DICHROMATIC AFRICAN CATFISH *Clarias gariepinus* LARVAE AND JUVENILEs HAVE COLOUR VISION BUT LOSE IT IN LOW LIGHT INTESITY AT 1 LUX

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THIS DISSERTATION IS SUBMITTED IN FULFILLMENT OF THE REQUIREMENTS TO GRADUATE AS A BACHELOR OF SCIENCE WITH HONOURS

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Abstract

DICHROMATIC AFRICAN CATFISH *Clarias gariepinus* LARVAE AND JUVENILES HAVE COLOUR VISION BUT LOSE IT IN LOW LIGHT INTESITY AT 1 LUX

Freshwater catfish species have been believed to be dichromats which have only 2-photopigment system, green and red. However, ontogeny of colour vision of dicromats has never been studied. Using the classical conditioning method, this experiment determined colour vision in African catfish *Clarias gariepinus* larvae and juveniles and the effect of light intensity on colour vision. Farmed larvae and juveniles, 3 (6 ± 0.2 mm) days and 25 days old (26.6 ± 1.8 mm) respectively, were presented with a palette with 8 wells at the bottom of 4 aquaria (10 larvae or 10 juveniles per aquarium). One of the wells of each pallet was painted green or red (colour of conditioning, CC) and the other 7 wells were painted with different shades of grey. The fish were conditioned to associate the green or red well with a feed illumination. Conditioning trials were repeated 1 time daily under the illuminance of 337 – 2396 lux. After every 10 feeding trials, fish were presented with the pallet without the feed reward (conditioning test) and fish response to the pallet was video-recorded and frequency of visits by the fish to each well was counted. The larvae showed significantly biased conditioned response to CC after 20 trials in 2 aquaria and after 40 or 50 trials in the other aquaria ($P<0.05$ or $P<0.01$) and achieved Correct Respond Ratio (CRR) ranged from 36 to 41% after 100 trials. The juveniles were conditioned earlier and showed higher CRR than the larvae. The juveniles exhibited significantly biased response to CC after 10 feeding trials in the all aquaria (chi-square test, $P<0.01$), that is, they clearly discriminated CC based on chromaticity. The CRR ranged from 60 – 100% after 65 feeding trials. The conditioned juveniles of two aquaria did not exhibit the conditioned response under the illuminance of 1 lux in a dark room. They were retrained with the same conditioning method under 1 lux but the conditioning was unsuccessful even after 65 trials (13 days), indicating colour blind under this illuminance. It is concluded that the dichromatic African catfish have colour vision at 5 day old, and it is unlikely that the retinal tapetum formed in the pigment epithelial layer at 3 day old greatly increases the chromatic sensitivity of cones.

ABSTRAK

Spesies ikan keli air tawar dipercayai merupakan dikromatik yang mempunyai hanya 2 sistem photopigment, iaitu hijau dan merah. Walaubagaimanapun, pertumbujan penglihatan warna bagi ikan dikromatik tidak pernah dikaji. Dengan menggunakan kaedah klasik, eksperimen menentukan penglihatan warna larva dan juvenil keli Afrika *Clarias gariepinus* dan kesan keamatan cahaya pada penglihatan warna. Larvae yang berlahir selepas 3 hari (6± 0.2 mm) dan juvenil yang lahir selepas 25 hari (26.6 ± 1.8 mm) dikaji untuk memdiskriminasi warna hijau atau merah dengan kelabu. Palet bulat yang mempunya 8 cekung digunakan untuk melatih larva dan juvenil keli Africa untuk mendiskriminasi warna. Salah satu cekung dicat dengan warna hijau atau merah, manakala cekung yang lain dicat dengan warna kelabu yang berbeza (Kelabu 30 – 90%). Makanan ikan diletakkan dalam cekung yang berwarna hijau atau merah untuk membiasakan ikan keli mengkaitkan warna hijau atau merah dengan makanan. Cahaya di bawah bumbung hatcheri yang menternak ikan adalah dalam 337 – 2396 lux. Selapas setiap 10 kali memberi makanan dalam palet, palet tanpa ganjaran makanan akan dimasukkan dalam setiap tangki ikan. Tindak balas ikan terhadap cekung palet akan direkod dengan video dan kekerapan lawatan oleh ikan terhadap setiap cekung dikira. Larva menunjukkan tindak balas berat sebelah terhadap cekung yang berwarna selepas 20 percubaab dalam 2 akuarium dan selepas 40 atau 50 perbicaraan dalam akuarium yang lain ($P < 0.05$ atau $P < 0.01$) dan mencapai Correct Respond Ratio (CRR) di antara 36 – 41% selepas 100 percubaan. Juvenil dapat mendiskriminasi warna dan menunjuk nisbah tindak balas CRR yang lebih tinggi berbanding dengan larva. Juvenil bertindak berat sebelah kepada warna selepas 10 percubaan makan dalam semua akuarium (ujian chi-square, $P < 0.01$). CRR adalah di antara 60 – 100% selepas 65 perubaan melatih juvenil untuk makan di cekung palet yang berwarna. Juvenil dalam dua akuarium, G1 dan R1 tidak menunjukkan tindak balas terhadap warna dalam pencahayaan 1 lux di dalam bilik gelap. Ikan ini dilatih semula dengan kaedah yang sama seperti di hatcheri BMRI tetapi bawah cahaya 1 lux. Walaubagaimanapun, juvenil keli Afrika tidak berjaya menentukan warna selepas 65 ujian dalam tempoh 13 hari, menunjukkan buta warna dalam cahaya 1 lux. Larva keli Afrika yang lahir selepas 5 hari mempunyai visi warna dan retinal tapetum yang dijumpa dalam larva yang berlahir selepas 3 hari tidak membantu dalam meningkat sensitif mata terhadap warna dalam kegelapan 1 lux.

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LIST OF SYMBOLS

ppt	parts per trillion
%	percentage
µm	micrometer
DO	Dissolved Oxygen
mm	millimetre
L	Litre
cm	centimetre
°C	Degree Celcius
G	gram
ml	milliliter
sp.	species
nm	nanometer

LIST OF ABBREVIATIONS

dAH	Days After Hatching
CRR	Correct Respond Ratio

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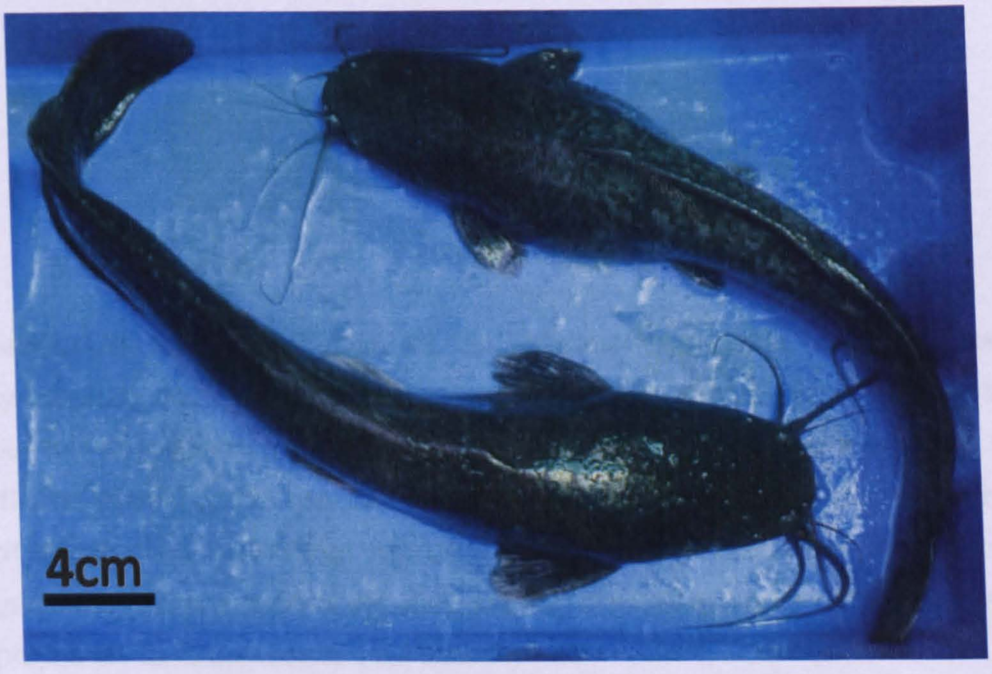
CHAPTER 1

INTRODUCTION

1.1 Targeted Species



Picture 1.1 African catfish (*clarias gariepinus)*

African catfish, *Clarias gariepinus* (Picture 1.1) is a fresh water species which can be found over the world. African catfish is also known as walking catfish as they are able to 'walk' over land. African catfish become one of the popular aquaculture species due to advantages such as fast growth, resistance to disease and can tolerate extreme water conditions (Huisman and Richter,1987; Moor and Bruton, 1988). It can survive in low oxygen, temperature extremes from 8 - 35°C with salinity levels between 0 and 10ppt as well as a wide tolerance of pH range (Safriel and Bruton, 1984). Due to this, efforts and studies are continuing to improve its growth, survival and production. The scientific classification for African catfish is as below.

1.1.1 Scientific Classification

Kingdom	: Animalia
Phylum	: Chordata
Class	: Actinopterygii (ray-finned fishes)
Superorder	: Ostariophysi
Order	: *Siluriformes*
Family	: Clariidae (airbreathing catfish)
Genus	: *Clarias*

1.2 Fish Behaviour

Behaviour is the manifestation of an organism's response to both internal (physiological) and external (environmental) signals. Animals acquire information about the environment through sensory cells that are specialized to respond to a particular stimulus, and use this information to accomplish behavioral objectives, such as feeding, swimming, schooling, predator avoidance, net avoidance, or migration. Successful survival depends upon an animal's ability to sense and respond appropriately to its immediate environment.

Understanding fish behaviour can contribute to improvements in aquaculture technologies and production. Fish in farms are able to detect unfavorable stimuli, but are unable to avoid unfavorable conditions and move to favorable locations, unlike the wild fish populations at sea. Many of the problems encountered in farms stem from the intended or unintended interference with the natural behavior of the fish. Interventions and solutions to these problems could be derived from an understanding of why the fish behave as they do in the particular farm. What sensory information is obtained by the fish, how is it obtained (the mechanism). And how is the information useful or detrimental to them? Research in the application of behavioural concepts to aquaculture has intensified recently (Huntingford *et al.*, 2012).

Aquaculture facilities include hatchery tanks and a variety of nursery, grow-out, and broodstocks tanks, cages, ponds, and pens. In hatcheries and nurseries, the ontogenetic development of the sense organs is crucial for early feeding, swimming, avoidance, survival, and growth. Most fish species hatch with poorly developed sense organs that are rapidly elaborated by the time of first feeding, and further improved with growth in time with gross changes in anatomy and behaviour (Kawamura *et al.*, 2003). Hatcheries must have appropriate lighting and water current conditions that allow proper development of the senses and behaviour of larvae. In grow-out farms, it is crucial that the environmental conditions are kept at optimum or tolerable levels for the fish stocks.

1.3 Colour Vision

Eye is a sensory organ allowing us to see and discriminate objects. Colour vision can affect the behaviour of organisms. Studies examine the developed behavioural criteria for establishing that non-human animals see colour (Lubbock, 1888; von Frisch 1913 & 1914). The eyes of human and water organisms are difference in terms of structure, shapes, types and distribution of cone cells and sensitivity towards light wave. Due to this, water organisms may have difference sense of visual compare to human.

Colours have the qualities of hue, saturation and brightness. Brightness is the value on the dark to light scale. Saturation is a colour's similarity to a neutral grey or white. Hue refers to colour differences other than those of brightness and saturation, and is the attribute denoted by terms such as red, yellow, green or purple (Wyszecki and Stiles, 1982; Bryne and Hilbert, 1997; Backhaus *et al.*, 1998).

Varying sensitivity of different visual cells in the retina to light of different wavelength is need for an organism to distinguish for colours. Colour vision is use in the phototaxis task, to identify light sources, detecting and discriminate objects

(Menzel, 1979; Jacobs, 1981; Mollon, 1989). There are two types of photoreceptor responsible for colour vision in the eye, the cone and rod cells. Cones are active at higher light levels, responsible to detect colour at day time while rods are not able to detect colours, but it can sense light intensity which responsible in grey, white and black vision. Colour vision can be classified as dichromatic (2 types of cones cell in retina) or trichromatic (3 types of cones cell in retina), depends on the number of types of photoreceptor cells (cones cell) in the retina. For a living organism to able to discriminate colours, it must require at least two types of cones cell which is called as dichromatic vision (Kaiser & Boynton, 1996).

1.4 Tank Colour

Experiments on the effects of tank colour are often designed on the assumption that the test fish have colour vision. Difference tank colours influence fish with different contrasts against background colour and influence the efficiency of detecting and catching the feeds by sight. A high contrast leads to higher visibility of feeds and better feed consumption (Dendrinos *et al.*, 1984; Kawamura *et al.*, 2010; El-Sayes and El-Ghobasgy, 2011).

1.5 Catfish Colour Vision

Most fish larvae are dichromatic or trichromatic at the onset of first feeding and become trichromatic or quadrichromatic after acquisition of the duplex retina (Britt *et al.*, 2001). Catfishes from family Ictaluridae such as channel catfish (*Ictalurus punctatus)*, white bullhead catfish (*Ictalurus catus*) and black bullhead catfish (*Ictalurus melas*) have single cones with peak absorption either at 530nm or 608nm (Sillman *et al.*, 1993). Brown bullhead catfish (*Ameiurus nebulosuss)* are reported to have green–sensitive and red-sensitive single cones (Lythgoe and Partridge, 1989). Ancistrys catfish and Kryptopterus catfish have only red-sensitive single cones (Douglas and Wagnerf, 1984; Lythgoe and Partridge, 1989) and are not likely to discriminate colour.

While Clarias catfishes have not been studied for spectral sensitivity of visual cells, Lee *et al,*. (2013) successfully conditioned *C. gariepinus* juveniles to discriminate two colours, red and green, and demonstrated the possession of colour vision in this fish. The retinal tapetum can be seen as eye shine in adult *C. gariepinus* in the dark and enable fish to forage in dim light by increasing the sensitivity of the retina by a factor of about 1.5 (Takei and Somiya, 2002). Mukai *et al.* (2008) examined the development of the retina of *C. gariepunus* larvae but did not mention the presence of the retinal tapetum. Therefore, the contribution of the retinal tapetum on the vision is not known in larval *C. gariepinus.*

1.6 Problem Statement

In an aquatic environment, most species of fish have used their eyes for vision. However, in a deeper water level or muddy environment, lower light penetration and limited colour spectrum is believed to seen by the fish. In the natural habitat of *C. gariepinus*, visibility is low and vision is nearly useless.

C. gariepinus is now an important fresh water species in aquaculture in the developing world, efforts are continuing to improve its growth and survival. Tank colouration was designed which found out to give affect in growth and survival of fish. It gives an assumption that fish is able to see colour. However, tank colour experiments were carried out with clear conditions, we do not know if fish is still able to see the tank colour in a dark condition. The tank colour selection may not be necessary if the rearing water is cloudy and has high turbidity is found out to affect the growth and survival of fish.

Eyes shine was found in the eye of teleost fish in river with high turbidity indicate role of dim-light vision (Arnott *et al.*, 1970). Eye shines is due to the present of components, retina tapetum located at the retina to allow more light to enter the eye. It is an adaptation of nocturnal species to recognize images as prey, predator and safety are essential. The eye of mormyrid fishes, *Marcusenius* and *Gnathonenius* contains a retinal tapetum composed of guanine crystals which is believe to involve in nocturnal activities. *C. gariepinus* may able to detect colour in dim light with the help from eyes shine.

Freshwater catfish species are believed to be dichromate which has only 2 Photopigment systems, green and red. Lee (2013) has examined 6 weeks old African catfish juvenile (42.9 ± 2.4mm) able to discriminate colours red and green based on chromatically. Besides, Fatollahi and Kasumyan (2006) examined feed colour preference in the laboratory and reported a blue bias among 4-month old juvenile *C. gariepinus* indicating colour vision. These studies indicated that African catfish have colour vision.

Many colours of tank were designed for aquaculturist to rear fish, the choice of tank colour become one of the question in fish rearing. The common culture method for African catifsh rearing is normally using green water which gives a very low light intensity in the water (surface illuminance was 2465 - 2684 lux and 10 – 20 lux in 40cm depth of tank with density of 176,000 green algae cell/ ml). Green water may reduce the background colour seen by fish. Since African catfish is well known with chemosense and taste buds on the body, we do not know if they are still depend on vision under dark. If they have vision in the dark, we do not know if the colour vision able to function in the dark. This experiment is carried out to investigate the African catfish eyes and to find out if African catfish is able to see colour in a dim water condition.

1.7 Hypothesis

During African catfish larvae stage, they have colour vision. Both larvae and juveniles are able to discriminate colour and they are able to see colour in dim water condition.

1.8 Objectives

The objectives of this study are as follows:

a. To study which stage do African catfish start to have colour vision.
b. To determine colour vision of African catfish in dim light condition.

CHAPTER 2

LITERATURE REVIEW

2.1 African Catfish Characteristic

African catfish has small eyes, no scale, elongate cylindrical body, flat head and small eyes. Four pairs of barbels are present. The dorsal and anal fin is long, no dorsal fin spine and adipose fin present. It has a smooth rounded tail fin and does not have scale on its body. The colour range from sandy-yellow through gray to olive with dark greenish-brown and belly is white in colour. Taste bud was found on its barbels and body (Mukai *et al.*, 2008).

2.2 African Catfish Distribution

The African catfish is the most widely distributed fish in Africa. This species can be found in rivers, dams, weirs, lakes, swamps, muddy waters and floodplains. The most common habitats are floodplain swamps and pools. They are able to survive during the dry season due to their accessory air breathing organs (Bruton, 1979; Clay, 1979). It is an omnivorous fish which is fast in growth and highly resistance to disease, but exhibit strong cannibalism starting from larvae stage.When there is a decrease in water or drought occurring, it is able to bury itself in the river bed or wet mud, to survive for a temporary in period dried pond. They can stay in muddy ground of ponds and gulping air directly using their accessory breathing organ instead of their gills.

2.3 Studies on African Catfish

African catfish live in the habitats of muddy water and deep river from 0 – 80m (Witte and Winter, 1995). In the deep river, visibility is low and vision is nearly useless. They have small eyes (Weisel and Mc Larury, 1964) and generally considered to be nocturnal scavenger (Rodriguez *et al.*, 1990; Pohlmann *et al.*, 2001).

The growth rate of African catfish was higher at low densities and in reduced light conditions where shelter was provided (Hossain *et al.*, 1998). In a continuous light period, a strong negative phototaxis, long rest periods, refuge seeking behaviour, and increasing incidence of territorial aggression were observed. In continuous darkness, the fish displayed a high level of active swimming with fewer rest periods, and a lower incidence of disturbed rest activity. Continuous light probably increase the rate of cannibalism, while continuous darkness can apparent reduce stress level and reduce rate of cannibalism (Britz and Pienaar, 1992).

Studies indicated that African catfish showed better feed ingestion and growth in the dark than in the light (Appelbaum and Kamler, 2000; Mukai and Lim, 2011). African catfish is well known with chemosense which taste buds all over the body (Mukai *et al.*, 2008). The feeding behaviour of *C. gariepinus* depends on chemo-sensory senses rather than visual or mechanical senses (Mukai *et al.*, 2013). All this information suggests limited importance of vision in African catfish.

Based on the previous studies, four month old juvenile African catfish showed blue bias on feed colour preference, indicating colour vision (Fatollahi and Kasumyan, 2006). African catfish juvenile able to discriminate colours of red and green based on chromaticity (Lee *et al.*, 2013). Studies suggest African catfish use vision quite well in clear water conditions. In 2 day-old larvae, the eyes were fully pigmented and retina of the African catfish eyes had developed (Mukai *et al.*, 2008). However, there was no information on development of retinal tapetum and colour vision of African catfish.

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