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Effects of delayed first feeding on nutritional condition of tiger grouper, *Epinephelus fuscoguttatus* (Forsskål, 1775) larvae

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ABSTRACT

The effects of delayed first feeding on the nutritional condition of tiger grouper, *Epinephelus fuscoguttatus* (Forsskål, 1775), larvae were examined under controlled conditions. Larval gut epithelium development and morphometric changes of the larvae fed at different first times (0, 6, 12, 18 and 24 h after mouth opening stage; h AMO) were compared. Gut epithelium height ($14.81 \pm 0.24\,\mu$m) of larvae first fed at 0 h AMO was significantly higher ($P < 0.05$) compared to other treatments and gut was morphologically well developed. A continuous reduction of gut epithelium height was observed in larvae first fed beyond 0 h AMO and severe damage on connective tissue surrounding larval gut was observed in larvae fed at 24 h AMO. All morphometric growth on each body proportion of larvae first fed at 0 h AMO was gradually increased as they developed, while larvae first fed at 6, 12, 18 and 24 h AMO experienced slow development and degradation of entire body proportions. This study concludes first feeding at mouth opening stage to the tiger grouper is essential to enhance larval nutritional condition that is important to maximize larval survival and growth at subsequent stage.

1. Introduction

The tiger grouper *Epinephelus fuscoguttatus* (Forsskål 1775) is among the widely distributed tropical groupers in Southeast Asia region (Liao et al., 2001). In recent years, the tiger grouper aquaculture has expanded rapidly and is the most popular cultured grouper owing to their remarkably fast growth and higher market price. However, mass mortality at early larval stage remains a foremost constraint to the development of the industry (Ching et al., 2012) mostly due to first feeding-related complications.

Generally, groupers initiated first feeding at mouth opening stage and at this time, larvae still have small yolk sac, however its absorption period is short, resulting in a nutritional transition period (NTP), described by Fhyn (1989) as the period between the onset of exogenous feeding and the end of yolk sac absorption, that is relatively shorter. Presuming larvae are able to rely on yolk sac to provide food or energy sources throughout the NTP, to initiate first feeding at mouth opening stage has often been neglected. To date, however, no work has been done on the effect of delayed first feeding time within the NTP on nutritional condition of the tiger grouper larvae.

Evaluating larval nutritional condition is of significant, since the physical and physiological condition of fish larvae throughout their development particularly at early stage influences their survival, growth and feeding performances, which eventually contributes to the success of its production. In this study, gut development and morphometric changes were used to evaluate larval nutritional condition since the digestive tract; particularly the gut epithelium height is among the most sensitive tissues affected by feeding performance of fish larvae (Yin and Blaxter, 1987), whereby starvation usually results in shrinkage in the height of fish larval gut. Meanwhile, larval morphometric changes are the most immediate indicators and easy to be performed to characterize nutritional condition of fish larvae under different feeding performances (Dou et al., 2005), in which larval development

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2. Materials and methods

2.1. Egg collection

Eggs of the tiger grouper were collected from broodstock tank that spontaneously spawned at the marine hatchery of the Centre of Collaborative Research in Aquaculture (Universiti Malaysia Sabah-Kinki University) in Sabah, Malaysia. Eggs were incubated in a 1000 L fiberglass-reinforced plastic tank at the stocking density of 30 eggs L^{-1}. Water temperature, pH, dissolved oxygen and aeration rate were in the range of 28.5 ± 0.5 °C, 6.8–7.2, 6.5–6.9 mg L^{-1} and 250 mL min^{-1} respectively. Hatching rate was recorded at 86.7%.

2.2. Larval rearing and experimental treatment

The tiger grouper larvae initiated first feeding at 54 h AH (27.5 ± 0.5 °C) and defined as 0 h after mouth opening (0 h AMO) as generally used to defined first feeding time in several grouper species (Yoseda et al., 2006). First feeding time was delayed by 6-h intervals, ranging 0, 6, 12, 18 and 24 h AMO. Five of 7 L sampling tank were prepared to resemble 5 different first feeding times treatment and larvae were stocked at 200 tails per tank. At first feeding, rotifer, *Brachionus plicatilis* sp. complex and cultured *Nannochloropsis oculata* were supplied at a density of 30 individual L^{-1} and 0.5×10^{-6} cells mL^{-1} respectively.

2.3. Larval gut epithelium height

Histological section was used to observe and compare larval gut epithelium development in each treatment. Larvae were sampled ($n = 5$) from 0 h AMO at 6-h intervals within the NTP. Sampled larvae were initially fixed for 1 day in Bouin's fluid, dehydrated in graded alcohols and embedded in paraffin and cut in serial sagittal sections (6 μm) and slides were stained by the Haematoxylin and Eosin (HE). The section was examined and measured under a light microscope and photographed using image analysis (ImageJ1.44P, Wayne Rasband, USA).

2.4. Morphometric changes

Larvae ($n = 5$) were similarly sampled from 0 h AMO at 6-h intervals within the NTP. Fresh samples were slightly anaesthetized (25 ppm) with Transmore (Nika, Nika Trading, Malaysia) and then immediately measured under a profile projector individually. The following body proportion including body length (BL), body height (BH), pectoral angle height (PH), eye height (EH), gut height (GH) and head height (HH) was measured.

2.5. Statistical analysis

Statistical analysis was performed using SPSS 15.0 software (SPSS Incorporation, Chicago, IL, USA) and a significance level of $P < 0.05$ was applied. One-way-ANOVA was performed to compare larval gut epithelium height and each body proportion examined. When a significant difference was found, a post hoc test using Tukey's HSD was performed to ascertain any significant differences between treatment means.

3. Results

3.1. Gut epithelium height

At 0 and 6 h AMO, all larvae had a slightly developed gut coupled with peristaltic movement (Fig. 1A) and the average gut epithelium heights were 7.53 ± 0.41 and 9.43 ± 0.71 μm respectively (Fig. 2) and remained no significant different in all treatments

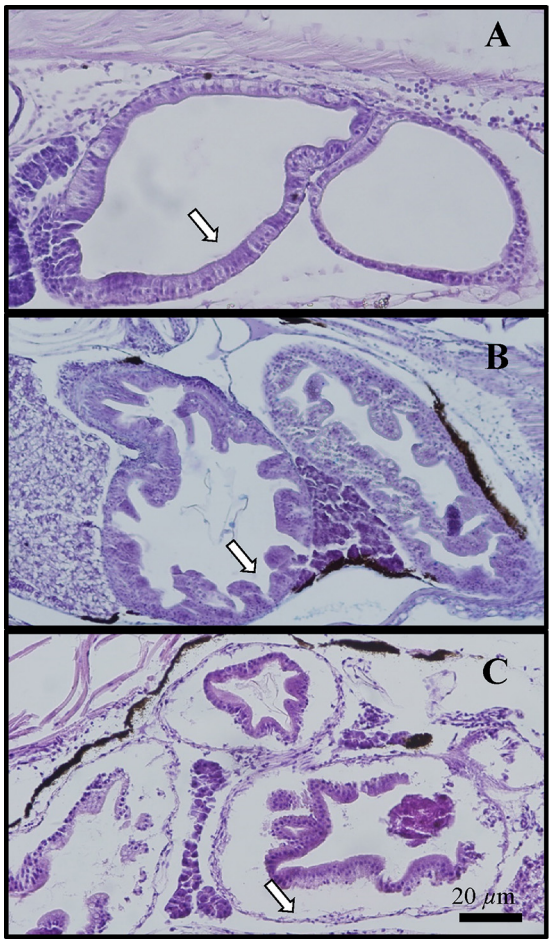

Fig. 1. Gut epithelium development of the tiger grouper *Epinephelus fuscoguttatus* fed at different first feeding times. (A) Gut was undifferentiated and straight at first feeding stage in all treatment; (B) rapid development on larvae first fed at 0 h AMO at 72 h AH; (C) severe damage on connective tissues surrounding gut of larvae first fed beyond 6 h AMO at 72 h AH.

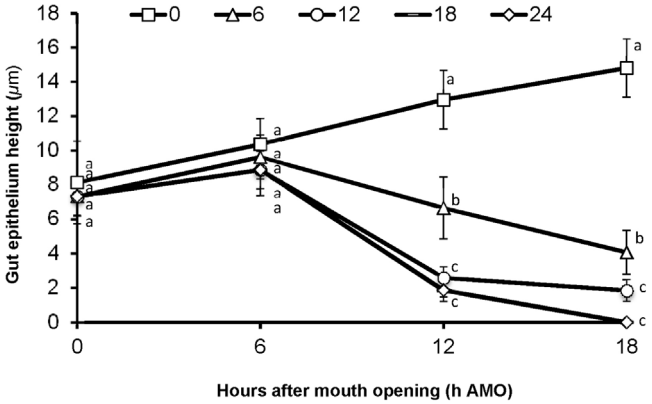

Fig. 2. Gut epithelium height (μm) of the tiger grouper *Epinephelus fuscoguttatus* fed at different first feeding times. Values are the mean ± SD and different superscript letters (a–c) in each vertical column indicates significant differences.

($P > 0.05$). However, at 12 h AMO, a remarkable increased ($P < 0.05$) of gut epithelium height was observed in the larvae first fed at 0 h AMO (12.96 ± 0.65 μm), while a rapid reduction was observed in those larvae first fed at 6, 12, 18 and 24 h AMO (6.66 ± 0.87, 2.59 ± 0.19, 1.92 ± 0.06 and 1.85 ± 0.02 μm) respectively (Fig. 2). As larvae aged at 18 h AMO, gut epithelium height of larvae first fed at 0 h AMO had significantly increased to 14.81 ± 0.24 μm