ONTOGENETIC DEVELOPMENT OF DIGESTIVE SYSTEM IN TIGER GROUPER, *Epinephelus fuscoguttatus* (Forsskal, 1775) LARVAE

SITI ARIZA ARIPIN

MASTER OF SCIENCE
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Chairperson : **Assoc. Prof. Abol Munafi Ambok Bolong, Ph.D**

Committee Members : **Prof. Mohd Effendy Abd. Wahid, Ph.D**

: **Prof. Mohd Azmi Ambak, Ph.D**

Institute : **Institute of Tropical Aquaculture**

Information on the ontogeny of digestive system related with food digestion processes is crucial for understanding the physiology of larval fish. Although the larval rearing techniques in marine aquaculture have been improved progressively over recent years, its grouper larvae production has been facing problems. The limited success of intensive rearing of *Epinephelus fuscoguttatus* is due to a poor knowledge of nutritional feeding, morphological and functional development of the digestive tract and digestive enzyme in the larvae. A study on the ontogenetic development of digestive system of *E. fuscoguttatus* was conducted in Institute of Tropical Aquaculture of Universiti Malaysia Terengganu from first day after hatching until 35 days after hatching (DAH) by morphological, enzyme assay and histological techniques.

Morphological development of digestive tract showed that *E. fuscoguttatus* larvae digestive tract appear as a straight undifferentiated tube with mouth and the anus that were still closed at first day after hatching. During the endogenous feeding phase, yolk was assimilated while the digestive tract was divided into a buccal cavity, oesophagus and intestine. At the end of the 2 DAH, the mouth and the anus were opened. By 3 DAH, the yolk was completely absorbed. Coiling intestine was observed on 6 DAH (2.82 ± 0.05 mm). By 9 DAH (3.68 ± 0.05 mm), the dorsal and pelvic fin spine started to appear. The notochord had started to bend upward at 18 DAH (7.25 ± 0.04 mm) and the notochord had disappeared by 29 DAH (17.14 ± 0.07 mm), indicates that the end of flexion stage. At hatching day, the mean total length was 2.44 ± 0.28 mm and reaching 24.09 ± 0.01 mm by 35 DAH. The mean growth rate of the larvae was 0.62 $mm.day^{-1}$. The mouth was open on 3 DAH where the mouth opening was 0.29 ± 0.05 mm and 0.16 ± 0.03 mm for the opening angles of 90 degree and 45 degree, respectively.

Trypsin and chymotrypsin activities were firstly detected on 2 DAH, at 0.0006 mU $mg^{-1}protein^{-1}$ and 0.0005 mU $mg^{-1}protein^{-1}$, respectively. The highest activity for trypsin was at 0.0073 mU $mg^{-1}protein^{-1}$ by 20 DAH and the highest activity for chymotrypsin was 0.0597 mU $mg^{-1}protein^{-1}$ by 18 DAH. The average value of trypsin (0.0022 mU $mg^{-1}protein^{-1}$) is lower than chymotrypsin (0.0164 mU $mg^{-1}protein^{-1}$) for *E. fuscoguttatus* larvae. The higher chymotrypsin activity level might suggest that the fish might be omnivores at larval stages. A few fluctuations were detected at 6 DAH, 20 DAH and 29 DAH for trypsin activity. As for chymotrypsin activity, the fluctuations were detected on 8 DAH, 20 DAH and 29 DAH. The fluctuations in enzyme activities explained the

differentiation in larval stages, period of morphological differentiation in the digestive tract and also associated organs. Using the statistical Analysis of Variance (ANOVA), there was no significant different between trypsin and chymotrypsin where $P>0.05$. There were a strong correlation between the mouth opening and enzyme activities observed where as the mouth opening getting bigger, the less enzyme activities were produced in *E. fuscoguttatus* larvae. Using Pearson correlation (2-tail) test, the correlation value is $r= -0.666$ where $p<0.01$ for trypsin and the correlation value for chymotrypsin is $r= -0.562$ where $P<0.01$.

Histological analysis in this study showed that the mouth and the anus were closed at 1 DAH (22.44 ± 0.28 mm). At 2 DAH (2.68 ± 0.01 mm), the mouth had opened but not fully functioning. The rudimentary gill arches were noticeable under the epithelium of the posterior buccal cavity. By 3 DAH, the digestive tract had differentiated from the mouth to the anus. In *E. fuscoguttatus*, primordial pyloric caeca was first appeared as a protrusion of the anterior intestine by 7 DAH. By 12 DAH, mucous cells and gill rakers had appeared in the pharyngeal cavity and the pharyngeal teeth had formed. The taste buds were also observed in the epithelial folds. In this experiment, pyloric caeca, gastric gland and blind sac were formed completely at 26 DAH. Although the larvae were not as fully developed as adult, the digestive system was distinguished and functioning toward the end of the study. Based on the results, we suggest that *E. fuscoguttatus* larvae would start to be capable of intensively digesting and assimilating compound diets from around 23 DAH until 26 DAH. In this study, the functioning of the digestive tract is fully achieved at 29 DAH. Changes in the activity of digestive enzymes were definitely

associated with the morphological development of *E. fuscoguttatus* larvae's digestive system.

Abstrak tesis yang dikemukakan kepada Senat Universiti Malaysia Terengganu sebagai memenuhi keperluan untuk ijazah Master Sains.

PERKEMBANGAN ONTOGENETIK BAGI SISTEM PENCERNAAN DALAM LARVAL KERAPU HARIMAU, *Epinephelus fuscoguttatus* (Forsskal, 1775)

SITI ARIZA ARIPIN

May 2010

Pengerusi : **Prof. Madya Abol Munafi Ambok Bolong, Ph.D**

Ahli jawatankuasa : **Prof. Mohd Effendy Abd. Wahid, Ph.D**

: **Prof. Mohd Azmi Ambak, Ph.D**

Institut : **Institut Akuakultur Tropika**

Maklumat mengenai ontogeni sistem pencernaan berkaitan dengan proses penghadaman makanan adalah penting bagi memahami fiologi larva ikan. Meskipun teknik penternakan larva dalam akuakultur marin sudah diperbaiki secara progresif kebelakangan ini, namun pengeluaran larva kerapu telah berdepan pelbagai masalah. Kejayaan terhad dalam penternakan intensif *Epinephelus fuscoguttatus* adalah kerana kurang pengetahuan dalan nutrisi pemakanan, morfologi dan kefungsian perkembangan salur pencernaan dan enzim penceranaan dalam larva. A study on the ontogenetic development of digestive system of *E. fuscoguttatus* w Satu kajian berkaitan dengan perkembangan ontogenic di dalam sistem pencernaan bagi *E. fuscoguttatus* telah dijalankan di Institut Akuakultur Tropika, Universiti Malaysia Terengganu dari hari pertama selepas penetasan hingga 35 hari selepas penetasan (HSP) dengan menggunakan teknik-teknik morfologi, ujian enzim dan histologi.

Perkembangan morfologi saluran pencernaan menunjukkan bahawa saluran pencernaan larva *E. fuscoguttatus* kelihatan seperti salur lurus yang tidak membeza dengan mulut dan anus yang masih tertutup pada hari pertama selepas penetasan. Semasa fasa pemakanan endogenous, yolka diasimilasi semasa saluran pencernaan membahagi kepada rongga mulut, esofagus dan usus. Pada penghujung 2 HSP, mulut dan anus telah terbuka. Ketika 3 HSP, yolka telah diserap sepenuhnya. Lingkaran usus telah diperhatikan pada 6 HSP (2.82 ± 0.05 mm). Pada 9 HSP (3.68 ± 0.05 mm), spina sirip dorsal dan pelvis mula terbentuk. Notokord telah mula membengkok ke atas pada 18 HSP (7.25 ± 0.04 mm) dan notokord telah menghilang pada 29 HSP (17.14 ± 0.07 mm), menunjukkan penamat bagi peringkat fleksi. Pada hari penetasan, purata jumlah panjang adalah 2.44 ± 0.28 mm dan mencapai 24.09 ± 0.01 mm ketika 35 HSP. Purata kadar tumbesaran larva adalah 0.62 $mm.day^{-1}$. Pembukaan saiz mulut pada hari ketiga selepas penetasan ialah 0.29 ± 0.05 mm dan 0.16 ± 0.03 mm, masing-masing bagi sudut bukaan 90 darjah dan 45 darjah.

Aktiviti tripsin dan kimotripsin pertama kali dikesan pada 2 HSP, masing-masing pada 0.0006 mU $mg^{-1}protein^{-1}$ dan 0.0005 mU $mg^{-1}protein^{-1}$. Aktiviti tripsin tertinggi adalah 0.0073 mU $mg^{-1}protein^{-1}$ pada 20 HSP dan tertinggi bagi kimotripsin adalah 0.0597 mU $mg^{-1}protein^{-1}$ pada 18 HSP. Nilai purata bagi tripsin (0.0022 mU $mg^{-1}protein^{-1}$) adalah rendah berbanding kimotripsin (0.0164 mU $mg^{-1}protein^{-1}$) bagi larva *E. Fuscoguttatus.* Tahap aktiviti kimotripsin yang lebih tinggi mungkin mencadangkan bahawa ikan ini

adalah omnivor pada peringkat larva. Beberapa turun naik telah dikesan pada 6 HSP, 20 HSP dan 29 HSP bagi aktiviti tripsin. Bagi aktiviti kimotripsin, turun naik telah dikesan pada 8 HSP, 20 HSP dan 29 HSP. Turun naik dalam aktiviti enzim menerangkan pembezaan dalam peringkat larva, tempoh bagi pembezaan morfologi dalam salur pencernaan dan juga organ-organ berkaitan. Menggunakan statistik Analysis of Variance (ANOVA), tiada berbezaan bererti di antara tripsin dan kimotripsin di mana $P>0.05$. Terdapat korelasi yang kuat di antara bukaan mulut dan aktiviti-aktiviti enzim diperhatikan dimana semakin besar bukaan mulut, semakin kurang aktiviti enzim yang dihasilkan dalam larva *E. Fuscoguttatus*. Menggunakan ujian korelasi Pearson (2-hujung), nilai korelasi adalah $r= -0.666$ dimana $p<0.01$ untuk tripsin dan nilai korelasi untuk kimotripsin adalah $r= -0.562$ dimana $P<0.01$.

Analisis histologi di dalam kajian ini menunjukkan bahawa mulut dan anus adalah tertutup pada 1 HSP (22.44 ± 0.28 mm). Pada 2 HSP(2.68 ± 0.01 mm), mulut telah terbuka tetapi masih tidak berfungsi sepenuhnya. Lengkungan insang rudimen telah kelihatan dibawah epitelium pada belakang rongga mulut. Ketika 3 HSP, saluran pencernaan telah membeza dari mulut hingga anus. Bagi *E. fuscoguttatus*, pyloric caeca primordial pertama kali muncul sebagai bonjolan pada anterior usus pada 7 HSP. Pada 12 HSP, sel mukus dan pencakar insang telah muncul di rongga farinks dan gigi farinks telah terbentuk. Tunas rasa juga diperhatikan di lipatan-lipatan epitelium. Di dalam eksperimen ini, pyloric caeca, kelenjar gastrik dan kantung buta telah terbentuk sepenuhnya pada 26 HSP. Walaupun larva tidak sepenuhnya membesar seperti dewasa,

sistem pencernaan dapat dibezakan dan berfungsi apabila semakin menghampiri akhir kajian. Berdasarkan kepada keputusan, kami mencadangkan bahawa larva *E. fuscoguttatus* boleh bermula untuk mampu mencerna dan mengasimilasi makanan sebatian secara intensif sekitar 23 HSP hingga 26 HSP. Di dalam kajian ini, saluran pencernaan yang berfungsi dapat dicapai pada 29 HSP. Perubahan di dalam aktiviti enzim pencernaan nyata sekali berkaitan dengan perkembangan morfologi bagi sistem pencernaan larva *E. fuscoguttatus*.