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Larval Rearing and Feeding Behavior of African Catfish, *Clarias gariepinus* under Dark Conditions

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ABSTRACT

The present study was conducted to elucidate the relationship between the larval feeding behavior of African catfish, *Clarias gariepinus* and their sensory organs. We also sought to reduce the cannibalism and improve larval rearing methods for this species. The feeding behavior of intact and streptomycin-treated larvae was examined under light and dark conditions using *Artemia* nauplii. The ingestion rates under light versus dark conditions did not vary significantly; furthermore, no difference was observed between intact and streptomycin-treated larvae. These results showed that the feeding behavior of *C. gariepinus* depends on chemo-sensory senses rather than visual or mechanical senses. In addition, the larvae were reared under normal light conditions (following a diurnal cycle) and continuous dark conditions from the hatching day until they were 20 days old. Although, there was no significant difference, the trials under continuous dark conditions tended to have higher survival rates than those under light conditions. Moreover, the larvae reared under dark conditions showed the same growth rate as those under light conditions. The observations of larval behavior in the present study showed that larval activity increased under dim light conditions; the number of fish resting on the bottom of the aquaria decreased and fewer larvae were bitten by other individuals than under light conditions. The results of this study suggest that higher activity of *C. gariepinus* under dark conditions reduced the rate of cannibalism and resulted in higher rates of survival compared to light conditions. This study suggests that the survival rate of *C. gariepinus* larvae can be improved under dark or dim light conditions with low larval densities and adequate access to food.

Key words: Cannibalism, chemosense, ingestion rates, survival rates, taste buds

INTRODUCTION

The African catfish, *Clarias gariepinus* is an important species in the aquaculture industry throughout the world. The species has several advantages such as good taste, fast growth, resistance to low oxygen and considerable ease in farming (Pillay, 1990; Appelbaum and Mcgeer, 1998; Appelbaum and Kamler, 2000; Van de Nieuwegiessen *et al*., 2009). However, the larval rearing methods for *C. gariepinus* still have several problems. For instance, the larvae exhibit strong cannibalism, which results in low survival rates in hatcheries.

According to previous reports, *C. gariepinus* larvae and juveniles show unusual behavior. They have the ability to feed under dark conditions and can be reared under dim light or dark conditions (Hossain *et al*., 1998; Appelbaum and Kamler, 2000), indicating that their behavior must be dependent on sensory organs other than the eyes. Hecht and Appelbaum (1988) conducted behavioral experiments. The study showed that the ingestion rate of the larvae with their barbels removed decreased in comparison to the untreated control group and the eye-cauterized group. Mukai *et al.* (2008) demonstrated that *C. gariepinus* has numerous taste buds and assumed that the taste buds play an important role in their feeding behavior.

When fish larvae detect stimuli through their sensory organs, they exhibit various behaviors in order to survive in rearing tanks and in their natural habitats; therefore, their behavior is closely related to their sensory organs (Blaxter, 1986; Kawamura and Washiyama, 1989; Mukai and Kobayashi, 1995; Mukai *et al.*, 1994, 2007, 2010; Poling and Fuiman, 1997, 1998; Kawamura *et al.*, 2003). However, there have only been a few studies on the *C. gariepinus* focusing on the relationships between feeding behavior and sensory organs (Hecht and Appelbaum, 1988; Mukai *et al.*, 2008). This study investigated the effects of dark conditions on survival rates and determined which sense plays the important role in feeding behavior.

MATERIALS AND METHODS

Larval specimens and observation of their feeding behavior: Fertilized eggs from African catfish *C. gariepinus* were obtained from the brood fish reared in a hatchery at the Borneo Marine Research Institute, Universiti Malaysia Sabah at 2007. The eggs hatched 24 h after artificial fertilization. The larvae were reared in a 10-ton fiber-reinforced plastic tank with sufficient aeration and a water temperature of 28.0-30.0°C. The same group of larvae was also reared in a 40 L glass aquarium to observe their behavior. From the second day after hatching, the larvae were fed with rotifer (*Brachinonus* sp.) and *Artemia* nauplii and their behavior in the tank was observed.

Feeding experiments: Feeding experiments were conducted under light and dark conditions using *Artemia*. 18 and 19-day-old larvae were used for the experiments. Prior to the experiments, the larvae were starved for 24 h and single larvae were then transferred to 500 mL beakers containing 300 mL fresh water. The larvae were left for 20 min to recover from the stress of handling. Following the recovery period, 30 *Artemia* were placed inside each beaker and the larvae were left in the beakers to feed for 20 min. The larvae were subsequently anesthetized with Transmore (Nika Trading Co.) and the remaining *Artemia* were counted in order to determine the number of *Artemia* consumed by each larva (n = 10). The experiments under dark conditions were carried out in a completely darkened room (n = 10). The number of *Artemia* eaten by a larva was expressed as the ingestion rate of *Artemia* per 20 min.

To examine the role of free neuromasts in feeding behavior, a group of larvae was immersed in 11 mg L^{-1} streptomycin solution for 24 h before the feeding experiments to block the functioning of free neuromasts (Kaus, 1987). The trials using treated larvae were conducted under light and dark conditions (n = 10, for each trial).

The water temperature during the feeding experiments was 28.0-30.0°C. Statistical analyses between groups were performed by two-way ANOVA (SPSS Ver. 17).

Larval rearing experiments: Groups of 50 (larval density, 10 individuals L^{-1}), 100 (20 L^{-1}), or 150 (30 L^{-1}) individual newly-hatched larvae were introduced into 5 L plastic aquaria in triplicate. The larvae were reared under light conditions following a diurnal cycle (n = 900) or under continuously dark conditions (n = 900). Under light conditions, the larvae were reared under 600-1000 lx during the daytime and without light at nighttime (<0.01 lx). Under dark conditions, the larvae were reared continuously under 0.00 lx (measured by Light meter No. 401036, Extech Instruments Co.) in aquaria covered by black plastic sheets. The same amount of *Artemia* was provided twice daily, with some of *Artemia* remaining until the next feeding time. The water in the aquaria was changed once daily. The water temperature during the experiments was 27.0-28.0°C. The aquaria were exposed to light for less than 10 min to feed with *Artemia* and to change the water. At 20 days, the larvae remaining in each aquarium were anesthetized with Transmore (Nika Trading Co.), counted and weighed. Statistical analyses between groups were performed by two-way ANOVA (SPSS ver.17).

RESULTS

Larval behavior: The newly hatched larvae (TL±SD n = 10; 3.9±0.2 mm) were observed at the bottom of the aquaria.

One day-old yolk-feeding larvae (5.4±0.2 mm) moved continuously at the bottom of the aquaria and sometimes swam vertically and horizontally in the middle layers of the water column.

Two-day-old larvae (6.7±0.2 mm) commenced feeding on rotifers, *Artemia* and artificial compound feed, under light and completely dark conditions. Larvae swam most often on the bottom, in contact with the bottom substrate of the aquaria and against the water flow.

Three-day-old larvae (7.2±0.6 mm) displayed cannibalism such that when a larva rested on the bottom of an aquarium, other individuals bit the resting larva.

Five-day-old larvae (8.1±0.6 mm) swam mostly at the bottom of the aquaria and sometimes in the middle and surface layers at daytime. They swam throughout the surface and middle layers more actively at nighttime under dim light conditions, then cannibalistic behavior was fewer than under the light conditions.

Seven-day-old larvae (9.0±0.7 mm) exhibited discriminative resting behavior under light conditions; they suddenly stopped moving and appeared unconscious under light conditions. This behavior continued until they were 20 days old at the final day of observation. The fish displayed higher activity at nighttime, thus resting behavior was less prevalent during the nighttime than during the daytime.

The behavior of older stage (up to 20-day-old) larvae was similar to the behavior of 7-day-old larvae.

Feeding experiments: Figure 1 shows the ingestion rates under light and dark conditions for intact and streptomycin-treated larvae. The ingestion rates under the four conditions showed nearly identical values. The results of two-way ANOVA showed no significant differences among the various lighting (light or dark) and treatment (intact or streptomycin-treated) conditions; in addition, there were no interactions between the conditions (Table 1).

Larval rearing experiments: Survival rates under both light and dark conditions are shown in Fig. 2. Although, there was no overall significant difference among the groups (Table 2; two-way ANOVA, $p>0.05$), the trial with 20 individuals L^{-1} under dark conditions displayed the highest survival rates among all trials. In addition, the survival rates under dark conditions in the three trials had a tendency to be higher than those of paired light conditions.

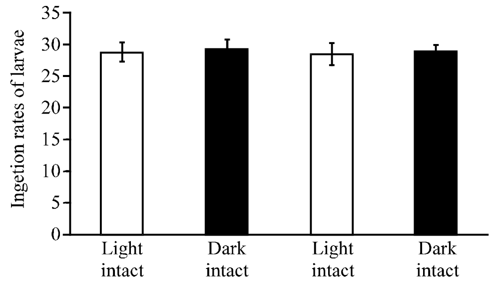

Fig. 1: Ingestion rates (the number of *Artemia* eaten by a larva in 20 min, Mean±SD). Intact: non-treated larvae, Treated: streptomycin-treated larvae

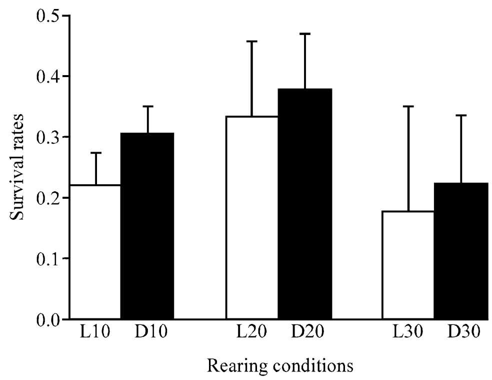

Fig. 2: Survival rates from the larval rearing experiment (Mean+SD). Larval density: 10, 20, 30 individuals L^{-1}. L: light conditions (following diurnal cycle), D: Dark conditions (continuous dark conditions)

Table 1: Results of two-way ANOVA of ingestion rates on lighting conditions and treated conditions

Source	df	M S	F	p-values
Treated conditions	1	0.9	0.406	0.528
Lighting conditions	1	1.6	0.722	0.401
Treated conditions×Lighting conditions	1	0.1	0.045	0.833
Error	36	2.217		

Table 2: Results of two-way ANOVA of survival rates on density and lighting conditions

Source	df	MS	F	p-values
Density	2	0.036	3.085	0.083
Lighting conditions	1	0.016	1.321	0.273
Density×Lighting conditions	2	0.001	0.074	0.929
Error	12	0.012		

Table 3 shows the mean individual weights of 20-day-old larval fish on the final day of rearing experiments. The results of two-way-ANOVA (Table 4) showed no significant differences between lighting conditions; however, significant differences were observed between density conditions. Tukey's post-hoc test showed that groups raised at a density of 20 individuals L^{-1} had significantly lower weights than other groups ($p<0.05$).

Table 3: Larval body weight (Mean±SD mg) under different conditions

$10\ L^{-1}$		$20\ L^{-1}$		$30\ L^{-1}$	
Light	Dark	Light	Dark	Light	Dark
221±71.8	194±17.4	80.9±15.5	88.7±31.5	192±157	99.2±31.9

Table 4: Results of two-way ANOVA of body weight on density and lighting conditions

Source	df	MS	F	p-values
Density	2	22658	4.175	0.042*
Lighting conditions	1	6339.4	1.168	0.301
Density×Lighting conditions	2	3897.5	0.718	0.507
Error	12	5427.6		

*Tukey post hoc test showed that 20 individuals had significant lower than other groups ($p<0.05$)

DISCUSSION

The results of feeding experiments showed that the ingestion rates under light and dark conditions were not significantly different (Fig. 1). This result suggests that feeding behavior did not depend on visual senses. There were also no significant differences in the ingestion rates between intact larvae and streptomycin-treated larvae. Streptomycin blocks the functioning of free neuromasts (Kaus, 1987). In the case of *Gnathopogon elongates caerulencens*, the larvae can detect *Artemia* under dark conditions by means of free neuromasts; however, streptomycin treated larvae show significantly lower ingestion rates than intact larvae (Mukai *et al*., 1994; Mukai, 2006). Although, the larvae of *C. gariepinus* have well-developed free neuromasts (Mukai *et al*., 2008), the results of the present experiments (Fig. 1) suggest that free neuromasts do not play a role in their feeding behavior.

Mukai *et al*. (2008) suggested that the taste buds of *C. gariepinus* play an important role in their feeding behavior. Hecht and Appelbaum (1988) demonstrated that blinded *C. gariepinus* larvae can capture *Daphnia*; however, larvae from which the barbels have been removed capture fewer *Dapnia*. In the case of sutchi catfish *Pangasianodon hypophthalmus*, the early-stage larvae (2- to 10-day-old) detect food under dark conditions using chemosensory organs such as taste buds located on their skin surfaces and on their barbels (Mukai *et al*., 2010; Mukai, 2011). Therefore, the feeding behavior of *C. gariepinus* in the present study most likely depends on their chemosensory organs, including taste buds.

Although, the larval rearing experiments caused no significant differences on the survival rates (Table 2), the results showed that these rates tended to be higher in dark versus light conditions (Fig. 2). In addition, the body weight of 20-day-old larvae (final day of experiment) showed almost the same value under light and dark conditions (Table 3); however, the weight of larvae grown at a density of 20 individuals L^{-1} was significantly lower than that of the other groups (Table 4). The difference was most likely due to the survival rate of the 20 individuals L^{-1} group, which tended to be higher. The results of a two way ANOVA showed that only the differences among density conditions were statistically significant (Table 4); therefore, the larvae under dark conditions showed the same growth rates as those under light conditions.

Based on the observations made in this study, the larvae were more active under dim light conditions at night than under well-lit conditions. Thus, the larvae are most likely nocturnal in habit. Under light conditions, the larvae showed resting behavior on the bottom of the aquarium during daytime; these larvae were then bitten by other larvae. Moreover, 7-day-old larvae displayed discriminative resting behavior, in which they suddenly stopped moving and appeared to be unconscious under light conditions. Hecht and Appelbaum (1988) also mention the resting behavior of *C. gariepinus* larvae; hence, under light conditions, the resting larvae will be more likely to be bitten by other individuals. Consequently, survival rates probably decrease under light conditions. Accordingly, the survival rates under dark conditions were slightly higher than those under light conditions.

Previous studies also demonstrated that *C. gariepinus* larvae, juveniles or fingerlings can be reared under continuously dark conditions and that survival rates under dark conditions are better than under continuous light, or alternating light and dark conditions (Britz and Pienaar, 1992; Appelbaum and Mcgeer, 1998; Hossain *et al*., 1998; Appelbaum and Kamler, 2000; Almazan-Rueda *et al*., 2008; Adewolu *et al*., 2008). The results of the present study consistent with these results.

On the one hand, Britz and Pienaar (1992) demonstrated that the activity of *C. gariepinus* larvae and juveniles under dark conditions is higher than under light conditions. On the other hand, many other studies mention that the activities of *C. gariepinus* larvae or juveniles are reduced under dark or dim light conditions; consequently, fish cannibalism decreases and survival rates increase. In our observations, larval activity became higher under dim light conditions as the number of fish resting on the bottom of the aquaria decreased. Accordingly, the present study suggests that the higher activity of *C. gariepinus* under dark conditions results in reduced rates of cannibalism and higher rates of survival than under light conditions.

In the case of sutchi catfish (Mukai unpublished data), reduced larval activity under light conditions was closely related to their cannibalism. Under light conditions, larvae remain close to the bottom of the aquarium and are more likely to encounter other individuals, thereby increasing cannibalism. Thus, under dark conditions, fewer larvae would be bitten by other larvae.

The present study showed that reduced larval activity under light conditions made the larvae more vulnerable to cannibalism. When the larvae are reared in the hatcheries, their survival rate is an important factor. The data presented here suggest that *C. gariepinus* larvae can be reared at low density under dark or limited light conditions with adequate feeding to increase larval survival rates in the hatcheries.

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