TOTAL NITROGEN BUDGET IN DEVELOPING PUPS OF THE DOGFISH SQUALUS ACANTHIAS (L.).

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Embryos of the dogfish, Squalus acanthias (Linnaeus), complete their development in a uterine environment that can be considered quite unusual, where the ammonia concentration may approach 22 mM and the pH is about 6 (Kormanik and Evans, J. Exp. Biol. 125:173-179, 1986). While the acidic environment serves to protect the pups from this high ammonia concentration, it also serves to promote ammonia acccumulation, the source of which is the mother (Kormanik, Kremer and Patton, Bull. MDIBL 25:142-145, 1985). Why then are the pups exposed to such a high concentration of ammonia during their development? While each developing embryo has its own source of nutrients, the yolk sac, one possible explanation is that the external ammonia acts as an additional source of nitrogen for the developing pups. This aspect of pup nutrition was examined by measuring the total nitrogen in pups and yolks at several stages during the two year period of development. If uterine ammonia acts as an exogenous source of nitrogen, then total nitrogen in the pups and yolks should increase throughout the gestation period.

Embryos of the spiny dogfish (Squalus acanthias) were collected from sacrificed females taken from Frenchman Bay in different stages of the two year gestation period, at the Mount Desert Island Biological Laboratory, Salsbury Cove, Maine, during the summers of 1985 through 1987. Since it was impossible to determine the exact age of the embryos, they were arbitrarily placed in groupings of early-, mid-, and late-term depending on the embryo weight. Yolk sacs were ligated and separated from the anesthetized pups, each was weighed separately, frozen, and then returned to UNCA for analysis. Total nitrogen was determined after the methods of McKenzie and Wallace, Aust. J. Chem. 7:3-39, 1954, using a micro-Kjeldahl apparatus (Labconco). Statistical comparisons were made using Student's t-test, one-tailed, for unpaired data.

The weights of the pups and yolks at the three successive stages of development used are reported in Table 1. While the exact ages of these samples collected from early to late summer are unknown, the various ranges of weights given do not overlap, and these groupings therefore represent reasonable approximations of different embryo ages during the nearly two year period of development. The early-term embryos represent Stage 'A', while the mid- and late-term embryos probably represent Stage 'C', using the classification system of Hisaw and Albert, Biol. Bull. 92:187-199, 1947. Stage 'A' and 'C' represent the first and second summers of development, and therefore embryos approximately six and eighteen months of age, respectively. This grouping of animals by weight also helps to minimize the variability encountered in embryo size (Hisaw and Albert, op. cit.). As one might expect, the weight of the pup increases and the weight of the yolk sac decreases as nutrients are absorbed and used as the pup grows.

The total nitrogen recovered by digestion of the pup and yolk samples is also reported in Table 1. The total nitrogen content of the pups increased during maturation and that of the yolks decreased. Thus nitrogen moves from the yolk stores into the pups as development proceeds and the pups increase in size. More instructive are the data from the sum of nitrogen in the pups and yolks.

Table 1 Pup weight, yolk weight and total Kjeldahl nitrogen at three successive stages of development in embryos of the dogfish (Squalus acanthias). Data are presented as mean ± s.e., n = 4 to 5. Comparisons are made to the previous term.

		early-term	mid-term	late-term
weight (g)	pup	0.114 ± 0.011	23.38 ± 1.07	41.32 ± 5.86
	yolk	47.44 ± 3.21	24.27 ± 0.93	16.46 ± 1.13
Total N (mg)	pup	2.19 ± 0.036	588 ± 13**	1210 ± 140*
	yolk	2300 ± 130	1380 ± 30**	730 ± 73**
	pup + yolk	2300 ± 40	1970 ± 40**	1940 ± 130$^{n.s.}$

* – $p < 0.01$
** – $p < 0.001$
n.s. – $p > 0.1$

The sum of total nitrogen in the pup and yolk pairs certainly did not increase, and actually showed a decrease between the amounts found in the early and mid-term pups. These data indicate that the nitrogen found in the yolk stores is more than sufficient to account for the amounts found in the mid-term pups. However, the amount of total nitrogen found in the mid- and late-term pups was not significantly different. Thus there was no nitrogen lost from the pups during this latter portion of the developmental period. From a consideration of these data it is evident that the pups are probably not using the external ammonia as a significant nitrogen source during development. However, since Evans and Kormanik (J. Exp. Biol. 119:375-380, 1985) have demonstrated that the mid- and late-term pups excrete nitrogen in the form of urea and ammonia (unpublished observations) at least in normal fresh sea water, and since the total nitrogen in mid- and late-term pups is not significantly different, the external ammonia may represent at least a minimal source of nitrogen for the pups during development. A simple calculation will suffice. Stage 'C' pups excrete urea at a resting rate of 20 umol $100g^{-1}$ h^{-1} (Evans and Kormanik, op. cit.) which represents a nitrogen excretion rate of 0.56mg N $100g^{-1}$ h^{-1}, or about 0.4g N $100g^{-1}$ $month^{-1}$ in the latter months of gestation. Since the pups + yolks at this stage contain only 3.3g N $100g^{-1}$ (from Table 1) urea excretion alone would represent a substantial loss of nitrogen. Thus uterine ammonia, while not augmenting total pup nitrogen, might serve as a source to replace that nitrogen which is lost through excretion. In conclusion, the role of the external ammonia remains unclear. It certainly does not augment total nitrogen for the developing pups, as the nitrogen that is supplied by the yolk stores appears to be more than sufficient to account for that found in the developing pups. On the other hand, it may serve to replace nitrogen lost during the course of development. (Supported by NSF DCB-8502251 to GAK; UNCA UGR grant to WAK).