MOUTH AND GUT DEVELOPMENT OF MALAYSIAN RIVER CATFISH MYSTUS NEMURUS (CUVIER AND VALENCIENNES) LARVAE

GHADA AHMED EL HAG

FP 2000 2
MOUTH AND GUT DEVELOPMENT OF MALAYSIAN RIVER CATFISH
MYSTUS NEMURUS (CUVIER AND VALENCIENNES) LARVAE

By
GHADA AHMED EL HAG

Thesis Submitted in Fulfilment of the Requirements for the Degree of
Master of Science in the Faculty of Agriculture
Universiti Putra Malaysia.

January 2000
DEDICATION

To my ever dearest husband, Isam for his love, support

and encouragement throughout this study
Abstract of the thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirements for the degree of Master of Science.

MOUTH AND GUT DEVELOPMENT OF MALAYSIAN RIVER CATFISH
*Mystus nemurus* (CUVIER AND VALENCIENNES) LARVAE

By

GHADA AHMED ELHAG

January 2000

Chairman: Dr. Mohd. Salleh Kamarudin

Faculty: Agriculture

Ontogenetic changes in mouth and gut during larval developmental stage of Malaysian river catfish, *Mystus nemurus* were studied for 21 days to facilitate and determine the suitable feed and feed particle size for the growing larvae. The eggs began to hatch at day 2 after fertilization (DAF) and most of the larvae hatched within 2-4 DAF. The larval mouth was opened at the end of the first day after hatching (DAH) and the commencement of exogenous feeding was began on the 4 DAH after the movement of jaws. The barbels began to appear on the upper jaw and lower jaw on 3 DAH, and two small barbels appeared around olfactory pits by 5 DAH. Free neuromasts were observed under the lower jaw on 7 DAH and around the olfactory pits, the eyes, and upper operculum by the 9 DAH. Strong

\[ Ma = 5.5108 \, TL + 47.16, \, R^2 = 0.9302 \] at 45° opening and \[ Ma = 10.138 \, TL + \]
87.141, \( R^2 = 0.9302 \) at 90° opening) relationships were found to exist between mouth size and total length of fish.

The morphological and histological development of the gut in *M. nemurus* larvae was observed using a profile projector and a light microscope. During the yolk absorption period, the gut was a simple, straight, undifferentiated tube throughout its length. By 4-5 DAH, the gut differentiated to the oesophagus, stomach, and intestine. At the first feeding, the larval gut was functional, but was structurally and functionally less complex than that of adults. By the 13 DAH, four developed tissue layers (mucosa, submucosa, muscularis and serosa) were observed in the larval gut which are the characteristics of the gut of an adult vertebrate.
Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains.

PERKEMBANGAN MULUT DAN SALURAN PENGHADAMAN LARVA MYSTUS NEMURUS (CUVIER AND VALENCIENNES)

Oleh

GHADA AHMED EL HAG

Januari 2000

Pengerusi : Dr. Mohd. Salleh Kamarudin

Fakulti : Pertanian

Perubahan ontogenik pada mulut dan saluran penghadaman semasa peringkat perkembangan larva ikan baung, Mystus nemurus telah dikaji selama 21 hari untuk membantu dan menentukan makanan yang sesuai dan saiz partikel makanan yang terbaik untuk larva yang sedang membesar. Telur menetas pada hari ke 2 selepas persenyawaan (DAF) dan kebanyakan larva menetas dalam masa 2-4 DAF. Mulut larva terbuka pada akhir hari pertama selepas menetas (DAH) dan pemakanan eksogenous bermula pada 4 DAH selepas pergerakan rahang. Sesungut (barbel) mula kelihatan pada rahang atas dan rahang bawah pada 3 DAH, dan dua barbel kecil kelihatan di sekitar liang olfaktori pada 5 DAH. Neuromast bebas diperhatikan di bahagian bawah rahang bawah pada 7 DAH dan di sekitar liang olfaktori, mata dan operkulum atas pada 9 DAH.
Perhubungan linear yang kuku (Ma = 5.5108TL + 47.16, R² = 0.9302 pada bukaan 45° and Ma = 10.138TL + 87.141, R² = 0.9302 pada bukaan 90°) didapati wujud di antara saiz mulut dan panjang keseluruhan ikan.

Perkembangan morfologi dan histologi saluran penghadaman larva *M. nemurus* diperhatikan dengan menggunakan projektor profil dan mikroskop cahaya. Semasa tempoh penyerapan yolka, saluran penghadaman terdiri dari satu tiub yang ringkas, lurus, tanpa pembezaan pada keseluruhan panjangnya. Pada 4-5 DAH, salur penghadaman membeza kepada esofagus, perut dan usus. Pada pemakanan pertama, saluran penghadaman larva telah berfungsi tetapi secara struktur dan fungs, saluran itu adalah kurang kompleks berbanding dengan ikan dewasa. Pada 13 DAH, saluran mempunyai empat lapisan tisu yang sempurna perkembangannya (mukosa, submukosa, muskularis dan serosa) iaitu ciri yang terdapat pada saluran penghadaman vertebrata dewasa.
ACKNOWLEDGEMENTS

First and foremost, I am indebted to the Chairman of my Supervisory Committee, Dr. Mohd. Salleh Kamarudin of the Department of Agrotechnology, Faculty of Agriculture, UPM. His valuable contribution, intellectual guidance, input, careful supervision and constructive comments throughout the course of this study and during the preparation of the manuscript are greatly acknowledged.

I would like to express my most gratitude and appreciation to the members of my Supervisory Committee, Dr. Che Roos Saad (Institute of Bioscience) and Dr. Siti Khalijah Daud (Faculty of Science and Environmental Studies) for their valuable ideas, guidance and review of my work.

Furthermore, I would like to express my deep appreciation to the staff of Aquatic Resources Technology Laboratory, Institute of Bioscience, UPM particularly Jasni, Krishnan, Nordin and Zaidi for their help. I wish to acknowledge Universiti Putra Malaysia and the Malaysian Government for supporting the research and providing the Graduate Assistantship through Intensification of Research Priority Areas (IRPA) project No. 49-01-02-04.

It is a particular pleasure to express my sincere appreciation and special thanks to my colleague and friends Annie, Abeer, Paymon, Abdusamad, Asaduzzaman, Manuel Laron, Hamid Khoda Bakhsh, Albert Gabriel and Hanib for their hospitality and assistance. My special thank also goes to the staff of
Electron Microscopy Unit, Institute of Bioscience and Aquatic Animal Health Unit, Faculty of Veterinary Medicine, UPM for providing necessary facilities for histological studies.

Words are not enough to express my heartfelt feelings to my sons Mohamed and Razi who serve as my inspiration to strive for more. Last, but definitely not the least, I wish to express my utmost appreciation and love to my parents, brothers and sisters for their profound encouragement, and moral support throughout my course of study.

And above all, thanks to the God Almighty for all the blessings.
TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>TABLE OF CONTENTS</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>DEDICATION</td>
<td>ii</td>
</tr>
<tr>
<td>ABSTRACT</td>
<td>iii</td>
</tr>
<tr>
<td>ABSTRAK</td>
<td>v</td>
</tr>
<tr>
<td>ACKNOWLEDGEMENTS</td>
<td>vii</td>
</tr>
<tr>
<td>APPROVAL SHEETS</td>
<td>ix</td>
</tr>
<tr>
<td>DECLARATION FORM</td>
<td>xi</td>
</tr>
<tr>
<td>LIST OF TABLES</td>
<td>xiv</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td>xv</td>
</tr>
<tr>
<td>LIST OF ABBREVIATIONS</td>
<td>xvii</td>
</tr>
</tbody>
</table>

CHAPTER

I INTRODUCTION ................................................................. 1
   Background of the Study .............................................. 1
   Statement of the Problem ............................................ 2
   Objectives of the Study .............................................. 4

II LITERATURE REVIEW .......................................................... 5
   Mouth Morphology and Prey Selection ................................ 5
   Weaning of Larval Fish ................................................ 7
   Larval Nutrition .......................................................... 9
   Digestive Enzymes of Larval Fish .................................... 13

III GENERAL MATERIALS AND METHODS ..................................... 17
   Culture Tanks ............................................................ 17
   Experimental Animals ................................................... 17
   Feeding Protocol ......................................................... 18
   Water Management ........................................................ 19

IV MOUTH DEVELOPMENT OF MYSTUS NEMURUS (CUVIER AND VALENCIENNES) LARVAE .............................................. 20
   Introduction ............................................................. 20
   Materials and Methods .................................................. 22
   Results ................................ ..................................... 23
   Discussion ................................ .............................. 35
   Conclusion ................................ .............................. 39

V GUT MORPHOLOGY OF DEVELOPING MYSTUS NEMURUS (CUVIER AND VALENCIENNES) LARVAE .............................................. 40
   Introduction ............................................................. 40
   Materials and Methods .................................................. 42
   Results ................................ ..................................... 44
   Discussion ................................ .............................. 52
   Conclusion ................................ .............................. 55

xii
VI GUT HISTOLOGY OF *Mystus nemurus* (Cuvier and Valenciennes) Larvae

- Introduction: 56
- Materials and Methods: 57
- Results: 58
- Discussion: 69
- Conclusion: 73

VII GENERAL DISCUSSION: 74

- BIBLIOGRAPHY: 78
- APPENDICES: 94
- BIODATA OF AUTHOR: 100
LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Mean TL, UJ, LJJ, mouth gape (45° and 90° openings) and number of neuromasts</td>
<td>25</td>
</tr>
</tbody>
</table>
### LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Development in the mouth morphology with growth of <em>M. nemurus</em> larvae</td>
<td>26</td>
</tr>
<tr>
<td>2</td>
<td>Linear relationship between upper jaw length and total length of <em>M. nemurus</em> larvae</td>
<td>32</td>
</tr>
<tr>
<td>3</td>
<td>Linear relationship between lower jaw and total length of <em>M. nemurus</em> larvae</td>
<td>33</td>
</tr>
<tr>
<td>4</td>
<td>Linear relationship between mouth size and total length of <em>M. nemurus</em> larvae</td>
<td>34</td>
</tr>
<tr>
<td>5</td>
<td>Development in the gut morphology with growth of <em>M. nemurus</em> larvae</td>
<td>46</td>
</tr>
<tr>
<td>6</td>
<td>Linear relationship between gut length and day after hatching of <em>M. nemurus</em> larvae</td>
<td>50</td>
</tr>
<tr>
<td>7</td>
<td>Linear relationship between gut length and total length of <em>M. nemurus</em> larvae</td>
<td>51</td>
</tr>
<tr>
<td>8</td>
<td>Longitudinal section of the esophagus on 5 DAH. (Bouin’s; H&amp;E; X320)</td>
<td>61</td>
</tr>
<tr>
<td>9</td>
<td>Longitudinal section of the esophagus on 7 DAH. (Bouin’s; H&amp;E; X285)</td>
<td>61</td>
</tr>
<tr>
<td>10</td>
<td>Longitudinal section of the esophagus on 9 DAH. (Bouin’s; H&amp;E; X322)</td>
<td>62</td>
</tr>
<tr>
<td>11</td>
<td>Longitudinal section of the esophagus on 13 DAH. (Bouin’s; H&amp;E; X311)</td>
<td>62</td>
</tr>
<tr>
<td>12</td>
<td>Longitudinal section of the esophagus on 15 DAH. (Bouin’s; H&amp;E; X281)</td>
<td>63</td>
</tr>
<tr>
<td>13</td>
<td>Longitudinal section of the stomach on 5 DAH. (Bouin’s; H&amp;E; X285)</td>
<td>64</td>
</tr>
<tr>
<td>14</td>
<td>Longitudinal section of the stomach on 7 DAH. (Bouin’s; H&amp;E; 400)</td>
<td>64</td>
</tr>
</tbody>
</table>
15  Longitudinal section of the anterior stomach on 9 DAH. (Bouin’s; H&E; X258) ................................................................. 65

16  Longitudinal section of the posterior stomach on 9 DAH. (Bouin’s; H&E; 233) ................................................................ 65

17  Longitudinal section of the posterior stomach on 13 DAH. (Bouin’s; H&E; X333) .............................................................. 66

18  Longitudinal section of the intestine on 5 DAH. (Bouin’s; H&E; X 266) .......................................................... 67

19  Longitudinal section of the intestine on 7 DAH. (Bouin’s; H&E; X 266) .......................................................... 67

20  Longitudinal section of the intestine on 9 DAH. (Bouin’s; H&E; X 364) .......................................................... 68

21  Longitudinal section of the intestine on 13 DAH. (Bouin’s; H&E; X 311) .......................................................... 68
LIST OF ABBREVIATIONS

BW = Body Weight
C&V = Cuvier and Valencinnes
DAF = Day After Fertilization
DAH = Day After Hatching
DO = Dissolved Oxygen
G = Goblet cell
GL = Gut Length
H & E = Haematoxylin and Eosin
GS = gastric gland
L = Lamina propria
LJ = Lower Jaw
M = Mucosa
MA = mouth size
MU = Muscularis
RGI = Relative Gut Index
S = Submucosa
SCR = Specific Growth Rate
SE = Serosa
SEM = Scanning Electron Microscopy
TL = Total Length
UJ = Upper Jaw
UPM = Universiti Putra Malaysia
CHAPTER I

INTRODUCTION

Background of the Study

Malaysian river catfish, *Mystus nemurus* (C & V) or locally known as “baung” is an edible species preferred by all ethnic races in Malaysia (Khan et al., 1990). Seven other species of *Mystus* found in Malaysian waters are *M. nigriceps*, *M. planiceps*, *M. micranthus*, *M. wyckii*, *M. guhio*, *M. wolffii* and *M. bimaculatus* (Lim et al., 1993). *M. nemurus* is the most popular and is the largest of local Mystides (Smith, 1945). The species has a wide range of distribution, from the East Indies to South-east Asia including Peninsular Malaysia, Indo-China and Thailand. The fish is a bottom feeder and feeds extensively on a wide range of food items which include teleosts, crustaceans, benthic invertebrates and detrital materials (Khan, 1987). *M. nemurus* are monogenous and sexes cannot be differentiated in fishes less than 18 cm in sizes (Khan et al., 1990).

Recently, the interest has been growing rapidly in both its intensive and extensive domestication (Kamarudin, 1999; Khan et al., 1990). However, inadequate seed supply coupled with a relatively high fingerling price limit its production. Large scale rearing *M. nemurus* larvae has yet to be refined in terms of husbandry techniques and nutritional requirements of the larvae.
Statement of the Problem

The demand for fish is expanding rapidly and exceeding the supply due to a steadily increase of population and income. Aquaculture is the logical answer to meet that demand as wild resources are declining due to over fishing, pollution, etc. while the cost of fishing is increasing. With the increasing numbers of grow-out ponds and changes towards intensive and semi-intensive culture system, a greater and more consistent supply of seed from hatcheries has to be obtained.

In recent years an increasing attention has been given to the survival of early stages of fish larvae as a successful mass-production is the principal requirement in fish culture (Senoo et al., 1994). In some species, high mortality during the egg and larval stages is a fundamental aspect for the assessment of fish production (Lasker, 1984). Fish larvae are the smallest autonomous actively feeding vertebrates (Wieser, 1995). Heavy mortality usually occurs early in the life history if the suitable larval food is scarce, especially when the yolk sac is exhausted and the larvae must begin active feeding (Iwai, 1980). Therefore, one of the most important factors in fish larviculture is the provision of a suitable food. Good quality feeds can give poor results, unless proper feeding practices such as the right ration, feeding frequency, and feeding methods are used. In the absence of adequate food, fish larvae eventually reach a point of no return, which is defined by Blaxter and Ehrich (1974) as the point at which only 50% of larvae
are still able to feed if sufficient food become available. Even under optimum conditions, the other 50% are no longer capable of taking up food.

Feeding of fish is strongly influenced by food particle size in relation to mouth size and gut structure of larvae. Mouth size appears to be the limiting factor in larval feeding of both natural and artificial diets (Hyatt, 1979). Once the prey has been located, it must be captured and the first problem to be overcome is getting the food item into the mouth. Strong relationships between the mouth size and the suitable size of food during larval stages have been documented (Shiota, 1970, 1978a, 1978b; Dabrowski and Bardega, 1984).

There is also a relationship between the structure of alimentary canal and the nature of feeding (Bond, 1996). The poor growth of some fish larvae is due to the under development of their digestive tract (Kaushik and Luquet, 1993). Preferred food types require different sets of morphological and physiological adaptations for ingestion, digestion and absorption of nutrients (Hirst, 1993). There may be marked differences in the morphology and histology of the guts among fish species, and there may be differences in the profiles of the digestive enzymes present. There are also differences in the morphology and physiology of fish species that feed upon different prey types (Horn, 1989). It is well established that the main objective of fish culture is to maximize survival and growth, which accordingly are measured to evaluate the effects of rearing technologies.
Reports on the relationships between the mouth size, gut morphology and the suitable size of food during larval stages are not available for most fish species. However, there is no available information in the literature that suggests such relationships in *M. nemurus* larvae. There is also no published information on the mouth and gut development of larval fish among the close relatives of *M. nemurus* or genous *Mystus*.

Although the seed production of *M. nemurus* has been routinely carried out in a few Malaysian hatcheries, there is still a need for more research on the early stages of fish to solve most of the problems related to its feeding during those stages. It is envisioned that this study will pave the way towards to facilitate a better understanding of the development of mouth and gut of *M. nemurus* and its feeding behaviour, which in turn will lead to the development of larval diets and an increase of its seed production to ensure sustainable and adequate supply of juveniles.

**Objectives of the Study**

(1) To establish information on the mouth development of *M. nemurus* larvae, which will help to prepare a suitable feed size during this stage.

(2) To study the development of gut morphology and histology of *M. nemurus* larvae.
CHAPTER II
LITERATURE REVIEW
Mouth Morphology and Prey Selection

Prey size and the availability of prey of suitable size are two important factors for a successful initiation of exogenous feeding of larval fish (Hunter, 1980, 1981). With most predator-prey relationship, morphological characteristics of predator feeding apparatus are important constraints that determine the maximum prey size (Holling, 1964; Dabrowski and Bardega, 1984; Kusano et al., 1985).

Mouth gape is the physical limitation for prey selection for larval fish (Pepin and Penney, 1997). Adult rotifers are too large for most species of groupers (Lim, 1993) including *Epinephelus tauvina* (Hussain and Higuchi, 1980) and *E. amblycephalus* (Tseng and Chan, 1985) since epinephelid larvae have a relatively small mouth (Fukuhara, 1989). Ross (1978) reported that a switch to larger prey by leopard searobin *Prionotus scitulus* is preceded by a rapid increase in mouth size and intestinal length. To facilitate prey capture by smaller and weaker larvae, a mixed prey regime may improve nutritional quality of prey (Watanabe et al., 1996). For artificial diets, the feed particle size must be chosen by considering the small mouth size of fish larvae. However, the selection
of a very small feed particle can lead to nutrient leaching problems caused by a very high ratio of surface area-volume (Kim et al., 1996).

The size and number of prey increase with larval size in two species of co-occurring fish larvae: Callinoymidae and Bothidae (Sanchez, 1998). Similarly, Boube and Ward (1997) noted that the mean size of the dominant prey of common smelt (Retropinna retropinna) larvae increases as smelt mouth gape increases. Ross (1978) reported that the selection of prey by the larvae of leopard searobin P. scitulus depends upon the size of prey and larval age or size. They found that the larvae below 4.3 mm total length (TL) prefer small rotifers while larvae above 5.1 mm TL prefer larger rotifers. The importance of an optimum food particle size as a factor that influences feeding efficiency has been reported by several authors (Wankowski, 1979; Wankowski and Thorpe, 1979; Knights, 1983; Dabrowski et al., 1983 cited in Hasan and Macintosh, 1992; Hasan and Macintosh, 1992). Hasan and Macintosh (1992) reported that the size of food particle most quickly consumed by carp larvae increases with body size. Thorpe and Wankowski (1979) found that the most preferred food particle size for juvenile Atlantic salmon, Salmo salar L., is about 0.3-0.4 of mouth size. Coregonid, Coregonus pallan Thompson, larvae select zooplankton equivalent to 0.4-0.6 of fish mouth size under laboratory condition (Dabrowski, 1984). According to Khadka and Rao (1986) common carp larvae (8 days old) prefer intermediate size prey when offered zooplankton of three different sizes (83 ± 10, 198 ± 10 and 277± 40 μm).
Walford et al. (1991) showed that at the first feeding seabass (*Lates calcarifer*) larvae only ingested the microcapsule diets of 40-60 μm from the range of 15-150 μm offered. Thorisson (1994) found that 10-14 mm long cod larvae seems only able to eat relatively small prey but the size and amount of prey increases with the larval size.

**Weaning of Larval Fish**

Weaning is a critical stage in larviculture because a successful transition from a live food to a prepared feed is dependent on feed quality and the larval themselves (Deversse et al., 1991). Weaning is important for aquacultural purposes as it is economically advantageous and dry feed can be stored at room temperature or refrigerated or frozen for a long period (Lee, 1996).

Much of the labour, cost and time spend in rearing and dispensing of live food can be eliminated as commercial dry feed can be delivered by automatic feeders (Bromely, 1981). The best time to initiate weaning is at the start of exogenous feeding. However, this is not always feasible for a number of reasons. Among them are many larval species have incomplete digestive systems and there is no available dry diet which is thoroughly digestible and nutritionally balanced (Person-Le Ruyet, 1991; Walford et al., 1991; Person-Le Ruyet et al., 1993).