

URICOLYSIS AND THE ORNITHINE-UREA CYCLE
DURING ONTOGENY
OF THE AFRICAN CATFISH, *CLARIAS GARIEPINUS*

B.F. Terjesen,
Dept. of Zoology, University of Bergen, Allègt. 41, N-5007, Bergen, Norway.
Tel: +47 55 58 35 91; Fax: +47 55 58 96 73;
email: bendik.terjesen@zoo.uib.no

Chadwick, T.², Verreth, J.³, Rønnestad, I.¹, Wright, P.A.².
1). Dept. of Zoology, UoB; 2). Dept. of Zoology, University of Guelph, Guelph,
Canada; 3). Dept. of Fish Culture and Fisheries, Wageningen Agricultural
University, Wageningen, The Netherlands.

EXTENDED ABSTRACT ONLY – DO NOT CITE

Due to the high degree of utilisation of amino acids for energy and assumed absence of specialised excretory organs for voiding ammonia, urea may be an alternative vehicle for nitrogen excretion or storage during early teleost development. In investigations of the facultative air-breathing African catfish *Clarias gariepinus* (Burchell 1822), we found that around metamorphosis as much as 44% of the total nitrogen excretion was due to urea-N (Terjesen *et al.*, 1997). This species is tolerant to variations in water availability, temperature and salinity. We consequently studied the pathways for urea production in this species. Although the ornithine-urea cycle (OUC) in embryonic teleosts has received increasing attention the last few years (Wright *et al.*, 1995; Chadwick and Wright, 1999; Terjesen *et al.*, 2000), little is known about uricolysis.

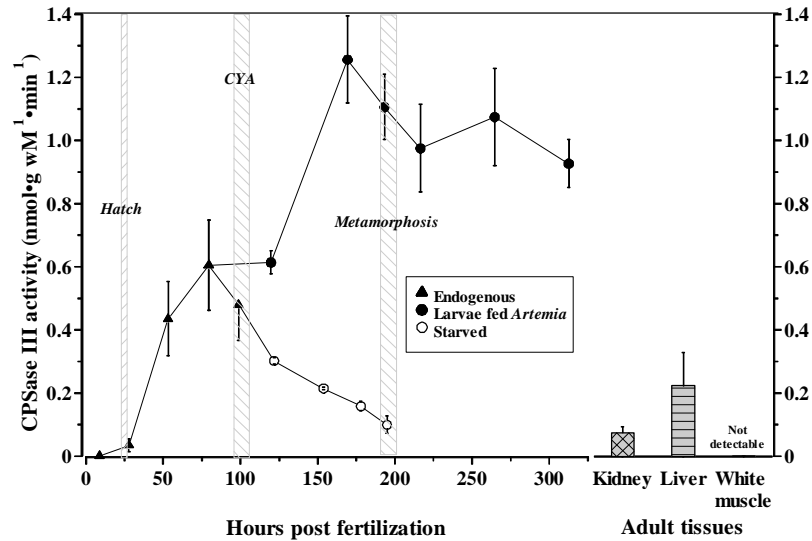


Figure 1. Carbamoyl phosphate synthetase III (CPSase III) activity during ontogeny of *Clarias gariepinus*. Each datapoint refers to the average (\pm SD) of three samples, except at 79 and 120

Rearing and sampling procedures were conducted after Terjesen *et al.* (1997). The OUC was analysed after Chadwick and Wright (1999) and Terjesen *et al.* (2000). Uricase was measured with a continuous assay at 293 nm. Allantoinase activity was estimated by an end-point assay employing differential analysis of glyoxylate derivatives. Allantoicase and ureidoglycollate lyase (UGL) activity were estimated with continuous assays employing LDH and NADH and followed at 340 nm.

The key regulatory OUC enzyme carbamoyl phosphate synthetase (CPSase) III was detected throughout development of *C. gariepinus* (Fig. 1). Arginase, ornithine carbamoyl transferase, and glutamine synthetase were also detected (data not shown). Argininosuccinate synthetase and lyase were not measured but are generally found in teleost tissues. The OUC appears therefore to be expressed in embryos and larvae of *C. gariepinus*. Adult *C. gariepinus* expressed only low levels of CPSase III, which was surprising since *C.*

batrachus express significant levels of CPSase III, even when not challenged by water deprivation or ammonia exposure (Saha and Ratha, 1989). It is possible that in adult *C. gariepinus*, the OUC is only expressed during harsh environmental conditions.

Unlike the CPSase III of larval Atlantic halibut (Terjesen *et al.*, 2000), *C. gariepinus* CPSase III showed virtually no activity without its positive effector *N*-acetyl-L-glutamate (AGA) present in the reaction mix, even at high glutamine concentrations. We suggest that this enable the OUC to be more efficiently controlled by AGA in face of varying glutamine concentrations, in contrast to the Atlantic halibut. Experiments were also conducted where *C. gariepinus* larvae were dissected for liver and tail (*i.e.* muscle) pieces. These samples were subjected to gel filtration chromatography. The muscle elution showed two peaks with CPSase activity, characterised to be CPSase II and III, while the liver elution was devoid of CPSase activity. This suggest that CPSase III is only expressed in muscle in larval *C. gariepinus*. By extending the suggestion by Lindley *et al.* (1999), it may be that the liver is too small for accommodating a substantial increase in expression during periods of adverse environmental conditions.

C. gariepinus larvae expressed high levels of CPSase III compared to other studied larval teleosts, but magnitudes lower than in elasmobranchs and batrachoid fishes. Furthermore, by comparing total urea production rates of *C. gariepinus* larvae with the CPSase III activity, it was found that the OUC at no stage in development could account for more than 20% of the produced urea. Hydrolysis of arginine by arginase can account for an additional 65%. Consequently, additional urea producing system(s) must be present. All four uricolytic enzymes were detected in embryos and larvae as well as in adult liver (Fig. 2: uricase). Uricase appears to be the rate limiting step of uricolysis. Starved larvae showed the highest activity, possibly because of increased cell degradation liberating nucleotides for breakdown. UGL showed considerable activity, suggesting that urea production is not limited to the allantoicase reaction but is a two-enzyme process in which UGL splits of a second urea molecule. Uricolysis could account for all urea produced. However, the present study reports the maximum *in vitro* capacity for urea production through the pathways, and clearly, *in vivo* experiments employing radiotracers should be conducted to confirm these findings.

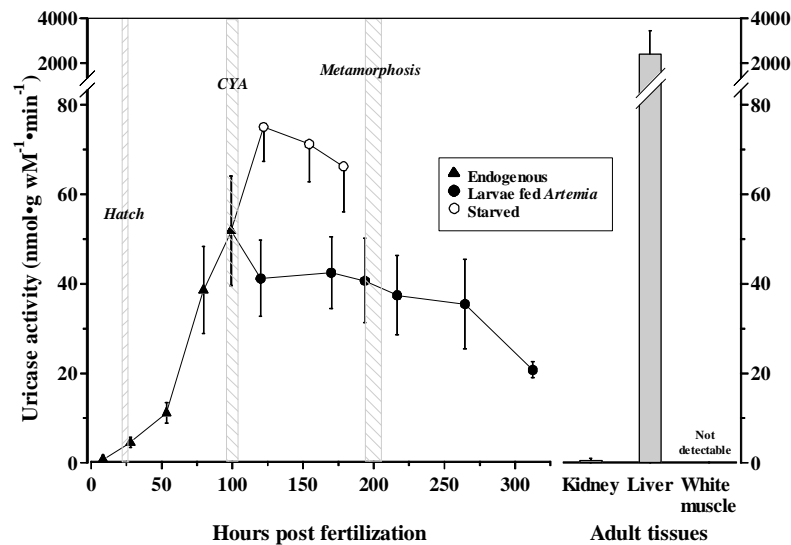


Figure 2. Uricase activity during ontogeny of *Clarias gariepinus*. Each datapoint refers to the average (\pm SD) of 3-6 samples. wM= wet mass; CYA = complete yolk

In conclusion, the observation that the maximum *in vitro* capacity of CPSase III is below that of urea production, suggests that other pathways predominate during early development. Possibly, the OUC is expressed at higher levels, and is quantitatively more important during periods of adverse environmental conditions.

Acknowledgements

This study was supported in part by the Grant for Biological Research provided by the University of Bergen. The first author wish to thank Dr. Armando Garcia-Ortega and the staff at the Dept. of Fish Culture and Fisheries, Wageningen Agricultural Univeristy for assistance with catfish reproduction and rearing.

References

- Chadwick T.D., Wright P.A. 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.
- Lindley T.E., Scheiderer C.L., Walsh P.J., Wood C.M., Bergman H.L., Bergman A.L., Laurent P., Wilson P., Anderson P.M. 1999. Muscle as a primary site of urea cycle enzyme activity in an alkaline lake-adapted tilapia, *Oreochromis alcalicus grahami*. *J. Biol. Chem.* 274:29858-29861.
- Saha N., Ratha B.K. 1989. Comparative study of ureogenesis in freshwater, air-breathing teleosts. *J. Exp. Zool.* 252:1-8.
- Terjesen B.F., Verreth J., Fyhn H.J. 1997. Urea and ammonia excretion by embryos and larvae of the African Catfish *Clarias gariepinus* (Burchell 1822). *Fish Physiol. Biochem.* 16:311-321.
- Terjesen B.F., Rønnestad I., Norberg B., Anderson P.M. 2000. 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., 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.

