THE DEVELOPMENT OF SENSORY ORGANS IN AFRICAN CATFISH, *Clarias gariepinus* DURING LARVAL TO JUVENILE STAGE

MAZFARINA BINTI MUSTAFA

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Alamat Tetap: LOT 60, KG. BIAH JLN PEROL, 16010 KOTA BHARU, KELANTAN

DR. YUKINORI MUKAI
Nama Penyelia

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DECLARATION

I declare that this dissertation is the result of my own independent work except where otherwise stated.

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MAZFARINA MUSTAFA

HS2005-1755

AUTHENTICATION

I/We, members of the Dissertation Supervisory Committee, certify that this dissertation has fulfilled all the research requirements for the Bachelor Degree in Science with Honours, has been verified and is qualified for examination.

Members of Dissertation Supervisory Committee

Signature

1. **SUPERVISOR**

   Dr. Yukinori Mukai

2. **EXAMINER 1**

   (Muhammad Ali Syed Hussein)

3. **EXAMINER 2**

   (Masaru Tanaka)

4. **DEAN OF SST**

   Supt/Ks. Prof. Madya. Dr. Shariff A. Kadir S. Omang

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ABSTRAK

Kajian ini menjelaskan perkembangan organ deria larva keli Afrika, *Clarias gariepinus* dan bertujuan untuk memahami ekologinya serta memperbaiki kaedah pengkulturan larva. Telur diperoleh dari Institut Penyelidikan Marin Borneo dan larva keli Afrika dikultur dari peringkat penetasan sehingga juvenil. Penyampelan dilakukan setiap hari dan perkembangan organ derianya diperhatikan dengan menggunakan mikroskop cahaya. Larva diberi makan rotifer, Artemia *nauplii* dan serbuk makanan tiruan. Panjang keseluruhan larva keli Afrika pada peringkat 20, 25 dan 30 hari adalah 16.85±1.53mm, 19.17±1.48mm dan 24.19±2.61mm (purata ± S.D.). Dalam usia ini, larva telah mempunyai mata berpigmen. Nisbah cone-nuclei bertambah dengan pertumbuhan larva. Nisbah mencapai 1.0 semasa larva berusia 20 hari. Peningkatan nisbah cone-nuclei menunjukkan kewujudan sel rod di dalam retina keli Afrika. Peningkatan ketebalan lapisan nuklear luar dengan pertumbuhan larva menunjukkan peningkatan dalam kepadatan rod. Sebagai hasil daripada pertambahan kelebaran lapisan pigment epithelium, lapisan sel penglihatan dan lapisan nuklear luar, kawasan dasar menjadi lebih tebal berbanding dengan kawasan lain di dalam retina kerana nuklear sel penglihatan di kawasan dasar menjadi lebih panjang. Berkait dengan kelakuannya, larva keli Afrika sentiasa tinggal di kawasan tengah dan dasar tangki. Mereka menunjukkan fototaksis negatif. Kewujudan sel rod menunjukkan larva keli Afrika lebih suka tinggal di persekitaran yang gelap. Pertumbuhan organ olfaktori meningkat dengan pertumbuhan larva menunjukkan larva keli Afrika bertindak balas dengan rangsangan kimia pada peringkat awal larva. Larva menunjukkan kelakuan kemotaksis yang kuat apabila larva diberi makan dengan serbuk makanan tiruan. Reseptor perasa dijumpai di bahagian barbel, mulut, rongga mulut dan di bahagian ranker insang menunjukkan larva keli Afrika dapat memperbaiki kelakuan pemakanannya kerana reseptor perasa membenarkan larva untuk memilih antara jenis makanan. Larva keli Afrika juga menunjukkan reotaksis positif yang kuat. Pertumbuhan deria organ yang disertai dengan perubahan kelakuan membawa implikasi penting untuk ekologi larva di habitat semulajadinya dan pengkulturan larva keli Afrika.

ABSTRACT

DEVELOPMENT OF SENSORY ORGANS IN AFRICAN CATFISH, *Clarias gariepinus* DURING LARVAL TO JUVENILE STAGE

This study was conducted to clarify the development of the sensory organs with larval growth in African cafish, *Clarias gariepinus* for the purpose of understanding its ecology as well as to improve the larval rearing techniques. Eggs were collected from Borneo Marine Researched Institute and the larvae of African catfish were reared from hatching to juvenile stage, sampled every day and observed under light microscope for the development of the sensory organs. Larvae were fed with rotifers, *Artemia nauplii* and artificial powder feed. The 20, 25 and 30-day-old larvae were 16.85±1.53mm, 19.17±1.48mm and 24.19±2.61mm (mean ± S.D) in total length (TL). During these days after hatching, the eye already deeply pigmented. The nuclei-cone ratio was increase with larval growth. The ratio exceeds 1.0 when the fish were 20-day-old. The cone-nuclei ratio was increase showed that the appearance of rods cell in the retina of the African catfish. The thickness of the outer nuclear layer in the retina also increases with larval growth showing an increase in rod density. As a result of the width added from the pigmented epithelium layer, visual cell layer and outer nuclei layer, the bottom area became thicker than other areas of the retina because the visual cell nuclei of the bottom area became longer than those in other areas. Related to its behaviour, African catfish larvae mostly stayed at the middle and bottom part of the tanks and they showed negative phototaxis. The appearance of rods cell indicates that the larvae of African catfish preferred to stay in dark environment. The development of olfactory organs increased with larval growth showed that African catfish response to chemical stimuli in the early larval stage. They showed strong chemotaxis behaviour when they were fed with artificial feed powder. The taste buds were found in barbels, mouth, buccal cavity and gill rankers showed that the African catfish larvae can improve their feeding behaviour because taste buds allow larvae to choose among food items. The African catfish larvae also showed the positive rheotaxis behaviour in this larval stage. The development of the sensory organs is accompanied by behavioural changes that have important implications for larval ecology at their natural habitat and African catfish larval rearing.

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LIST OF ABBREVATIONS

cm	centimetre
µm	micrometre
d	day
dAH	days after hatching
DO	dissolved oxygen
kg	kilogramme
g	gramme
x	multiply
λ_{max}	wavelength maximum
L	litre
ml	mililitre
mm	millimetre
no.	number
ppt	part per thousand
TL	total length
BL	body length
SL	standard length
HL	head length
BH	body height
BR	body round
BWidth	body width
BWt	body weight
/	per
S.D	standard deviation
µm	micrometre
%	percentage
^{0}C	degree celcius
±	more or less
$IUkg^{-1}$	international unit per kilogramme
ONL	outer nuclear layer
INL	inner nuclear layer
PE	pigmented epithelium
PR	photoreceptor cell
L	lens
hc	horizontal cell
ipl	inner plexiform layer
gl	ganglion layer
vc	visual cell

CHAPTER 1

INTRODUCTION

1.1 Aquaculture Industry in Malaysia

Aquaculture industry in Malaysia considered as new, contributing nearly 12% of total landings. Production from the aquaculture was recorded at 169,449 metric tonnes valued at RM 1,047.2 million in 2001. The production is from a culture area of 23,227 ha. In Third National Agricultural Policy (NAP3) it is expected that the production would increase to 600,000 metric tonnes by 2010. To achieve this target aquaculture has to be streamlined in terms of investment of capital, technology and human resources. Thus, in NAP3 greater attention has been to aquaculture production through commercialization of the sector with private and corporate sectors participation.

The environmentalists have constantly criticized aquaculture development as a sector of non-sustainable and environmental degradation. So, the industry possess critical constrains in its development. The key issues are limitation of land resources and the water bodies as uses for the other purposes. Other issues are pollution and environmental degradation, healthy and quality of seeds, disease, high production costs, indiscriminate use of chemicals, drugs and antibiotics. Government has identified certain areas for sustainable aquaculture production and makes it environmental friendly. These are, (i) zoning of potential aquaculture areas (AIZ), (ii) legislation for aquaculture, (iii) guidelines for aquaculture development, (iv) code of practice for aquaculture, (v) farm accreditation and (vi) better extension services.

1.2 African Catfish, *Clarias gariepinus*

Photo 1.1: African catfish, *Clarias gariepinus*.

Catfishes constitute a large group of primarily freshwater fishes which are widely distributed throughout the world. They reach their greatest diversity in the continents spanning the equator, namely South America, Africa and Asia. They are especially diverse in the larger rivers such as the Amazon and Zaire, in each of which several hundred species are found. Catfish are classified in the order Siluriformes which includes such familiar fishes as the bullheads, squeakers, electric catfish, sea barbel and armoured catfishes, as well as less familiar forms such as the doradids, plotosids, pimelodids and callichthyids. There are about 2000 species of catfishes in the world (about 8% of the total number of fishes). Most African catfishes are either too small or too difficult to culture or would encounter too much consumer resistance to be successful aquaculture candidates.

There are only three African Siluroidea families which contain some species which could be considered suitable for food fish aquaculture; one of which is *Clarias gariepinus*. The distinguishing characteristics of *Clarias gariepinus* are: Head large and bony with small eyes. Dorsal and anal fins long. No adipose fin. Pectoral fin with stout serrated spine, used for defence or "walking" overland. Mouth terminal, large. Four pairs of barbels present. Colour varies from sandy-yellow through gray to olive with dark greenish-brown markings, belly white. Well developed suprabranchial organ present. The principal feature which distinguishes *Heterobranchus longifilis* from *Clarias gariepinus* is its prominent adipose fin.

Clarias gariepinus can best described as an omnivore, often feeding on vegetable matter, aquatic invertebrates, small fish and detritus. Though it normally survives on dissolved oxygen, it comes to the surfaces and breathes atmospheric air when the oxygen concentration becomes low. A high degree of hardiness, the ability to feed on a variety of foodstuff, good growth and survival in poorly oxygenated water have made it an attractive fish for aquaculture. It is also resistant to diseases. So, the survival rate of this species is considered high but it is depends on environmental factors and cultural methods.

The species can also grow in brackish water in salinities of 10 ppt and survive in salinities up to 29 ppt. The species is one of the well-known freshwater species in Malaysia. It tastes good and Malaysian of all races, like to eat this fish. It can be cooked in Malay, Chinese and Indian dishes.

1.3 Problems on Larval Rearing of African Catfish

Although African catfish is suitable for mass production, there are several problems faced in culturing it. Main problem is they show cannibalism behaviour. This problem occurred because of feed sufficiency. When there are not enough feed, they will eat their own kind. This caused the amount of fish cultured to decrease and the percentage of survival rate does not high. So, the production of the seeds can not be maximised. It is important to improve larval rearing methods in African catfish to develop the aquaculture industry.

Other problem is African catfish faced the air bubbles in their stomach. It happens in the juvenile stage of African catfish. The air in the stomach can see be seen clearly. Their swimming behaviour would change. The air in the stomach prevent them to swim as usual because they had lost their balance in swimming. They gather at the surface of the tank and their activity will decrease. So, we have to find a way how to prevent this problem. We have to study the morphological features, their behaviour and also their sensory organs to successfully culture of this species.

In larval to juvenile stage, they are very sensitive and easily die. It is called critical period for the fish. We called this stages is critical period because during this time their organs growth rapidly. Their organs fully develop during this period. They easily get stress because of handling, feeding management or water management. So, the suitable condition is very important to maintain the health of the fish. Regular observation must be done to ensure the condition of the fish.

1.4 Study on Sensory Organ and Development and Behaviour Changes in African Catfish Juveniles

This study is conducted to find possible relationships between developmental change in morphology of sense organs and behaviour during early stages of African catfish, *Clarias gariepimus*.

Accompanying changes in behaviour such as feeding, schooling, phototactic and rheotactic behaviour, of the fish with growth were observed. For the histological observation of sense organs, the fish were fixed in Bouin's solution. Employing standard methods, embedded fish were cut 6 μm thick transversally and longitudinally. All sections were stained by standard method and observed under a microscope. This study is supported by The Ministry of Higher Education under Fundamental Research Grant. The research title: Studies on development of sensory organs and behavioural changes in the larvae of Grouper and other species of aquaculture importance. The project code is FRG0002-ST-1/2006.

1.5 Objectives of Study

All the three factors are correlative; behavior, sensory organ and morphological development. The objectives are:

1) This study is conduct to understand the morphological changes in African catfish juveniles.
2) To understand how behaviour changes with larval growth.
3) To study the function of sensory organ in African catfish that is related to its behavior.

CHAPTER 2

LITERATURE REVIEW

2.1 Sensory system

2.1.1 Eyes

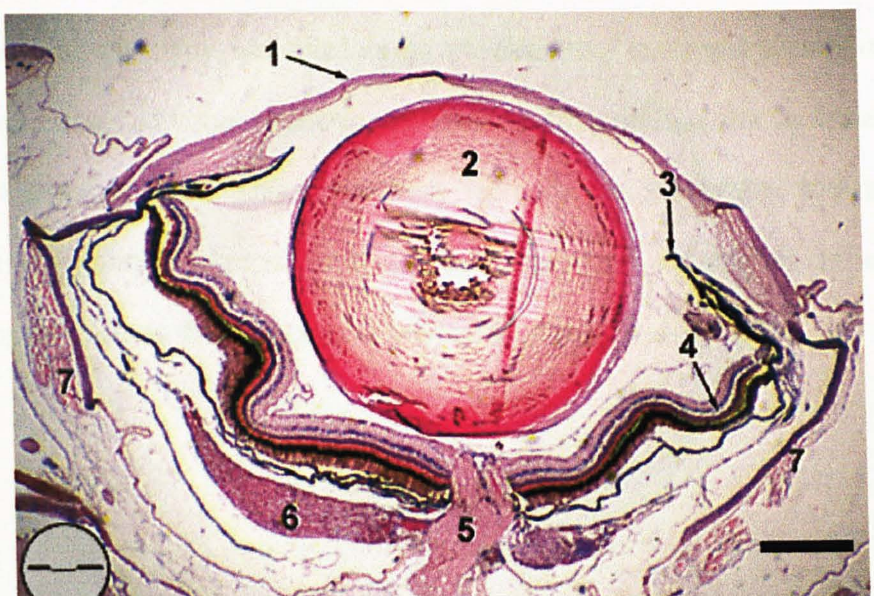

Photo 2.1: Eye, transverse section (Formalin, H&E, Bar = 335). 1, cornea; 2, lens; 3, iris; 4,retina; 5,optic nerve; 6, choroid body; 7, sclera. Scale bar, 335µm

The eyes of most teleosts are oriented and desirable so that light can enter from almost any direction, above, below, forward, or behind. In front, a fish view an object with both eyes to create binocular vision and improved depth perception. Many species can use ocular movements to adjust size of the binocular field. Most fishes can see objects with only one eye, where depth perception is personably compromised.

Light entering the eye is focused on the retina, a spherical lens that moves away from or toward retina to accommodate objects near or far. The central circuitry from the brain extends through the optic nerve to the retina, which is composed of layer distinctive neural cells (ganglion, bipolar, photoreceptors). The major classes of photoreceptors are cones and rods. Vision under bright conditions (photopic vision) immediated by cones, and rods take over the visual function when light levels are low (scotopic vision). Both types of photoreceptors operate by photoactivation of visual pigments. Visual pigments generally respond to a broad range of wavelengths but have a peak of sensitivity, identified as the wavelength of maximum absorption, λ_{max}. up to four classes of visual pigments, each with a different $\lambda_{max,}$ can be found in the different types of cones. This is the basis for color vision. Species with a single pigment discriminate objects from the background by differences in contrast; wavelengths closer to their λ_{max} appear brighter than other wavelengths.

2.1.2 Olfactory organs

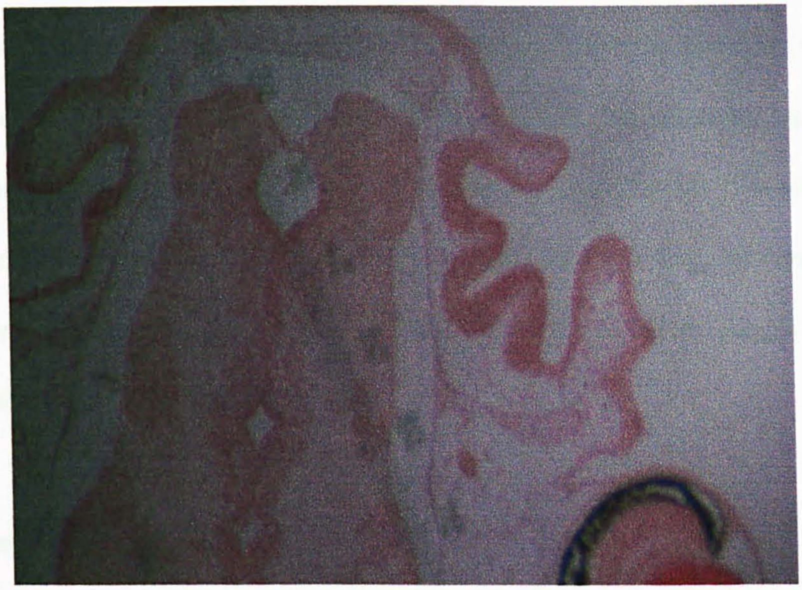

Photo 2.2: Olfactory organ in fish

The olfactory organ in fishes, as in other vertebrates, is located in the snout, where water, often aided by a groove or skin flap, passes over receptors in the epithelium, which is arranged in folds or lamellae, often resembling a rosette (Kleerekoper, 1969). Water passes through the organ either by movements of cilia located on supporting cells in the ephithelium or by muscular pumps associated with accessory sacs (Bardach and Villars, 1974; Doving *et al.*, 1977). The ultrastructure of the epithelium, bulb, and tract of the olfactory system is reviewed in detail by Hara (1993). For teleosts larvae, several studies have demonstrated that the receptor cells in the olfactory epithelium develop quickly (summarized by Doving and Knutsen, 1993) The sensory regions of the epithelium, in the majority of teleosts studied, contain at least two types of bipolar neurons, ciliated and microvillous, which are classified on the basis of their dendrites.

Differences in physiological function among the cell types are unclear. The axons of these bipolars, forming the olfactory nerve fibers, lead to the rostral end of the olfactory bulb, where they spread across its surface. The bulb, thought to process the bulk of olfactory signals (Bruckmoser, 1973), projects via olfactory tract to the telencephalon and minor components to the ventral diencephalon (Finger, 1988). Olfactory acuity appears to be correlated with the numbers and arrangement of olfactory lamellae in the nasal as well as the area covered by sensory supporting units in the epithelium (Bardach and Villars, 1974).

2.1.3 Taste buds

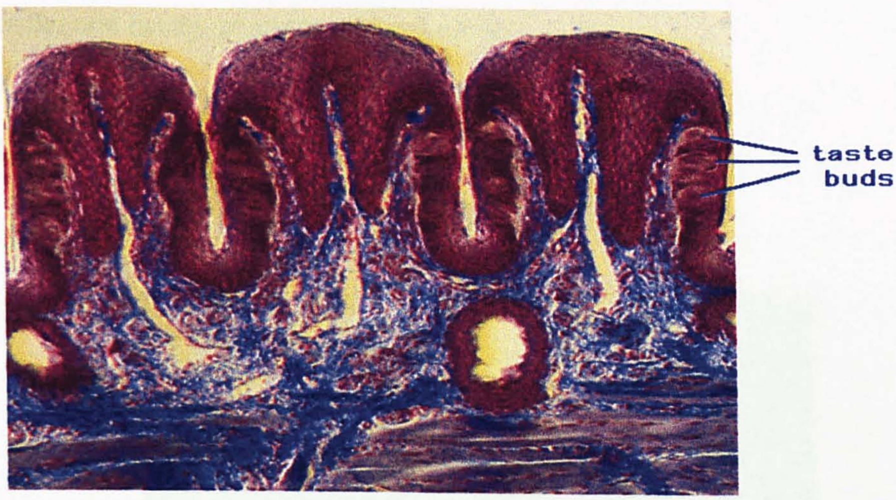

Photo 2.3: Section of taste buds in epithelium tissues.

Fish taste buds, located in the epidermis; vary between 30 and 80 μm in length and 20 and 50 μm in width. Generally, two types of microvillous cells, along with a basal cell, are routinely described in the taste buds of teleosts. One of the former, the so-called light cell, possesses a large club-shaped microvillus at its apical surface and is generally accepted as a gustatory receptor cell because of its synaptic contacts with nerve processes within the taste bud (Caprio, 1988). The other type of cell, the so-called dark cell, possesses the numerous small microvilli and has been considered a supporting cell. Reutter (1978) found, however, that dark cells in catfishes also form synapses with nerve terminals within taste buds. Basal cells, two to five per taste bud, are located in the base of the bud, resting against the basal lamina. Their function is unknown, but they may identify gustatory receptor activity through synapses with both light and dark cell, at least in ameirid catfishes (Reutter, 1982).

Although the gross morphology of the taste buds of fishes is similar, caution about generalizations regarding vertebrate taste buds is suggested based on differences in their ultrastructure (Reutter and Witt, 1993). Taste buds are generally distributed within the oral cavity, pharynx, and gill rakers.

2.1.4 Lateral line

Photo 2.4: Free neuromast from lateral line system.

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Foto sensory organ ; Source from Miss Diyana bt Ramli. Free neuromast and olfactory organs of patin, *Pangasius hypopthalmus*, Borneo Marine Reseach Institute, University Malaysia Sabah, Kota Kinabalu, Sabah. Pp. 46-49.

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