



Are Pre Spawning Stressors Affect Reproductive Performance of African Catfish *Clarias gariepinus*?

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Abstract

The influence of administered pre spawning stressors on female African catfish, *Clarias gariepinus* was investigated throughout induced spawning. It was found that no significant difference ($P > 0.05$) in ovulation percentages among stressed and control groups. Significant differences were recorded for eggs weight and eggs numbers produced by stressed females and control one. Stressed females show low fertilization and hatching rate compared to the control group. Starved females progeny showed significantly higher deformity rate, dead larvae and low survival rates compared other groups. African catfish exposed to stressor have the ability to spawn successfully, but there appears to be a negative influence of this stress on their breeding output, particularly through the production of abnormal larvae. Thus, there is a limit to the ability of these fish to tolerate stressor as revealed. Proper feeding, handling and management of female African catfish is recommended for proper induced spawning.

Keywords: Stressors, African catfish, reproductive performance, induced spawning.

Introduction

Clariid catfishes have a distinguished feature as one of the most important categories of farmed catfish in the world. *C. gariepinus* is the fundamental species cultured in Africa (Teugels, 1984). This species is known for its high growth rate, resistance to handling, stress, poor water quality, amenability to high stocking densities. It has also been known as the most suitable one for aquaculture throughout its distributional area (Hecht *et al.*, 1996). In Europe, *Clarias* sp have been farmed in Germany, Netherlands and Belgium (Verreth *et al.*, 1993). The Republic of Czech (Adamek and Sukop, 1995) has begun to culture this species on both an extensive and intensive basis. In South America, catfish is mainly farmed in Brazil (Hecht *et al.*, 1996).

Effective fish reproduction under controlled conditions depends on high gamete quality and progeny performance (Bromage, 1995). Number of eggs spawned, egg mortality, size of larvae, and percent of larval mortality are considered attributes with high relative value when selecting brood fish (Leitritz and Lewis, 1980). Despite the need for high quality gametes, the environment under which the brood fish are maintained before reproduction is often stressful (Schreck *et al.*, 2001; Gowaty *et al.*, 2007;

Schreck, 2010).

Although many studies on catfish have generated a body of information on spawning and hatching, and larval and juvenile rearing (Appelbaum and Mcgeer, 1998; Brzuska, 2002; Han *et al.*, 2004; El Naggat *et al.*, 2006; Olumuji and Mustapha, 2012; Marimuthu *et al.*, 2015). Studies on the influence of environmental and stress factors in African catfish reproduction are few (e.g., de Graaf and Janssen, 1996; Boeuf and LeBail, 1999; Adebayo, 2006; Okanlawon, 2010).

Common management applied in hatcheries such as transportation, rough handling, poor cleaning, overcrowding, use of chemicals, and problems with poor water quality are stressors that may negatively influence fish breeding (Bromage, 1995; Schreck *et al.*, 2001; Adebayo, 2006; Okanlawon, 2010).

The hardly nature of *C. gariepinus* and its tolerance to adverse ecological condition make fish culturists believe that nothing can affect on *C. gariepinus* broodstocks and this persuasion bring them to ignore the effects of rough handling, poor feeding, hypoxia and crowding in their hatcheries. Research on the effect of stress such as starvation, handling, crowding on the reproduction of *C. gariepinus* may lead to the development of culture practices that minimize adverse effects of stress and enhance the

production of high quality gametes and progeny with the subsequent commercial and ecological benefits. Therefore, the objective of this study was to determine if stresses applied to *C. gariepinus* broodstock during hormonal induction affect their reproduction performance or progeny outputs.

Materials and Methods

Broodstock Care and Selection

C. gariepinus broodfish females were stocked in concrete pond belonging to the department of Animal Husbandry and Animal Wealth Development, Faculty of Veterinary Medicine, Alexandria University. Study starting about mid-April 2015 fish fed on a diet of 25% protein at 3% total body weight every day. Towards the end of May 2015, when the climatic conditions were suitable for breeding (average daily water temperatures of 25 ± 1 °C), females were examined and their readiness to breed assessed on the basis of external appearance and release of eggs upon gentle pressure on the abdomen. Broodfish were selected using this process, weighed, and assigned randomly to one of the experimental treatments.

Experimental Design

Forty-eight *C. gariepinus* females were randomly assigned among sixteen 200-liter indoor plastic tanks at a density of three fish per tank supplied with tap water provided with air through air stones distributed evenly in the tanks. Experimental groups consisted of two replicates each (i.e., 6 females per treatment). Each group was randomly assigned to a particular stressful condition allocated in the experimental design Table 1. Experimental fish were injected gonadotropin releasing hormone analogue GnRH α (Cystorelin®, 40 μ g kg $^{-1}$ female $^{-1}$) and dopamine antagonists Domperidone (Gastromotil®, 10mg kg $^{-1}$ female $^{-1}$) intramuscularly of the dorsal fin to artificially induce them for spawning (Shourbela *et al.*, 2014).

Stripping and Collection of Eggs

Female fish were checked for ovulation by making gentle pressure to the abdomen starting ten

hrs post injection and continued at one-hour intervals. The fish that produced stream of transparent green brown eggs were rated as ovulated (Richter *et al.*, 1987). Meanwhile, Non-ovulated fish were backed to the tanks and kept under the respective treatment design. Eggs were obtained by applying gentle pressure to abdomen of ovulated females.

Fertilization and Incubation

Eggs from individual treated females were fertilized with pooled milt obtained from macerated testes of three killed males Rothbard (1981). Pooled milt was added and the gametes were gently mixed. Water was added to activate sperm and the mix was allowed to stand for two minutes, then rinsed and held for an additional 2 minutes. Eggs of each female were incubated in large trays, and kept separated from other females eggs.

Reproduction and Performance Parameters

Female body weights (gm) were recorded at the start of the experiment. Weight of the ovulated eggs was estimated also. The number of ovulated eggs were calculated by multiplying egg weight in grams by 700 (1 gm=700 eggs) (Viveen *et al.*, 1985). The latency period (the time between treatment and ovulation) was recorded. The percentage of fertilized eggs was determined by sampling 100 eggs per female at 24 h after fertilization, using a dissecting microscope. Likewise, mean percentage of embryos hatched was calculated for each female by counting three replicates of random samples of hatchlings inside petri dish (Rothbard, 1981). At two days post-hatching fry survival rate, dead larvae percentage and deformity percentage were recorded per female according to De Leeuw *et al.* (1985).

Statistical Analysis

A one way analysis of variance (ANOVA) was performed at the beginning of the experiment for fish weight to ensure randomization. Spawning percentage was analyzed with the Chi-square test, other reproductive parameters were analyzed by one-way analysis of variance (ANOVA), using the general linear models procedure using Statistical Analysis

Table 1. The experimental design

Treatments	Stressors
T1	Good condition without any stress (Control).
T2	Starvation for ten days before hormonal injection.
T3	Harvesting stress (bruises due to poor handling before hormonal injection).
T4	Netting (fish chased with a dip net in the tank every one hour for 2 minutes after hormonal injection).
T5	Oxygen stress (one hour out of water before hormonal injection).
T6	Oxygen stress (five hours out of water before hormonal injection).
T7	Crowding stress (two-fold increase in density after hormonal injection)
T8	Crowding stress, (four-fold increase in density after hormonal injection).

System software (SAS Institute Cary, North Carolina, USA, 2004).

Results

Chi-square test indicated no significant differences ($P > 0.05$) were found in ovulation percentage among stressed and control brood fish. Meanwhile, the brood fish females of different treatment groups did not spawn completely with the exception of those of T1 and T5 (Table 2). At stripping, the latency period was within the range of 11.3 to 13 hrs for the eight experimental groups (Table 2). Significant differences were found among the experimental groups. The longer latency period reported for T7 (13 hrs). In contrary, the lowest latency periods were recorded for T8 (11.3 hrs).

Weight of eggs produced per female showed no significant differences ($P > 0.05$) among all treated brood fish groups with the exception of T1 (77.2 gm) which showed significant difference ($P < 0.05$) from other experimental groups (Table 2). Likewise, Females of T1 had the highest mean number of ovulated eggs (54,935 eggs) with a significant difference ($P < 0.05$) from other experimental groups (Table 2). T1 and T5 brood fish had the highest significant ($P < 0.05$) fertilization and hatching rate (Table 3).

On the other hand, T8 brood fish was of the lowest fertilization rate (66.5 %) and T3 brood fish was the lowest hatching rate (60.9 %) (Table 3).

Additionally, T2 brood fish had significantly higher deformity, dead larvae and survival rate percentage ($P < 0.05$) compared to other brood fish groups (14.1%, 7.1%, 78.8%, respectively) (Table 3). Significant differences ($P < 0.05$) among treatments were found for survival rate in T1 (94.3 %) compared to other brood fish groups.

Discussion

Stress can affect breeding in many ways, depending on the severity and duration of the stressor. It can accelerate spawning or inhibit reproduction or may resulting in greater number of gametes to compensate for poor-quality of gametes (Schreck, 2000; Gowaty *et al.*, 2007). Low effective stress may both hasten and enhance acclimation to a stressor (Emlen *et al.*, 1998). Previous studies showed degenerative changes in the gonad, oocyte atresia and inhibition of spawning as a result of stress (Clearwater and Pankhurst, 1997; Schreck, 2000). Stress has been found to inhibit aspects of the fish endocrine system responsible for breeding (Pankhurst and Van der Kraak, 1997; Pankhurst and Van der Kraak, 2000). Our results indicated successful induction of spawning of *C. gariepinus* using the spawning agent (Ataguba *et al.*, 2009; Ndimele and Owdeindi, 2012; Marimuthu *et al.*, 2015). Meanwhile, stress caused cessation in reproduction (number of stressed females did not spawn after spawning induction, (Table 2). This result confirms previous

Table 2. Female weights (gm), ovulation %, latency period (hrs), egg weight (gm) as well as number of ovulated eggs of African catfish (*C. gariepinus*) as influenced by various Treatments (LSM \pm SD)

Treatments	Number	Fish weight (g)	Ovulation %	Latency period (hrs)	Egg weight (g)	Egg number
T1	6	714.2 \pm 36.0	100.0	11.5 \pm 0.5 ^c	77.2 \pm 7.8 ^a	54,935 \pm 4,735 ^a
T2	6	704.3 \pm 63.2	66.67	12.7 \pm 0.5 ^{ab}	50.5 \pm 6.5 ^b	36,002 \pm 4,262 ^b
T3	6	671.1 \pm 54.9	66.67	12.2 \pm 0.9 ^{abc}	51.7 \pm 8.9 ^b	34,955 \pm 5,054 ^b
T4	6	647.2 \pm 67.2	66.67	11.5 \pm 0.5 ^c	55.8 \pm 5.1 ^b	39,975 \pm 3,234 ^b
T5	6	692.1 \pm 66.3	100.0	11.8 \pm 0.7 ^c	53.2 \pm 7.3 ^b	37,071 \pm 4,855 ^b
T6	6	625.7 \pm 45.5	83.33	12.8 \pm 0.8 ^{ab}	50.9 \pm 5.3 ^b	35,184 \pm 3,527 ^b
T7	6	685.9 \pm 69.5	66.67	13.0 \pm 0.8 ^a	55.6 \pm 7.5 ^b	37,498 \pm 4,623 ^b
T8	6	657.3 \pm 68.2	50.00	11.3 \pm 0.6 ^c	47.5 \pm 6.5 ^b	32,730 \pm 3,943 ^b

Means in the same column with similar letters are not significantly different ($P > 0.05$).

Table 3. Percentage of fertilization (%) at 24 hrs of incubation, hatching rate (%), deformity (%), dead larvae (%) as well Survival rate (%) of African catfish (*C. gariepinus*) as influenced by various Treatments (LSM \pm SD)

Treatments	Fertilization (%)	Hatching (%)	Deformity (%)	Dead larvae (%)	Survival rate (%)
T1	81.1 \pm 5.7 ^a	83.7 \pm 5.7 ^a	2.70 \pm 1.5 ^{ef}	2.8 \pm 1.4 ^{cd}	94.3 \pm 2.1 ^a
T2	70.0 \pm 7.0 ^{bc}	64.1 \pm 3.6 ^d	14.1 \pm 4.9 ^a	7.1 \pm 2.5 ^a	78.8 \pm 4.8 ^c
T3	74.6 \pm 6.6 ^{abc}	60.9 \pm 6.6 ^d	5.60 \pm 1.6 ^{bcd}	5.2 \pm 2.7 ^b	89.1 \pm 3.6 ^b
T4	75.1 \pm 6.5 ^{ab}	76.7 \pm 7.2 ^{ab}	4.40 \pm 2.2 ^{de}	5.9 \pm 1.8 ^{ab}	89.6 \pm 3.8 ^b
T5	80.2 \pm 7.5 ^a	80.1 \pm 8.3 ^a	2.40 \pm 1.6 ^f	1.5 \pm 1.1 ^d	96.0 \pm 1.7 ^a
T6	71.2 \pm 5.8 ^{bc}	71.9 \pm 9.3 ^{bc}	5.10 \pm 2.0 ^d	4.3 \pm 2.3 ^{bc}	90.5 \pm 3.8 ^b
T7	70.1 \pm 7.3 ^{bc}	65.1 \pm 8.3 ^{cd}	7.55 \pm 1.1 ^b	4.2 \pm 2.3 ^{bc}	88.2 \pm 2.0 ^b
T8	66.5 \pm 8.4 ^c	71.8 \pm 5.1 ^{bc}	7.00 \pm 1.5 ^{bc}	5.1 \pm 2.2 ^b	87.9 \pm 2.8 ^b

Means in the same column with similar letters are not significantly different ($P > 0.05$).

findings in broodfish (Carragher *et al.*, 1989; Campbell *et al.*, 1992). Meanwhile, all stressed African catfish *C. gariepinus* were spawned completely (Adebayo, 2006; Okanlawon, 2010).

Concerning the latency period, all *C. gariepinus* brood fish spawning 11-13 h after hormones injection. These results were higher than those reported by Shinkafi and Ilesanmi (2014) for African catfish, but it was lower than those reported in previous studies on carp (Arabaci *et al.*, 2004; Falahatkar *et al.*, 2013). Furthermore, experimentally stressed females exhibited significant reduced egg weight and average number of ovulated eggs in comparison to control females.

A stressful reproductive environment negatively impacts reproductive performance in many ways (Jardine *et al.*, 1996; Janz *et al.*, 1997; Bowron *et al.*, 2009). Similarly, stressed catfish spawn fewer eggs numbers (Ayinla, 1991; Adebayo, 2006). Additionally, in other fish e.g. Cichlid *N. pulcher*, stressed females showed longer intervals between spawning clutch, and produce fewer eggs of smaller mass than control females (Mileva *et al.*, 2011). Also in mature jundia, (Soso *et al.*, 2008), red gunard, *Chelidionichthys kumu*, (Clearwater and Pankhurst, 1997), silver carp (Akar, 2011). Subsequent studies have shown that stress does indeed have a negative effect on reproduction performance (Campbell *et al.*, 1994; Contreras-Sanchez *et al.*, 1998; Schreck *et al.*, 2001). It often appeared that stressed broodfish ovulated more eggs than non-disturbed fish e.g. catfish (Okanlawon, 2010) and cod (Morgan *et al.*, 1999).

Results based on rates of egg fertilization and hatching were the higher in control group than stressed one. The differences observed in the current study may suggest that well-handled unstressed females produced better quality eggs than stressed. Similarly, eggs spawned by stressed Atlantic cod *Gadus morhua* females had lower fertilization and hatching rates than eggs from unstressed females (Lambert *et al.*, 2000; Bogevik *et al.*, 2012; Eriksen *et al.*, 2015). Additionally, broodfish with bruises (T3) had the lowest hatching rate, spawner with bruises had adverse effect on their reproduction performance (Alatise, 1988).

Furthermore, low dissolved oxygen for five hrs group (T6) produced lower fertilization and hatching percentage than in control group. Hypoxia reduces overall reproductive success by disturbing endocrine functions, which in turn affect gametogenesis, gamete quality, fecundity, fertilization success, hatching, and viability of larvae (Wu, 2003; Thomas *et al.*, 2015).

Starved brood fish (T2) had significantly higher percentages of deformity, dead larvae and the lowest survival rate ($P < 0.05$) compared to other brood fish groups (14.1 %, 7.1 %, 78.8%; respectively) (Table 3). Those results suggested that not only maternal fitness is influenced by stress, but also that maternal stress may have repercussions for progeny fitness

increasing generation of deformed larvae (Jensen, 1996). Nutritional status is an important factor in gamete quality and has been shown to be related inversely to nutritional condition and cod fecundity is reduced markedly by poor nutritional condition (Kjesbu *et al.*, 1990; 1991). Likewise, Cerda *et al.* (1994) reported in sea bass *Dicentrarchus labrax* L. that reducing the dietary protein rates decrease offspring quality resulting in large degree of larvae deformities.

Stressed groups show lower offspring survival rate than control. In line with this, previous studies reported that exposing fish females to various stressors can be deleterious to offspring survival (Campbell *et al.*, 1992; Eriksen *et al.*, 2008; Mingist *et al.*, 2007). In contrast, several experiments demonstrate no effect of parental stress on survival rates of the progeny (Small, 2004; Tierney *et al.*, 2009; Eriksen *et al.*, 2015). High density group show poor fecundity. Similarly, prolonged culture stress and high stocking density had adversely effect on reproduction (Bayunova *et al.*, 2002).

An interesting findings from this study were that the lowest percentages of deformity, dead larvae, highest fertilization percentage and survival percentage was found in low dissolved oxygen for one hr group (T5) and control group (T1). Suggested that short period of stress can positively affected gamete quality. A small amount of stress has a positive effect and more severe stressors have a negative effect on reproductive performance. For example, physical conditioning involving low levels of stress, yet sufficient to activate the HPI, can enhance a condition of stress-resistance in fishes (Schreck *et al.*, 1997; Schreck, 2000). Increased fecundity associated with short-term, low severity stress has been shown previously in other fish, e.g. rainbow trout (*Oncorhynchus mykiss*) (Contreras-Sánchez *et al.*, 1998) and zebrafish (Faught *et al.*, 2016).

Finally, it should be mentioned that African catfish offspring can be dramatically affected when their mothers have stressed during the induced spawning, revealed by lowered survival rates, increased frequency of morphological anomalies. These findings may be of interest both in conceptual and applied condition as they links the brood fish environment to subsequent viability of the progeny. In catfish hatcheries, brood fish are frequently disturbed during the breeding season and such practices may impact progeny quality. Thus, attempts to protect potential breeders from managerial disturbance during induced breeding would be secured because stressed fish mothers are likely to create a less predictable and profitable product.

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