg)
Egg cell	3.6-5.9	0.35-0.57	0.83-1.36	228-375	18.8-31.3
Hatchlings	5.5-7.8	0.35-0.47	1.55-2.33	136-256	21.4-30.0
%*	153 - 132	100 - 83	187 - 171	60 - 68	114 - 96

* -% is expressed as the ratio of hatchling to egg cell

above that endowed by the mother, losing a bit less (0 to 17%) through development than does Squalus acanthias (ca. 13-22%; Kormanik et al., 1992) which also cannot synthesize this osmolyte (Goldstein et al., Comp. Bioch. Physiol. 21:719-722, 1967). The ratio of urea to TMAO in the egg cell at oviposition (2.4:1) increases by the time of hatching to 4.4:1. Adult urea:TMAO for plasma (9.2:1), red blood cells (11.5:1) and muscle cells (6.22:1) are typically higher than seen in other elasmobranchs (Forster and Goldstein, Am. J. Physiol. 230:925-931, 1976). If neither R. erinacea nor S. acanthias is able to synthesize TMAO, then different levels in plasma and cells reflect acquisition by diet and rates of loss. Urea on the other hand obviously is synthesized, as the total content increases during development. Total nitrogen declines through development, with the embryo retaining less than 70% of the amount endowed in the egg. Scyliorhinus canicula, an oviparous species, retains a similar amount of nitrogen (69%) through development (Mellinger et al., in: Indo-Pacific Fish Biology: Proceedings of the Second International Conference on Indo-Pacific Fishes, Tokyo, Ichthyol. Soc. Japan, 1986, pp. 310-332). Thus R. erinacea, an oviparous species, loses more than twice the amount of nitrogen lost by the primitively viviparous S. acanthias (Kormanik, 1989; Kormanik et al., 1992). Embryos of S. acanthias are incubated in utero in a solution containing plasma-like levels of urea for the first few months, and high levels of ammonia during the latter months of gestation (Kormanik, 1989). These conditions may contribute to nitrogen retention by the embryo. (Supported by NSF DCB-8904429 to GAK)