International Research Journal of Biological Sciences ISSN 2278-3202
Vol. **3(1),** 36-41, January **(2014)** *Int. Res. J. Biological Sci.*

Colour vision in juvenile African catfish *Clarias gariepinus*

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Available online at: www.isca.in, www.isca.me
Received 2nd September 2013, revised 17th October 2013, accepted 18th November 2013

Abstract

This study determined colour vision in juvenile Clarias gariepinus by the classical conditioning method. Farmed juveniles about 6 weeks old were presented a pair of green and red plates at the bottom of six aquaria (5 fish per aquarium). In aquaria G1, G2, and G3, the fish were conditioned to associate the green plate with a feed reward, and in R1, R2, and R3, the fish were conditioned to the red plate with feed. After about 100 conditioning trials, the catfish were tested for colour discrimination by presenting them with the pair of green and red plates without the feed. The catfish showed significant bias for the colour of conditioning—for green in G1, G2, and G3, and for red in R1, R2, and R3 (binomial test, P<0.018). The fish were then tested for brightness discrimination by presenting the color of conditioning together with different shades of grey. The catfish in G3 and R2 showed significant bias for the colour of conditioning regardless of the brightness of the grey plates (χ^2 test, P<0.05), that is, they clearly discriminated colours based on chromaticity. On the other hand, (larger) juveniles in aquaria G1, G2, and R1 seemed to discriminate colours based on brightness even as they had color vision.

Keywords: *Clarias gariepinus*, African catfish, colour vision, classical conditioning.

Introduction

The African catfish *Clarias gariepinus* is now an important aquaculture commodity in the developing world given its amenability to hatchery and growout production[1] and efforts are continuing to improve its growth, survival, and production. Fatollahi and Kasumyan[2] examined feed colour preference in the laboratory and reported a blue bias among 4-month old juvenile *C. gariepinus*, indicating colour vision. Light can have several life-affecting characteristics: quality (colour), quantity (intensity) and periodicity. Different colours have 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[3-5].

Experiments on the effects of colour of light and tanks are often designed on the assumption that the test fish have 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[6]. At least two types of cones are required for colour discrimination. Ictalurid catfishes, channel catfish *Ictalurus punctatus*, white bullhead catfish *I. catus* and black bullhead catfish *I. melas* have single cones with peak absorption either at 530 nm or 608 nm[7]. Brown bullhead catfish *Ameiurus nebulosus* are reported to have green-sensitive and red-sensitive single cones[8]. *Ancistrus* catfish and *Kryptopterus* catfish have only red-sensitive single cones[8-9] and are not likely to discriminate colours.

Clarias catfishes have not been studied for spectral sensitivity of visual cells nor colour vision. In this paper, we report the colour vision of the juvenile African catfish *C. gariepinus* as determined behaviourally.

Material and Methods

Fish and holding condition: The experiment was conducted at the Borneo Marine Research Institute (IPMB), Universiti Malaysia Sabah (UMS). Juvenile African catfish, 6 weeks old, 42.9 ±2.4 mm in total length and 0.65 ±0.02 g in body weight, were obtained from the IPMB hatchery where they had been reared in a plastic tank (100 cm × 60 cm, 40 cm deep, with clean bottom) and fed UMS-formulated moist feeds twice a day.

Six transparent polystyrene aquaria (26.5 cm × 16.5 cm, filled to a water depth of 20 cm) were placed inside a roofed hatchery with natural illumination only. Maximum illuminance was 1472 lx (light meter No. 40103, EXTECH INSTRUMENTS) at the water surface in daytime. Each aquarium was supplied with a constant flow of non-chlorinated recirculating fresh water (0 ppt) and continuous aeration. The rearing water had temperatures of 25.4–27.9ºC, dissolved oxygen of 6.7–8.9 mgl^{-1}, and pH 5.8–8.0. Five juvenile African catfish were placed in each aquarium. The presence of conspecifics increases feeding motivation[10] and colour discrimination is learned faster in groups than singly[11].

Animal care and handling were in accordance with the guidelines set by the World Health Organization; by the Malaysian Animal Handling Code of Conduct; and by the National Research Council[12]. There was no mortality and no injuries or signs of disease throughout the experiments that lasted for 5 weeks.

Conditioning method: The classical conditioning method was employed. If a fish can be trained to choose between coloured stimuli rather than among a range of grey stimuli, then it is said to have colour vision[13-16]. For conditioning the African catfish, green and red were chosen as the stimulus colours, based on the finding that the eyes of the catfish *Ictalurus* are sensitive to green and red[7-8]. Three plastic plates (12.4 × 6.0 cm) were painted green, and three others were painted red (TENCO CRYSTAL AEROSOL PAINT). Under direct sunlight and beside the aquaria, the reflectance of the red plate and the green plate in the wavelength range of 320–800 nm was recorded with a spectroradiometer (HSR-8100, MAKI MANUFATURING Co., Ltd., Hamamatsu, Japan) and is shown in figure 1.

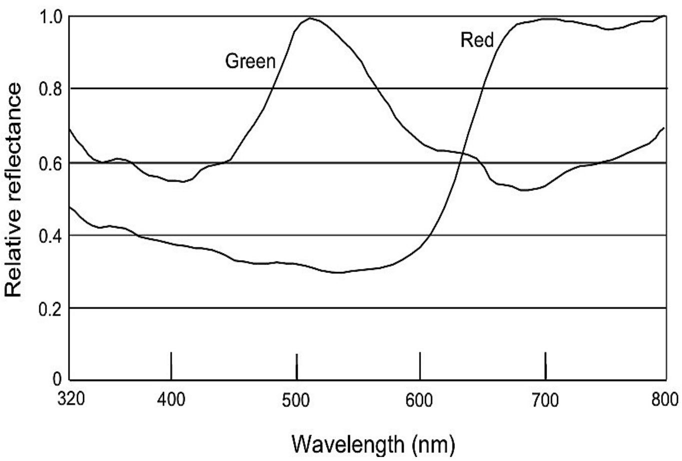

Figure-1
Reflectance spectra of the green and red plates used in conditioning African catfish

Conditioning was carried out as follows. A pair of green and red plates was simultaneously placed at the aquarium bottom to display the two colours to the catfish. A feed reward (UMS-formulated moist feed, 0.25 g in wet weight during the first two weeks and 0.5 g thereafter) was glued to one of the pair of displayed colour plates. The green plate was positive (+ feed) and the red plate.

ve in aquaria G1, G2, and G3; whereas the red plate was positive and the green plate was negative in aquaria R1, R2, and R3. The plate display positions were interchanged in a random way (by means of the random numbers table) to ensure that the catfish did not associate the reward with any particular plate position but rather with the plate colour. As soon as a pair of colour plates was placed at the aquarium bottom, the catfish approached the plates and licked them with the lips and barbels (figure 2) and consumed the reward feed within 30 s. They often repeatedly licked the plates until these were removed. Conditioning trials each took about 30 s and were repeated 4 times daily at about 2 h intervals. After each trial, all plates were withdrawn, washed in running tap water, air-dried, and prepared for the next trial. About 100 conditioning trials were done for fish in each aquarium.

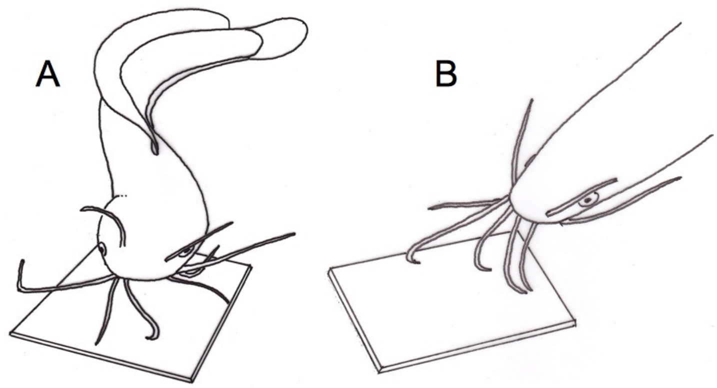

Figure- 2
The conditioned behaviour of juvenile African catfish towards the colored plates. A, licking with the lips; B, licking with the barbels

Colour discrimination test: To determine the success of the conditioning, the fish were subsequently tested for colour discrimination by displaying to them the pair of green and red plates without feed reward. To avoid the negative effect of hunger drive[17] on the colour discrimination test, feed of 1.0 g in wet weight was added into each tank every morning before the tests. The display positions of the color plates were interchanged in a random way. The fish visited the plates and licked one or both empty plates. The frequency of visits accompanied by licking was recorded by two observers over a 5 min period. This test was repeated 3 times per aquarium. When the total frequency of visits to the colour plate of conditioning was significantly higher than the visits to the negative colour plate (probability higher than at random choice level, binomial test), the fish were considered to have successfully learned to discriminate colours.

Brightness discrimination test: To determine whether juvenile catfish chose a colour plate based on the chromaticity per se or based on the difference in brightness of the two colour plates, discrimination tests without feed reward were done where the positive colour plate of conditioning was displayed with two grey plates of different brightness in the test aquaria. Grey colour was produced by mixing white paint (Kenlux Synthetic Enamel 9102) with black paint (Kenlux Synthetic Enamel 9103) at different ratios: 30%, 40%, 50%, 60% and 70% black, referred as Grey30, Grey40, Grey50, Grey60 and Grey70 hereafter. The display positions were interchanged at random.

Each combination of colour plate and grey plate was presented 3 to 8 times depending on the intensity of response of fish in each aquarium. The frequency of visits accompanied by licking was recorded by two observers over a 5 min period. When the frequency of visits to the colour plate of conditioning was significantly higher than to any of the grey plates, then the conditioned response was based on chromaticity and the catfish was deemed to have colour vision. Otherwise the conditioned response was based on brightness and the catfish was colour blind.

Statistical analysis: In a conditioning experiment, the random variable follows the binomial distribution. Therefore, the binomial test was employed for the statistical analysis of the color discrimination tests, on the hypothesis that the probability of choice for the colour plate of conditioning was 1/2. For the brightness discrimination tests, the χ^2 test was employed on the hypothesis that the probability of choice for the colour plate of conditioning was 1/3, and the probability of choice for the two grey plates was 2/3. The significance level was set at α=0.05.

Results and Discussion

After about 100 conditioning trials (total time taken 52~57 min), the catfish in aquaria G1, G2, and G3 learned to associate the green plate colour with the feed reward, and the catfish in R1, R2, and R3 learned to associate the red plate colour with the feed reward, that is, conditioning was successful in all six groups of catfish.

During the colour discrimination tests without feed reward, the catfish made somewhat less frequent visits to the colour plate of conditioning than during the conditioning session with feed reward. However, the choice was significantly biased toward the colour plate of conditioning (*P*<0.02; table 1), that is, the fish recognized green and red even without the feed reward.

During the colour discrimination tests, the larger catfish visited and licked the colour plate of conditioning while the smaller fish stayed at the corners of the aquarium, as if a size-hierarchy had formed in the aquaria. After the dominant fish left, the subordinate fish sometimes visited the colour plate of conditioning.

During the brightness discrimination tests, the juvenile catfish discriminated well the colour plate of conditioning from the grey plates of almost all different brightness combinations except green-Grey40-Grey60 in aquaria G1 and G2 and red-Grey40-Grey60 in R1 (table 2). In these combinations in G1, G2, and R1, the juvenile catfish did not discriminate the green or red colour of conditioning from Grey60. Among catfish in aquaria G3 and R2, the colour discrimination was clearly based on chromaticity rather than brightness (table 2). These results led to the conclusion that juvenile African catfish had colour vision. The brightness discrimination tests were not completed in G1 and R3 because of the weak response of the catfish to the colour plates of conditioning.

Table-1
Results of the colour discrimination tests carried out after repeated conditioning trials. Juvenile catfish visited and licked the colour plate of conditioning significantly more frequently in all aquaria (binomial test, α=0.05)

Aquarium	No. of coditioning trials	Plate color	Frequencies of visits in colour discrimination test	Binomial probability
G1	106	Green (conditioned) Red (distractor)	10 0	*P*=0.00098
G2	103	Green (conditioned) Red (distractor)	11 1	*P*=0.00292
G3	112	Green (conditioned) Red (distractor)	24 5	*P*=0.00022
R1	106	Red (conditioned) Green (distractor)	20 5	*P*=0.00022
R2	108	Red (conditioned) Green (distractor)	13 4	*P*=0.01816
R3	110	Red (conditioned) Green (distractor)	15 1	*P*=0.00024

Table-2
The results of the brightness discrimination tests where a trained-colour plate and two different grey plates were simultaneously presented. Visit frequencies to conditioned colour are significantly higher in all colour combinations in aquaria G3 and R2, and insignificant in the combinations of green-Grey40-Grey60 in G1 and G2, and red-Grey40-Grey60 in R1 (χ^2 test)

Aquarium	Colour and grey combination	No. of visits	$\chi2$
G1	Green	17	9.447
	Grey 30	3	$P<0.005$
	Grey 50	8	
	Green	6	0.30000
	Grey 40	3	$P>0.100$
	Grey 60	6	
G2	Green	14	10.50000
	Grey 30	2	$P<0.005$
	Grey 50	5	
	Green	20	*1.8*
	Grey 40	8	$P>0.100$
	Grey 60	19	
	Green	15	8.00
	Grey 20	4	$P<0.005$
	Grey 70	6	
G3	Green	16	12.71
	Grey 30	2	$P<0.005$
	Grey 50	3	
	Green	18	*12.07*
	Grey 40	4	$P<0.005$
	Grey 60	6	
	Green	40	*10.64*
	Grey 20	19	$P<0.005$
	Grey 70	20	
R1	Red	21	11.51600
	Grey 30	5	$P<0.005$
	Grey 50	6	
	Red	23	*0.68*
	Grey 40	15	$P>0.100$
	Grey 60	22	
	Red	26	*6.5*
	Grey 20	15	$0.010<P<0.015$
	Grey 70	11	
R2	Red	14	*9.09*
	Grey 30	5	$P<0.005$
	Grey 50	3	
	Red	20	*4.4*
	Grey 40	5	$0.025<P<0.050$
	Grey 60	16	
	Red	13	*12.25*
	Grey 20	3	$P<0.005$
	Grey 70	2	
R3	Red	13	*10.53*
	Grey 30	4	$P<0.005$
	Grey 50	2	

The juvenile African catfish conditioned to green colour and red colour with a feed reward later discriminated the green colour and red colour without the feed. The catfish in aquaria G3 and R2 clearly discriminated colours based on chromaticity rather than brightness, i.e., they had colour vision. On the other hand, catfish in aquaria G1, G2, and R1 discriminated colours based on brightness, i.e. they were colour blind. However, the latter result may be explained as follows: that some individuals, probably the larger dominant ones in G1, G2, and R1 learned colour discrimination based on brightness even as they had colour vision. Larger catfish frequently visited the grey 60% plate whereas the smaller fish stayed away even though they had been conditioned to discriminate two colours based on chromaticity. African catfish may be like most mammals with dichromatic vision that have comparatively poor colour discrimination, and prefer to use achromatic cues[15,18].

Freshwater catfishes populate the bottom of ponds, lakes and rather stagnant parts of rivers. Their habitat is often covered with vegetation in sand and mud, in which they dig small burrows or use natural shelters under logs and rocks. Generally, visibility is low and vision is nearly useless. Catfishes are generally considered to be nocturnal scavengers[19,20]. They have small eyes[21]. Bullhead *Ameiurus natailis* feed mostly at night, largely on benthic invertebrates and plants[22]. In laboratory experiments, African catfish larvae showed better feed ingestion and growth in the dark than in the light[23-26]. All this information suggests limited importance of vision to catfishes.

The natural behaviour of African catfish in fact involves vision to an important extent. The *Clarias* spp. catfishes are often called walking catfishes because they get out of the water and slither across dikes, rocks, and puddles to other bodies of water[27]. Colour vision would be of great advantage to African catfish out of its turbid water habitat. The African catfish is both a nocturnal and diurnal feeder[24] and demands almost two-thirds of the daily ration during the day[28]. In the field, the African catfish have two peaks in home range size, during midmorning (10h00) and after midnight (0h00) in the Great Fish and Sundays Rivers, Eastern Cape, South Africa[29]. Although the African catfish larvae hatch with immature unpigmented eyes, the eyes become functional by the onset of first feeding at 2 d of age[30]. African catfish prefers blue pellets to red and green pellets in the light[2]. Being able to discriminate green and red, juvenile African catfish may have green-sensitive and red-sensitive single cones, just like brown bullhead[8] and ictalurid catfishes[7]. Also, African catfish may have colour vision at all stages in the life cycle, given that in three ictalurid catfishes, the visual pigment complement does not change with age, as photoreceptors from adults contain visual pigments virtually identical to those of the larvae[6].

Conclusion

Farmed juveniles of *Clarias gariepinus* about 6 weeks old were successfully conditioned to discriminate two colours, green and red. In transposition tests, the juveniles showed significant bias for the colour of conditioning regardless of the brightness of the different shades of grey. Therefore, it is concluded that the *C. gariepinus* juveniles have colour vision.

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