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Development of the digestive tract, trypsin activity and gene expression in eggs and larvae of the bullseye puffer fish *Sphoeroides annulatus*

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Abstract

Hatchery-produced *Sphoeroides annulatus* were studied from fertilized egg until day 32 post-hatch to examine the digestive tract development and to evaluate its digestive capacity during the larval period. Fish larvae were progressively fed microalgae, rotifers, *Artemia* nauplii and a formulated microdiet. Digestive tract development, trypsin activity, and trypsinogen gene expression in fish samples were analyzed by histology, histochemistry and reverse-transcription coupled to the polymerase chain reaction (RT–PCR) respectively. The intestine and liver started to develop on day 1 after hatch, followed by the pancreas. The mouth opened at day 4 after hatch, which was the start of rotifer feeding. Trypsinogen gene expression was detected very early in development, starting in the fertilized egg, showing a sharp increase in eggs at 75 h after fertilization, and then a gradual increase after hatching as the larvae developed. Trypsin activity by histochemistry was first detected at day 2 post-hatch, and maximum expression and activity were observed at days 16 to 24 after hatch, which corresponded to the period of *Artemia* nauplii feeding. No gastric glands were observed during the whole period of study. From day 28 onwards the fish were fed with a formulated microdiet, at this time both trypsin synthesis and activity decreased, suggesting a more important role for other enzymes in the digestion process.
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Keywords: Trypsin; Gene expression; Digestive tract; Histochemistry; Bullseye puffer

1. Introduction

Live food organisms such as rotifers, copepods and *Artemia* are commonly used in marine fish larviculture. Since the major nutritional constituent in these organisms is protein (Watanabe et al., 1983), the proteolytic

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Table 1
Summary of digestive tract development and feeding scheme

Age	Digestive organs	Feeding scheme
Fertilized egg (24 h)	Yolk-sac	–
Fertilized egg (51–75 h)	Yolk-sac, undifferentiated digestive tract	–
Day 0 after hatch	Yolk-sac, liver and intestine start to develop	–
Day 1 after hatch	Liver and intestine developing	–
Day 2–8 after hatch	Mouth opens, liver, intestine, and pancreas (endocrine portion) are evident	Microalgae; Rotifers
Day 12–16 after hatch	Pancreatic excretory cells are observed	Rotifers; *Artemia* nauplii
Day 20–24 after hatch	Hindgut and midgut are visible	*Artemia* nauplii
Day 28–32 after hatch	Digestive tract fully developed except for stomach	*Artemia* nauplii; Formulated microdiet

capacity for food digestion can be regarded as very important during the early life stages of fish. Most teleost fishes are characterized by the lack of a functional stomach during the larval stage. Therefore, digestion of food in fish larvae is mainly dependant on trypsin-type alkaline enzymes (Hjelmeland and Jørgensen, 1985; Pedersen et al., 1987). This aspect has important implications in the type of protein the fish larvae are able to digest and consequently in determining adequate feeding strategies in fish larviculture.

Several studies have described the development of digestive tract organs in diverse marine fish species, including halibut (Blaxter, 1988), cod (Kjørsvik et al., 1991), turbot (Segner et al., 1994), Japanese eel (Kurokawa et al., 1995), European sea bass (Cahu and Zambonino Infante, 1995), gilthead seabream (Sarasquete et al., 1995), Senegal sole (Ribeiro et al., 1999), haddock (Hamlin et al., 2000) and California halibut (Gisbert et al., 2004). However, little information is available on digestive tract development and digestive enzymes capacities in larvae of tropical fish species. The bullseye puffer is a potential species for aquaculture in the Eastern Pacific. Controlled production of eggs, larvae and juveniles have been accomplished for this species (Duncan et al., 2003; García-Ortega et al., 2003) and optimization of the different culture phases is currently in progress.

The aims of the present study were to detect expression and activity of trypsin during larval development of the bullseye puffer, and relate trypsin synthesis and activity to the development of organs in the digestive tract and type of food ingested by the fish larvae.

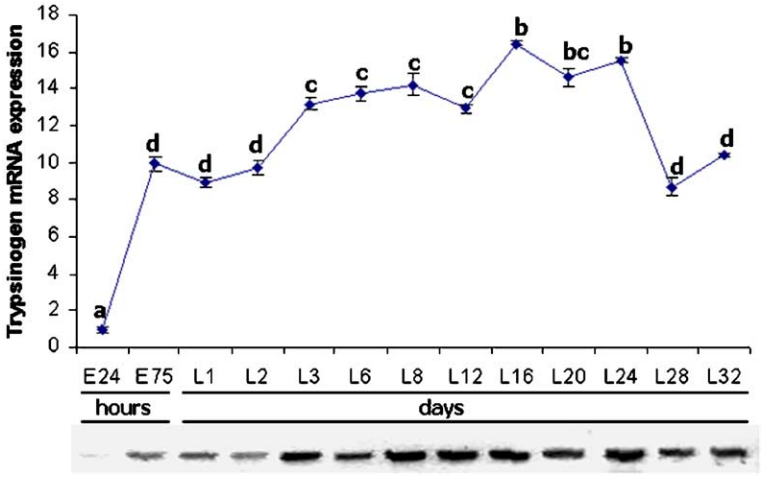

Fig. 1. Trypsinogen mRNA expression in eggs and larvae, relative to E24. The corresponding gel with the trypsinogen 272 bp PCR fragment is shown. E24 and E75: eggs of 24 and 75 h after fertilization respectively; L1 to L32: larvae of 1 to 32 days after hatch. Different letters indicate significant differences in trypsinogen expression levels.

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