

**ONTOGENY OF NITROGEN METABOLISM  
AND EXCRETION IN TELEOST FISHES**

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**EXTENDED ABSTRACT ONLY - DO NOT CITE**

Nitrogen metabolism and excretion during fish development is a critical aspect of early physiology because the major fuel source in most species is obtained endogenously through absorption of yolk proteins. Catabolism of proteins and amino acids results in the formation of ammonia, a potentially toxic nitrogenous end-product that must be either eliminated or modified to prevent damage to the developing embryo. Alternatively, if ammonia is sequestered or retained, this may occur in a compartment separate from the developing organs (eg., yolk sac). Nitrogen excretion during early life stages is not static but is influenced by developmental stage, environmental conditions, and in some cases, maternal factors.

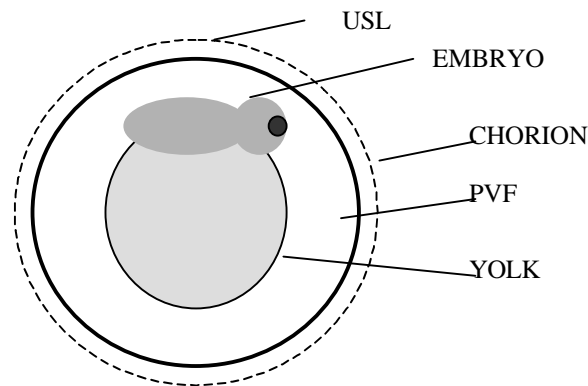
The physical properties of the embryo influence the rate of nitrogen elimination to the external environment. A typical teleost embryo is shown below, with the unstirred water boundary layer (USL), the developing embryo with the attached yolk sac, surrounded by the perivitelline fluid (PVF) and chorion or egg capsule. The development of gas and ion exchange surfaces are of primary importance to the excretion of nitrogenous wastes. Nitrogen excretion in embryos occurs in the absence of functional gill filaments, and even after hatch, cutaneous gas exchange may initially dominate.

In rainbow trout embryos, ammonia excretion is dependent on the  $\text{NH}_3$  partial pressure gradient ( $P_{\text{NH}_3}$ ) from the embryo to the water. (Rahaman-Noronha et al., 1996). There is an acidic USL that facilitates ammonia excretion by maintaining the  $P_{\text{NH}_3}$  gradient through conversion of  $\text{NH}_3$  to  $\text{NH}_4^+$  upon entry into the USL, as

in adult trout. Urea excretion constitutes a significant portion of total nitrogen excretion in some embryos. Diffusion of urea from the trout embryo was dependent, in part, on a bi-directional facilitated urea transporter with a  $K_m$  of 2 mM (Pillely et al., 2000). Urea analogs (i.e., thiourea, acetamide) and inhibitors (i.e., phloretin, NPTU) added to the external water reversibly inhibited urea excretion from the embryo. The tissue localisation (eg., yolk sac membrane, cutaneous surface) of this putative urea transporter is unknown.

Ammonia is excreted soon after fertilization, but it also steadily accumulates, peaking before or just after hatching (e.g. Wright et al., 1995; Chadwick and Wright, 1999). Is ammonia a trigger for hatching? Several environmental factors have been implicated in the hatching process, including oxygen availability, alkaline water and temperature. We tested the hypothesis that ammonia is a trigger for hatching in rainbow trout embryos. Eyed-up embryos (28 days post fertilization (dpf), 10° C) were exposed to external ammonia for 2 h (10 mM  $\text{NH}_4\text{Cl}$ ) or 4 days (0.2 mM  $\text{NH}_4\text{Cl}$ ), treatments that elevated internal ammonia concentrations. Elevated external ammonia, however, did not significantly effect the time to hatch, possibly because of efficient mechanisms that maintained low ammonia levels (see below).

In further studies we examined potential pathways for ammonia detoxification. It appears that urea is synthesized early in embryogenesis, because urea accumulates and/or it is excreted to the water. Urea cycle enzymes (carbamoyl phosphate synthetase (CPSase III), ornithine carbamoyl transferase (OCTase), arginase, and glutamine synthetase (GSase)) are induced in whole embryos of the freshwater rainbow trout (Wright et al., 1995; Korte et al., 1997), marine Atlantic cod (Chadwick and Wright, 1999) and Atlantic halibut (Terjesen et al., 1999). Recent



work on a variety of teleost species has demonstrated that CPSase III and other urea cycle enzymes may be located primarily in extra-hepatic tissues of adult fish (Felskie et al., 1997; Kong et al., 1998). Hence, we investigated the tissue location of urea cycle enzymes in rainbow trout embryos. Just-hatched embryos (38 dpf) were separated into three fractions using a dissecting microscope; the yolk, the liver, and the embryonic body. There was no CPSase III activity in yolk or liver tissue, but significant levels of activity were detected in the embryonic body. GSase, OTCase and arginase activities were present in yolk, liver and the embryonic body, but the highest activities were extra-hepatic (embryonic body).

In addition to urea synthesis, another possible pathway for ammonia detoxification is via the formation of nonessential amino acids, particularly glutamine (Gln) and glutamate (Glu). To examine the mechanisms of ammonia detoxification, trout embryos were exposed to environmental conditions that impaired ammonia elimination, that is alkaline water (pH 9.5; 12 C, hatched embryos, 31 dpf) or elevated ammonia (0.2 or 10 mM NH<sub>4</sub>Cl, 10 C) for 2 h or 4 days (embryos, 28 dpf). These environmental perturbations resulted in decreased rates of ammonia excretion and increased rates of urea excretion. There was no significant differences in either whole embryo Gln or Glu concentrations in ammonia-exposed relative to control fish. Embryonic tissue was separated from yolk using a new centrifugation technique recently developed in our group (A. Shahsahavarani, J. Ballantyne, and P. Wright, in preparation). By separating embryo from yolk, we can more carefully monitor changes in metabolite levels in the tissues which may be quite different from changes that occur in the yolk. Following acute or chronic exposure to NH<sub>4</sub>Cl, yolk ammonia levels increased by 2- to 4-fold but surprisingly, tissue levels were unchanged. At the same time tissue urea levels were significantly higher suggesting that excess ammonia was converted to urea as a detoxification mechanism. Urea cycle enzyme activities were not significantly different between the control and treated embryos. Thus, the level of activity of the urea cycle enzymes may have been sufficient to meet the increased flux through the pathway, or possibly urea was produced via another pathway, such as uricolysis.

Taken together, the data indicate that trout embryos are very capable of handling excess ammonia. Despite exposure to external ammonia for up to 4 days, there were no changes in the time to hatch compared to control embryos. Embryos maintain low tissue ammonia levels despite an inwardly directed (water-to-embryo) ammonia gradient. This may be achieved, in part, by sequestration of ammonia in the acidic yolk and detoxification by conversion to urea. Sensitivity to elevated environmental ammonia in rainbow trout is reported to be lower in embryos

relative to later stages of development. The results reported here indicate that trout embryos have efficient mechanisms to cope with excess ammonia and this may help to explain their relatively high tolerance of external ammonia.

## References

- Chadwick, T. D. and P.A. Wright. 1999. Nitrogen excretion and expression of urea cycle enzymes in the Atlantic cod (*Gadus morhua* L.): A comparison of early life stages with adults. *J. Exp. Biol.* 202: 2653-2662.
- Felskie, A.K., Anderson, P.M. and P.A. Wright. 1998. Expression and activity of carbamoyl phosphate synthetase III and ornithine urea cycle enzymes in various tissues of four fish species. *Comp. Biochem. Physiol.* 119B: 355-364.
- Kong, H. Edberg, D.D., Korte, J.J., Salo, W.L., Wright, P.A., and P.M. Anderson. 1998. Nitrogen excretion and expression of carbamoyl-phosphate synthetase III activity and mRNA in extra-hepatic tissues of largemouthbass (*Micropterus salmoides*). *Arch. Biochem. Biophys.* 350: 157-168.
- Korte, J.J., Salo, W.L., Cabrera, V.M., Wright, P.A., Felskie, A. and P.M. Anderson. 1997. Expression of carbamoyl-phosphate synthetase III mRNA during the early stages of development and in muscle of adult rainbow trout (*Oncorhynchus mykiss*). *J. Biol. Chem.* 272: 6270-6277.
- Pilley, C.M. and Wright, P.A. (2000). The mechanisms of urea transport in early life stages of rainbow trout (*Oncorhynchus mykiss*). *J. exp. Biol.* (in press).
- Rahaman-Noronha, E., O'Donnell, M.J., Pilley, C.M. and Wright, P.A.. (1996). Excretion and distribution of ammonia and the influence of boundary layer acidification in embryonic rainbow trout (*Oncorhynchus mykiss*). *J. exp. Biol.* 199:2713-2723.
- Terjesen, B., Rønnestad, I, Norberg. B., and Anderson, P.M. 1999. Detection and basic properties of carbamoyl phosphate synthetase III during teleost ontogeny: a case study in the Atlantic halibut (*Hippoglossus*

*hippoglossus* L.). Comp. Biochem. Physiol. [B] (in press).

Wright, P.A., Felskie, A. and Anderson, P.M.. 1995. Induction of ornithine-urea cycle enzymes and nitrogen metabolism and excretion in rainbow trout (*Oncorhynchus mykiss*) during early life stages. J. Exp. Biol. 198: 127-135.

