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Stages of embryonic development and changes in enzyme activities in embryogenesis of turbot (*Scophthalmus maximus* L.)

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Abstract The early development of turbot (*Scophthalmus maximus*) from fertilization to hatching was described. Hatching occurred at 108 h post-fertilization (hpf) in 14 °C. Yolk syncytial layer and blastocoel formed at morula stage and low stage, respectively. Neural rod derived from the ectoderm appeared and the first somite formed in the middle of the embryonic body at 90 % epiboly stage, and notochord primordium formed at complete epiboly stage. Kupffer's vesicle appeared at 59 h 35 min hpf and degenerated at 89 hpf. At 72 hpf, the digestive tract formed in the ventral side of the embryonic body, and the posterior digestive tract of embryo was ciliated at 89 hpf. Enzymes play a key role in the catabolism of yolk during embryogenesis of fishes. In this study, the main enzymes alkaline phosphatase (AP), leucine aminopeptidase N (LAP), pepsin, trypsin and Leucine-alanine peptidase (Leu-ala) were all observed in unfertilized eggs and embryo of *S. maximus*, but amylase was not detected, speculating that amino acids appear to be the main energy substrate during embryonic development of *S. maximus*, while carbohydrates is less essential. AP reached the lowest value at the gastrula stage and then increased rapidly reaching the highest value at hatching. LAP showed the highest value in unfertilized eggs and kept on decreasing until the blastula stage with the lowest value and then increased at the gastrula stage, followed by a gradual decline thereafter. Trypsin reached the lowest value at the blastula stage and then fluctuated with the maximal value at hatching. Pepsin reached the highest and the lowest values at the unfertilized eggs and the cleavage stage, respectively, but disappeared at hatching. Leu-ala had the maximum activity at the blastula stage and then declined to the minimum at the gastrula stage followed by a gradual increase thereafter.

Keywords Developmental stages · Embryogenesis · Enzyme · Histology · *Scophthalmus maximus*

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Introduction

The early development of fishes is a highly dynamic process, and researches on developmental mechanisms are imperative because it can provide knowledge on life history and is very useful for hatchery production (Martinez and Bolker 2003). Many literatures on developmental process of cultured fishes are available. Most of these studies focus on larval and juvenile development (Keefe and Able 1993; Schreiber and Specker 1998; van Maaren and Daniels 2000). In recent years, the process of embryonic development have been reported in several fishes, such as zebrafish (*Danio rerio*) (Hamilton) (Kimmel et al. 1995), medaka (*Oryzias latipes*) (Temminck and Schlegel 1846) (Iwamatsu 2004), loach (*Misgurnus anguillicaudatus*) (Cantor) (Fujimoto et al. 2006), summer flounder (*Paralichthys dentatus* L.) (Martinez and Bolker 2003) and Atlantic cod (*Gadus morhua* L.) (Hall et al. 2004).

Embryogenesis is an energetically demanding process and implies reliance on enzymatic systems for digestive physiology (Hall et al. 2004). Considering development of metabolic pathways commence during the embryonic period, egg quality as well as survival and growth performance can be linked to these traits. Accordingly, knowledge about the development of enzymes, which are involved in the catabolism of yolk, is essential. In recent years, ontogenesis of digestive enzymes and developmental features of the digestive tract have been well documented in larvae and juveniles of many species like Senegal sole (*Solea senegalensis*) (Ribeiro et al. 1999), red drum (*Sciaenops ocellatus*) (Buchet et al. 2000), yellowtail kingfish (*Seriola lalandi*) (Chen et al. 2006), sharpsnout seabream (*Diplodus puntazzo*) (Suzer et al. 2007) and common dentex (*Dentex dentex*) (Gisbert et al. 2009) and so on. However, few are known about the energetic requirements expressed by key enzyme activities in fish embryos besides several fishes such as the whitefish (*Coregonus* spp.) (Lahnsteiner 2005), sea bass (*Dicentrarchus labrax*) (Carnevali et al. 2001), *S. lalandi* (Moran et al. 2007), spotted wolffish (*Anarhichas minor*) (Desrosiers et al. 2008) and *G. morhua* (Sveinsdóttir et al. 2006).

Turbot (*Scophthalmus maximus*) is a commercially valuable teleost and useful for aquaculture because of rapid growth and quality of flesh. The embryonic development of turbot has been described in previous works. But these works mainly focused on the histological changes in embryo, and the digestive features were rarely studied. As for turbot, embryogenesis implies reliance on enzymatic systems for catabolism of yolk. Accordingly, knowledge about the development of enzymes is essential. Therefore, enzymatic activities of alkaline phosphatase (AP), leucine aminopeptidase N (LAP), pepsin, trypsin, Leu-ala and amylase were analysed in unfertilized eggs and embryo, which has not been conducted in previous works. The aim of this study was to study digestive physiology during embryonic development to provide knowledge of yolk utilization in relation to organogenesis and define 'normal' enzymatic profiles, which is valuable for the investigation of nutritional requirements and serve as a basis for future experimental studies.

Materials and methods

Eggs and embryos sampling

Eggs of *S. maximus* were obtained from several spawns of broodstock maintained at Oriental Ocean Sci-Tech Co., Ltd (Shandong Province, China) under controlled temperature. Following fertilization, viable buoyant eggs were transferred and incubated at 14 ± 0.5 °C.