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THE ACID–BASE STATUS OF PRENATAL PUPS OF THE DOGFISH, *SQUALUS ACANTHIAS*, IN THE UTERINE ENVIRONMENT

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SUMMARY

The acid–base status of late-term *Squalus acanthias* L. pups in the uterine seawater environment was examined. Blood values for pH, total CO_2, partial pressure of CO_2, urea and sodium concentrations in late-term pups were not significantly different from those of the mothers. Haematocrit was slightly lower, while total plasma lipid and ammonia concentrations were several times higher.

The uterine environment in which these pups reside and maintain normal acid–base status is nevertheless quite remarkable. In the later months of gestation, up to six pups (approx. 60 g each) reside in each horn of the uterus, in about 100 ml of seawater, in which they ventilate. While the major ion concentrations of the uterine fluid resemble normal seawater, the pH may be as low as 5·9, and the ammonia concentration as high as 22 mmol l^{-1}.

This system provides a unique opportunity to study acid–base balance, respiration and nitrogenous waste excretion in developing elasmobranchs under quite unusual conditions.

INTRODUCTION

The spiny dogfish, *Squalus acanthias*, possesses the unspecialized form of viviparity termed lecithotrophy (Wourms, 1981). Groups of fertilized eggs remain encapsulated for about 4–6 months *in utero*. These 'candles' then burst and the pups, which possess large yolk sacs, continue the period of gestation, which lasts nearly 2 years (Nammack, Musick & Colvocoresses, 1985; Woodhead, 1976). In the last months of gestation, pups still possessing yolk sacs can be removed from the uterus and will survive indefinitely in seawater (Evans, Oikari, Kormanik & Mansberger, 1982; D. H. Evans, unpublished data).

In the non-gravid female, the uterine lining is thin, but in the gravid female the uterine lining becomes highly vascularized (Jollie & Jollie, 1967) and the uterine artery increases in size to allow increased blood flow (Fuller, Griendling & Kent,

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1983). This high degree of vascularization and extensive blood flow has led some authors (Burger, 1967; Jollie & Jollie, 1967; see also Wourms, 1981) to suggest that the uterus (and therefore the mother) might play some role in supplying O_2 and removing waste products such as CO_2, and thus aid the pups in respiration. Late gestation pups, 2–15 per female (Nammack *et al.* 1985) and typically six per uterine horn, can be observed to ventilate *in utero* (G. A. Kormanik, personal observations). Burger (1967) has reported that the mother periodically flushes the uterus with seawater, however, which may also play a role in respiration and the removal of waste products. Thus the relative importance of the maternal uterine circulation *versus* flushing the uterine horns with seawater in maintaining the pups' environment is not clear.

The object of this study was to examine the role of maternal care for the pups by comparing the blood of the mother and pups. We also compared the uterine seawater environment and Frenchman's Bay seawater with respect to various parameters associated with respiration, acid–base balance and nitrogen excretion.

MATERIALS AND METHODS

Pregnant female *S. acanthias* were caught by gill nets from Frenchman's Bay, ME, and held in floating live cars for several days prior to use. Blood of females was sampled by puncture of the caudal dorsal aorta, within less than 30 s of removal from the seawater. The female was immediately killed and pups were removed from the uterine horns and placed briefly in fresh seawater while they awaited sampling. Pup blood was collected by caudal puncture, and all pups were bled within a few minutes of their removal from the uterus. Uterine seawater samples were collected through the surgically exposed uterine wall with syringe and needle, or by a blunt rubber catheter inserted into the uterine horns through the cloaca. All samples were placed on ice, and the blood was assayed immediately for haematocrit and pH. Seawater pH was determined as well. Blood was then centrifuged, and the plasma and seawater samples were assayed for total CO_2 content (T_{CO_2}). Both plasma and seawater samples were stored frozen for later assays.

Blood and seawater pH were determined with a Model 213 or a Micro 13 Blood/gas Analyzer (Instrumentation Laboratories) thermostatted to 15°C. T_{CO_2} was determined with a Capnicon Total CO_2 Analyzer (Cameron Instrument Co.). Ammonia in seawater was determined by the phenol–hypochlorite method (Solorzano, 1969) and in blood plasma, by an enzymatic method with Sigma Kit no. 170-UV (Sigma Chemical Co.). Ammonia was assayed within 24 h. Urea in plasma and seawater was analysed with the monoxime assay (Sigma Kit no. 535). Total lipids were assayed using the vanillin/phosphoric acid method (Tietz, 1976) with olive oil (Pompeian Co.) as a mixed lipid standard. Comparisons of the data were made on unpaired samples, using Student's t-test. All values are reported as mean ± standard error.

Table 1. *Selected blood acid–base values of* Squalus acanthias *pups and pregnant mothers*

	pH	T_{CO_2} (mmol l^{-1})	P_{CO_2} (mmHg)
Mothers ($N = 3$)	7·83 ± 0·02	8·55 ± 0·39	2·79 ± 0·19
Pups ($N = 7$)	7·91 ± 0·08	9·56 ± 1·48	2·34 ± 0·14
Significance	$P > 0·1$	$P > 0·1$	$0·1 > P > 0·05$

RESULTS

In the first series of experiments, we compared the blood of the mothers with that of the pups. We have also presented values for the partial pressure of CO_2 (P_{CO_2}), calculated from the pH and T_{CO_2} using the Henderson–Hasselbalch equation (Table 1). Values for 'apparent' pK and CO_2 solubility were calculated for elasmobranch blood from the data of Albers & Pleschka (1967), Albers (1970) and Pleschka & Wittenbrock (1971), using the empirically established polynomials of Boutilier, Heming & Iwama (1984) (Table 1). The temperature of the seawater measured during the experiments and used in the calculations was 15 ± 1°C. Blood values for pH, P_{CO_2} and T_{CO_2} are similar to those reported in the literature for rested sharks at 15°C (see Heisler, Neumann & Holeton, 1980). Blood pH and T_{CO_2} were not significantly different in the mothers and pups; P_{CO_2} in the pups was slightly lower, however, but the difference was not quite significant. Plasma values for urea, Na^+, ammonia, lipids and haematocrit are reported in Table 2. Plasma urea and Na^+ concentrations were not significantly different, corroborating the data of Evans *et al.* (1982). However, comparison of other values showed that the haematocrit of the pups was significantly lower whereas ammonia and total lipids were significantly higher than those of the mothers. The high values for total plasma lipids we observed in the pups are probably due to the lecithotrophic nutrition of these embryos.

We also examined the uterine environment, and compared values for the uterine seawater and the seawater from Frenchman's Bay (Table 3). The Na^+ concentration of the seawater from both sources is nearly the same, corroborating the data presented by Evans *et al.* (1982), which demonstrated that uterine seawater and ambient seawater are relatively similar, at least with respect to the concentration of

Table 2. *Plasma values for* Squalus acanthias *pups and pregnant mothers*

	Urea (mmol l^{-1})	Ammonia (μmol l^{-1})	Na^+ (mmol l^{-1})	Haematocrit (%)	Lipids (mg ml^{-1})
Mothers (N)	336 ± 8 (6)	334 ± 125 (3)	251 ± 4 (4)	20·9 ± 2·9 (3)	5·88 ± 0·52 (4)
Pups (N)	342 ± 5 (20)	1160 ± 95 (18)	250 ± 5 (14)	15·9 ± 0·9 (10)	18·2 ± 0·8 (6)
Significance	$P > 0·1$	$P < 0·005$	$P > 0·1$	$P < 0·05$	$P < 0·005$

Table 3. *A comparison of Frenchman's Bay seawater to uterine seawater removed from pregnant, late-term* Squalus acanthias *(where N = number of uteri sampled)*

	pH	T_{CO_2} (mmol l^{-1})	Urea (mmol l^{-1})	Ammonia (mmol l^{-1})	Na^+ (mmol l^{-1})	Volume (ml)	P_{CO_2} (mmHg)
Bay seawater	8·19 ± 0·02	2·04 ± 0·04	0	0	405 ± 0	—	0·21 ± 0·01
(*N*)	(9)	(9)	(2)	(4)	(2)	—	(9)
Uterine seawater	5·89 ± 0·08	0·20 ± 0·02	0·50 ± 0·32	9·74 ± 2·17	418 ± 4	103 ± 16	2·30 ± 0·35
(*N*)	(9)	(5)	(6)	(9)	(5)	(5)	(4)
Significance	$P<0·001$	$P<0·001$	$P>0·1$	$P<0·001$	$P>0·05$	—	$P<0·005$

the major ions. Nevertheless, the uterine seawater pH is over 2 pH units more acid than ambient Bay seawater and the T_{CO_2} is far lower than that of normal seawater. We have also presented values for seawater P_{CO_2} at 15°C, in Table 3 [P_{CO_2} of seawater was calculated after Boutilier *et al.* (1984), using the appropriate ionic strength for seawater]. The P_{CO_2} of the uterine seawater is not significantly different ($P>0·1$) from that of the blood of either the mother or the pups (Table 1). Nor, in these preliminary observations, is the P_{CO_2} of the pup blood significantly different ($P>0·1$) from the uterine seawater. While one might expect a P_{CO_2} gradient of a few mmHg from pup blood to uterine seawater, this slightly lowered pup blood P_{CO_2} is not unexpected. Since the pups were in fresh seawater with a low P_{CO_2} for a few minutes while they awaited sampling, they may have shown a slight respiratory alkalosis. Pup blood pH was slightly higher than that of the mother, but not significantly so. Nevertheless, the P_{CO_2} of the uterine seawater is substantially elevated above that found in Bay seawater and would appear to be in equilibrium with the P_{CO_2} of the blood of both the mother and the pups.

Uterine seawater has a very low and highly variable concentration of urea compared to that of the plasma. Nearly all of the values for urea in uterine seawater were higher than those for Bay seawater, whereas in all samples no urea was detectable (Table 3). Our observations of small quantities of urea in uterine seawater corroborate observations made earlier by Price & Daiber (1967) on *S. acanthias*. However, concentrations of urea found in our study of uterine seawater are less than 10% of those found by Evans *et al.* (1982).

Of great interest were the values we determined for the concentration of ammonia in uterine seawater, which ranged up to 22 mmol l^{-1}, far higher than Bay seawater, which was less than 1 μmol l^{-1}, typical of environmental waters. All our determinations of uterine seawater indicate that the pups were living in a very high concentration of ammonia and acidity *in utero*, with up to six individuals per uterine horn, and ventilating with their gills in about 100 ml of seawater (Table 3).

DISCUSSION

These pups appear to be in an environment which would normally be considered toxic to water-breathing organisms, yet they certainly thrive.

These data suggest two obvious questions: how do such high concentrations of ammonia and acid arise, and why would such conditions be permitted to occur? The high uterine ammonia concentration could arise for several reasons. The build-up of such a high concentration of ammonia and acid might indicate that uterine flushing with seawater, previously reported in the literature (Burger, 1967) is infrequent. The source of the ammonia and acid could be either the pups or the mother. To examine the first possibility, one can calculate how long it might take the pups to elevate the uterine seawater ammonia concentration to this level (Table 3). The average rate of ammonia excretion we measured in *S. acanthias* pups is about $0{\cdot}5\ \mu mol\ 100\,g^{-1}\,h^{-1}$ (Kormanik & Evans, 1984). With an average of six pups per horn of the uterus, and about 100 ml of seawater per horn (Table 3), it would take about 23 days to reach this ammonia concentration. Is the seawater held in the uterus for this period of time? If the seawater is not flushed, one can also calculate the urea concentration that would build up after 23 days, given the same conditions and an average resting urea excretion rate of $20\ \mu mol\ 100\,g^{-1}\,h^{-1}$ (Evans & Kormanik, 1985). After 23 days, the urea concentration in the uterine seawater would be about $400\ mmol\,l^{-1}$, and it certainly is not. Therefore, either the mother is removing urea to prevent its build-up in the uterine seawater, while not removing ammonia over the long period of time that the water is held *in utero*, or the water may be held for a relatively shorter period of time, and the mother might elevate the ammonia concentration by contributing to it, that is by the movement of ammonia from maternal blood into the uterus. To examine this latter possibility we have determined the ammonia gradients involved for both the ionic form, NH_4^+, and the gaseous form, NH_3, since ammonia can move across the tissues in either form (see Kormanik & Cameron, 1981). Values for pK′ and solubility for seawater and blood were obtained from the data of Cameron & Heisler (1983), and NH_3 and NH_4^+ were calculated from the Henderson–Hasselbalch equation (see Kormanik & Cameron, 1981). The gradients for pup blood, uterine seawater, mother's blood and Bay seawater (raw data from Tables 1, 2 and 3) are presented in Table 4. From these data it is apparent that the P_{NH_3} gradient is directed towards the uterine seawater from the blood of both the mother and the pups, while the gradient for NH_4^+ is directed away from the uterine seawater towards the blood of the mother and pups. The sum of the net gradients for both NH_3 and NH_4^+ is directed towards Bay seawater for both the mother and the

Table 4. *Summary of the gradients for NH_3 and NH_4^+ developed in the various seawater and blood compartments of* Squalus acanthias *pups* in utero *and mothers*

	Pup's blood	Uterine seawater	Mother's blood	Bay seawater
P_{NH_3} (nmHg)*	333 ± 28	30·7 ± 6·8	87·8 ± 31·9	0·41 ± 0·16
NH_4^+ ($\mu mol\,l^{-1}$)	1140 ± 93	9740 ± 2170	330 ± 124	0·67 ± 0·28
(*N*)	(18)	(9)	(3)	(4)

* = μmmHg or μTorr.

pups. The opposing gradients for NH_3 and NH_4^+ in the uterine seawater, along with the accumulation of ammonia in the form of the ion, NH_4^+, would support the suggestion that ammonia tends to move predominantly down the partial pressure gradient of P_{NH_3} (Kormanik & Cameron, 1981), and that the permeability of both the pup gill and maternal uterine tissue to NH_4^+ is quite low.

The low pH of the uterine seawater would serve to 'trap' ammonia in the form of the less diffusible NH_4^+, and therefore promote its accumulation in the uterus. NH_3 is also the far more toxic form of ammonia (see review by Colt & Armstrong, 1981). The acidity of the uterine seawater would also tend to protect the pups from the toxicity of NH_3, by lowering the partial pressure. Thus the mother appears to contribute to the build-up of uterine seawater ammonia by providing a favourable pH gradient for the conversion of NH_3 to NH_4^+. Recent data indicate that, even in the absence of pups, the uterine seawater pH and total CO_2 decrease in about 20 h to the levels we observed, while the ammonia concentration increases, albeit more slowly (Kormanik, Kremer & Patton, 1985*a*).

But why should a toxic waste product like ammonia accumulate in the uterine seawater in the first place? It would appear to be a simple matter to flush the uterus with seawater and eliminate the build-up of ammonia (as well as acid), but this apparently does not occur. Since both ammonia and acid, but not urea, build up it would appear to be a selective process. The only reason we might suggest, given these preliminary data, is that ammonia is present to act as a nitrogen source for the developing pups, and the concomitant build-up of acidity would serve not only to trap ammonia in the uterine seawater, but also to detoxify it. Our most recent evidence indicates that this low uterine seawater pH in the presence of a high total ammonia concentration prevents the build-up of ammonia and concomitant toxic effects in the blood of the pups (Kormanik, Kremer & Patton, 1985*b*). While elasmobranch eggs contain a significant amount of urea (5·9 %) and protein [approx. 25–28 %, depending on the species; see Wourms (1977) for a review] the use of an ordinarily toxic nitrogenous waste product as an additional source of nitrogen for a developing vertebrate embryo would be of considerable interest. This aspect is currently under investigation in our laboratory.

This system thus provides a unique opportunity to study acid–base balance, respiration and nitrogenous waste excretion in fishes in an unusual developmental environment, as well as to gain some insight into the developmental physiology of elasmobranchs.

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