

### **Ornithine urea cycle enzymes in holocephalan elephant fish (*Callorhinchus milii*)**

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Cartilaginous fish are comprised of two subclasses Holocephali (chimaeras) and Elasmobranchii (sharks, skates and rays). Little is known about osmoregulatory mechanisms in holocephalan fishes except that they conduct urea-based osmoregulation as in elasmobranchs. Therefore, we examined the ornithine urea cycle enzymes, which contribute to urea biosynthesis, in holocephalan elephant fish *Callorhinchus milii*. Since elephant fish is the only species of cartilaginous fish with a public genome database, it will serve as a useful model for the study of the biology of holocephalan fishes. Elephant fish have two glutamine synthetase (efGS) and arginase (efARG) genes. Molecular phylogenetic and tissue distribution analyses revealed that two efGSs are structurally and functionally separated into two types: brain/liver-type efGS1 and muscle-type efGS2. Most of the GS genes found in vertebrates, including mammalian GS, are grouped with the efGS1. Carbamoyl phosphate synthetase III (efCPSIII) mRNA expression was high in the liver, and was reduced by transfer of fish to a low salinity environment, which suggests that the liver is the primary organ for urea biosynthesis in elephant fish. The expression of efCPSIII, efGS1 and efARG mRNAs was increased during the course of embryonic development; whereas efGS2 mRNA showed a marked increase in expression after hatching.