

**ORNITHINE-UREA CYCLE IN THE
BRAZILIAN CATFISH, *PSEUDOPLATYSTOMA CORUSCANS*
DURING EARLY-LIFE STAGES**

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Introduction

To maintain the best physiological performance to face the toughness of environmental conditions the vertebrates selected the best strategies in the course of the evolutionary history. The outcome is the biological diversity as vast as the ensemble of molecular adaptations. The freshwater fishes in particular are usually exposed to many external changes and chemicals, as ammonia can be very stressing in some conditions. Internal ammonia is as harmful as the external one. Aquatic organisms can directly clear off this waste product from the special metabolism of nitrogenous substances. However, some environmental circumstances may impair that clearance. The main is the water availability but as much important as it is the concentration of H⁺. Conversion of ammonia (NH₃) to the ammonium (NH₄⁺) ion is a form to detoxify it. Moreover, the ammonium is usually rather excreted than ammonia. Any physiological condition accountable for the imbalance of the nitrogenous excretion forms should result in inappropriate cell medium and in the start of alternate steps to reestablish the equilibrium.

Growth and development bring a very special set of cell conditions, which impose a particular metabolic profile. Changes of nitrogenous metabolism during the fish embryonic development were early reported in rainbow trout *Oncorhynchus mykiss*, (Smith, 1947). While the fish dry weigh and the nitrogen excretion increased, the yolk content decreased. Recently, ammonia and urea

excretion were correlated to ornithine-urea cycle (Wright *et al.*, 1995) during the rainbow trout development. The higher activities of OUC enzymes take place in the larval period, between the 53th and 71st day. The expression of the genes for such enzymes would reduce the toxic effects of ammonia from the plentiful protein metabolism of the embryo. Those genes are expressed during the development and some are silent in adult fish. The OUC enzymes were quite active during the embryonic and larval stages of the Atlantic cod *Gadus morhua* L, but only arginase and glutamine synthase were kept very active (Chadwick & Wright, 1999) in adult fish. These should be expected for the most teleost species. However, those from warm waters present distinct physiological characteristics. The developmental period is very short compared to cold water fish. Such difference is positively related to the metabolic activity. Due to the faster processes concerning the ontogenesis we suppose the time of exposition to ammonia to be less injurious in tropical fish eggs and embryos than in cold water fishes, and this would imply that OUC enzymes are less active. We have studied the OUC enzyme expression in the Brazilian catfish “pintado” *Pseudoplatystoma coruscans*, in the whole developmental life stages.

Material and Methods

Eggs of *P. coruscans* were obtained from induced spawning. These experimental steps were performed at the Centro Nacional de Pesquisas Tropicais (CEPTA-IBAMA), Pirassununga, SP (21°59'46" S; 47°25'33" W). Two adult females and six adult males (15 ± 8 kg) were used. Eggs were fertilized at 24° C and incubated in well-aerated water in 100L fiberglass conical tanks, provided constant water renewing. Samples were collected by suction, transferred to special sieves, frozen at -10° C and lyophilized. The time interval of sampling are depicted in the table I. The OUC enzyme activity of lyophilized liver and kidney of adult fish were compared with eggs, embryos and larvae.

Biological samples (whole animal for eggs, embryos and larvae) were mechanically homogenized with a motor driven pestle into cold-water bath. Homogenates were done into 20mM glycine 10mM phosphate pH 7.0 in glycerol 1:1. Tissues, eggs and embryos were disrupted in the ratio 16.67mg per mL of homogenization buffer.

Table I.
Time interval of sampling *Pseudoplatystoma coruscans* during the early stages of life.

Δt	Observation
0h	---
2h	Morula
4h	Blastula
6h	Gastrula
8h	Blastopore
10h	---
12h	Neurula
14h	Embryo (no movements)
16h	Embryo (movements)
17h	Fast movements
17h:30	Hatching
23h:30	---
29h:30	---
35h:30	---
41h:30	Barbels and eyes
47h:30	Mouth
53h:30	Irregular opercular beats
59h:30	Fast swimming
65h:30	---
71h:30	---
77h:30	---
83h:30	Fins
89h:30	Feeding (<i>Artemia</i> sp)
113h:30	<i>Artemias</i> in the gut
Five-days from hatching	Larvae length 1.0 cm
12 day	Eating artificial feeding
19 day	Larvae length 2.5 cm
28 day	Larvae length 3.0 cm

The next OUC enzymes were assayed: ornithine carbamoyl transferase (OCT) as Nakamura & Jones (1970), arginase (ARG) as Rahmatullah & Boyde (1980) and carbamoyl-phosphate synthetase III (CPS_{III}) as Saha *et al*,1997. Protein contents of the samples were determined by UV-photometry (Warburg & Christian, 1941).

Results

Ornithine carbamoyl transferase was present in embryos since the egg phase. The enzyme activity in the ovule was 0.426 nmol of citrulline /min /mg of protein, and it oscillates (Fig. 1-a) until the larval stage (17: 30 min). From this moment to the 53h: 30 min the activity increased eight times. At this phase the first opercular movements were observed. Again, the activity decreases until 113: 30 min as the first artemias were observed in the gut. From then on, the activity enhances to the maximum values observed (Fig 2-a). Arginase activity was present since the unfertilized egg with a sharp peak around 18 h (beginning of the larval phase) and decreased to the lowest level. From this phase, the arginase activity trend was of constant raise. The activity of carbamoyl phosphate synthetase was observed from the fourth hour of development. This enzyme activity increased, oscillating with large peaks since the eighth to the hundredth hour. From that moment, it was observed a constant decrease to near by zero at the third week of development.

Discussion

At the present time, the ureogenesis during the initial phases of fish development has being generally accepted. This concept comes from the set of data for cold water fish (Wright *et al*, 1995; Chadwick & Wright, 1999). Just a few are available for tropical freshwater fish (Monzani, 1999; Terjesen *et al*, 2001). The Brazilian catfish pintado express the OUC enzymes at the first steps of development. However, compared with trout and Atlantic cod some differences are observed. Arginase and OCT of pintado are active since the unfertilized egg. Protein metabolism from adult fish during mating phase should be active enough to transfer these metabolic features to the generation.

To understand the OCT activity in the embryos of pintado three relevant events must be considered: the hatch, the beginning of opercular beats and the exogenous feeding. From the hatch to the opercular beats the OCT of pintado increased ten times. The hatch efforts should increase the metabolic demands and the absence of the gill apparatus during this period (Rombough, 1988) impairs the waste of ammonia propitiating the urea synthesis. The metabolic requirements at the opercular-beating phase also demand muscle work, and the consequent breakage of adenilates resulting in large amounts of ammonia. Similar biochemical response is also observed in adult fish submitted to exercise (Wright *et al*, 1988). Therefore, is reasonable to think that the increase of urea synthesis in this life phase of pintado occurs to detoxify ammonia. Decrease of

OCT is remarkable from the 53 h, precisely the moment of the beginning of the opercular beats. That means the gill started to work, probably in addition to ammonia excretion. Moreover, the yolk sack was practically reabsorbed. From the 113 h the enzyme activity of OCT raises constantly. That moment matches to a new event in the life cycle of pintado, it starts to feed on artemias. The large income of protein is likely the cause of such enzyme response. Correlation between OCT and CPS was observed particularly before the hatching.

Carbamoyl phosphate synthetase III must work in consonance with OCT as a substrate supplier. This enzyme is strictly related to urea synthesis and the presence in white muscle of many teleosts (Korte et al 97; Felskie *et al.* 1998; Chadwick & Wright 1999) reinforce the assumption of many fishes are ureogenic. However, under circumstances where ammonia toxicity does not threaten the organism, the spare of ureogenesis is expected. This should not be the case of many developmental phases (Felskie *et al.*, 1998). Like OCT ability to express the arginase activity seems to be bequeathed from the parental cell.

Arginase is a key enzyme of the intermediary metabolism. It is directly involved in the protein metabolism, in the synthesis of creatinine, nitric oxide, polyamines and proline/glutamate (Jenkinson *et al.*, 1996). The enzyme product of arginase is urea and ornithine. This amino acid is the precursor of polyamines (putrescine, spermine and spermidine), which are related to cell division and differentiation (Tabor & Tabor, 1984). In spite of the arginase activity and the developmental phases of pintado seem to be not straightly related its activity affords ornithine in the course of the growth and differentiation processes. Furthermore, the increase of arginase follows the food intake and larger growth of the larvae.

Comparison of warm and cold-water fish concerning the expression of OUC enzymes in the course of ontogenesis depicted the same pattern, and the specific enzyme activities are also similar. The time of residence of ammonia within the embryos is likely less important in the expression of OUC enzymes than are many other cell development mechanisms. The fishes' ancestors probably bequeathed the OUC enzymes and the species did not lose them in the course of history. They are silenced until new circumstances demand the use.

References

Chadwick, T. D.; Wright, P. A. (1999). Nitrogen Excretion And Expression Of Urea Cycle Enzymes In The Atlantic Cod (*Gadus Morhua*): A

- Comparison Of Early Life Stages With Adults. *J. Exp. Biol.* **202**, 2653-2662.
- Felskie, A. K.; Anderson, P. M.; Wright, P. A. (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.
- Jenkinson, C.P., Grody, W.W. And Cederbaum, S.D. (1996). Comparative Properties Of Arginases. *Comp. Biochem. Physiol.*, **114b** 107-132.
- Korte, J. J.; Salo, W. L.; Cabrera, V. M., Wright, P. A., Felskie, A. K., Anderson, P. M. (1997). Expression Of Carbamoyl-Phosphate Synthetase Iii Mrna During The Early Life Stages Of Development And In Muscle Of Adult Rainbow Trout (*Oncorhynchus Mykiss*). *J. Biol. Chem.* **272**, 6270-6277.
- Monzani, P. S. (1999). Estudo de alguns aspectos da excreção nitrogenada em *Piaractus mesopotamicus*, ao longo de seu desenvolvimento ontogenético. M.Sc Thesis. PPG-Gev. Universidade Federal de São Carlos. São Carlos – SP. Brazil.
- Nakamura, M. & Jones, M. E. (1970). Ornithine Carbamyltransferase (*Streptococcus Faecalis*). In: Methods In Enzymology (Colowick, S. P.; Kaplan N. O., Eds). New York. Vol. Xvii Part A. Section Iv, Chapter 32, 289-294. Academic Press.
- Rahmatullah, M. & Boyde, T. C. (1980). Improvements In The Determination Of Urea Using Diacetyl Monoxime: Methods With And Without Desproteinisation. *Clinica Chimica Acta* **107**, 3-9.
- Rombough, P. J. (1988). Growth, Aerobic Metabolism And Dissolved Oxygen Requirements Of Embryos And Alevins Of Steelhead, *Salmo Gairdneri*. *Can. J. Zool.* **66(3)** 651-660.
- Saha, N.; Dkhar, J.; Anderson, P. M.; Braja, K. K. (1997). Carbamoyl Phosphate Synthetase In An Air-Breathing Teleost, *Heteropneustes Fossilis*. *Comp. Biochem. Physiol.* **116** (B) 57-63.

- Smith, S. (1947). Studies In The Development Of The Rainbow Trout (*Salmo Irideus*). *J. Exp. Biol.* **23**: 357-378.
- Tabor, C. W., Tabor, H. (1984). Polyamines. *Annu. Rev. Biochem.* **53** 749-790.
- Terjesen, B. F.; Chadwick, T. D.; Verreth J. A. J.; Rønnestad, I.; Wright, P. A. (2001). Pathways For Urea Production During Early Life Of An Air-Breathing Teleost, The African Catfish *Clarias Gariepinus* Burchell. *J. Exp. Biol.* **204**, 2155-2165.
- Warburg, O.; Christian, W. (1941). *Biochim. Z.* **310**, 384-421.
- Wright, P. A., Felskie, A., Anderson, P. M (1995). Induction Of Ornithine-Urea Cycle And Nitrogen Metabolism And Excretion In Rainbow Trout (*Oncorhynchus Mykiss*) During Early Life Stages. *J. Exp. Biol.* **198**, 127-135.
- Wright, P.A., Randall, D.J. And Wood, C.M. (1988). The Distribution Of Ammonia And H⁺ Between Tissue Compartments In Lemon Sole (*Parophrys Netulus*) At Rest, During Hypercapnia And Following Exercise. *J. Exp. Biol.* **136** 149-175.

