

FULL PAPER

Does the Behavioral Stress Response of Nile Tilapia, *Oreochromis niloticus* Breeders During Isolation Influence Seed Production?

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ABSTRACT

This experiment assessed the effect of breeder's behavioral stress response [i.e., eye color pattern (ECP)] during isolation on *O. niloticus* seed production. ECP change was marked by fractional color changes of the iris and sclera, which was transformed into scores ranging from 0 (no darkening) to 8 (total darkening). After isolation, breeders were divided into two social groups: proactive breeders (PB) were those with a mean ECP score of <2, and reactive breeders (RB) with a mean ECP score of >6. Two breeding cycles were done in six (1 m x 2 m x 1 m) net enclosures. Mean spawning rates (SR) in PB during the two cycles were 38.89±14.70% and 33.33±8.87% while 3.33±9.62% and 22.22±2.48% in the RB group. Total seed productions (TSP) in PB were 1,906.22±733.72 and 1,681.19±1,070.48 fry, and those in RB were 996.35±218.11 and 461.39±151.37 fry. There were no significant differences between the two groups on SR and TSP in both cycles. On seed production per female that spawned, however, significantly ($P<0.05$) higher means (796.33±77.68 and 726.33±124.08 fry) were observed in the PB compared to those in RB (522.73±54.68 and 335.83±44.98 fry). These results demonstrated that seed production in *O. niloticus* could be increased by selecting proactive breeders through the evaluation of their ECP during isolation.

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1. INTRODUCTION

Reproduction in fishes is affected by several environmental factors that may impose stress on the fish. Stress is defined as “the nonspecific response of the body to any demand made upon it” (Selye 1973). However, this definition of stress as a biological term is controversial (Øverli et al. 2007). This is because what is stressful to an individual fish may not always be stressful to others belonging to the same species. The ability of individual fish to cope in a particular condition or situation has been referred to as coping style, behavioral syndrome, or behavioral stress response (Sluyter et al. 1996; Korte et al. 2005; Koolhaas et al. 2007). These various behavioral adaptations in a variety of situations have been described as either proactive or reactive. Proactive individuals are more aggressive and tend to be more

prone to take risks or put themselves to danger. On the other hand, reactive individuals are typically risk-avoiders and tend to hide and avoid dangerous situations (Huntingford et al. 2010). Using simplistic terms, these individuals exhibit different behavioral adaptations that may be described as either “bold” or “shy.”

Observations in teleost fish revealed that distinct behavioral stress-coping styles or responses are present, and they influence both social rank and levels of aggression (Øverli et al. 2006). In the wild and under culture conditions, adult fish are exposed to stressful situations. Depending on the fish's behavioral stress-coping styles, these stressful conditions may affect fish health and welfare and may reduce its reproductive success and fitness (Barton and Iwama 1991; Barton 2000; Schreck et al. 2001).

Since previous studies have revealed that the possible outcome of social dominance in Nile tilapia (*Oreochromis niloticus* L.) can be predicted using behavioral stress responses (i.e., eye color pattern, feeding response or duration of appetite inhibition) during isolation (Valdez 2010; Vera Cruz et al. 2011; Chinaman 2012; Tauli 2013), it is of great interest to investigate whether eye color pattern of the fish can be used in selecting breeders with reproductive advantage.

2. MATERIALS AND METHODS

2.1 Experimental fish

Male and female Nile tilapia breeders of similar ages were acquired from the Freshwater Aquaculture Center (15°44'8.9"N, 120°56'49.2"E), Central Luzon State University, Science City of Muñoz, Nueva Ecija. They were conditioned for two weeks, separately by sex, in 2 m x 1 m x 1 m CC17 mesh hapas at 6 to 10 fish m⁻². They were fed daily at 3% of their body weight divided equally into two and given at 0700 h and 1600 h.

2.2 Isolation and identification of social grouping

Twenty-five males and 50 females *O. niloticus* of similar size were isolated at random in seventy-five 30 cm x 15 cm x 30 cm aquaria for seven days. Three sides of each isolation aquarium were covered with white plastic boards to prevent the isolated fish from seeing other fish in other aquaria during the isolation period. The water temperature was maintained between 27°C and 29°C. The fish were fed, once a day, at 1% of the body weight. The eye color pattern (ECP) of each fish inside the aquarium was monitored every morning during the 7-day isolation period. Eye color was quantified as a darkened area of both iris and sclera (Volpato et al. 2003). The circular area of the eye was divided into eight equal parts using four imaginary lines. The ECP change was marked by fractional changes of the color of the iris and sclera around the pupil, which was transformed into scores ranging from 0 (no darkening) to 8 (total darkening) (see Fig. 1).

After the isolation period, the breeders were separated into two social groups, namely: proactive breeders (PB) and reactive breeders (RB), in relation



Figure 1. Fractional darkening of eye color

to their mean ECP during the 7-day isolation period (adapted from Vera Cruz and Tauli 2015). The PB were those that manifested a shorter period of adjustment in the novel environment, as indicated by lower ECP values (between 0 and 2) at the end of the isolation period. On the other hand, the RB exhibited a longer period of adjustment in the new environment as manifested by higher ECP values (between 6 and 8) at the end of the isolation period (Vera Cruz and Tauli 2015). Before the breeding period, breeders were conditioned separately by sex and by social group in net enclosures for one week. They were fed with commercial feed containing 30% protein twice daily (0700 h and 1600 h) at 3% of their body weight per day. After the conditioning period, the weight of each fish was taken, and males and females in each social group had mean body weights of 344.20 ± 25.30 g (PB males; $n = 6$), 338.80 ± 33.30 g (RB males; $n = 6$), 250.70 ± 12.20 g (PB females; $n = 18$), and 247.00 ± 12.10 g (RB females; $n = 18$). The broodfish's weight indicated that they were sexually mature, and the readiness-to-spawn condition was assessed by examining the genital papilla.

2.3 Experimental seed production

Breeding was conducted in six $2 \text{ m} \times 1 \text{ m} \times 1 \text{ m}$ fine mesh net enclosures installed in a 1000 m^2 earthen pond. Two groups of breeders, proactive breeders (PB) and reactive breeders (RB), each replicated three times, were used. Two seed production cycles were performed. Eight breeders, two males and six females, were stocked in each breeding net enclosure (El-Sayed 2006). Breeders were fed twice daily (0700 h and 1600 h) at 2% of their body weight using commercial feeds with 30% protein. After 14 days, the collection of eggs (from the female's mouth) and fry were done. The number of females that spawned (based on the examination of the genital papilla and abdomen; i.e., red genital papilla and shrunken to compressed abdomen) or where eggs had been collected was recorded. Spawning success was determined using the equation: Spawning rate (%) = (number of females that spawned / number of stocked females) \times 100. Eggs were artificially incubated in a downwelling incubation system (Fig 2), receiving a continuous water supply with temperature ranging from 27.10°C to 29.40°C . The total number of hatched eggs was determined, and the hatching rate was computed



Figure 2. The downwelling incubation system

using the equation: Hatching rate (%) = (number of eggs that hatched / total number of eggs incubated) x 100. Total seed production was determined by adding the total number of fry collected in each net enclosure and the total number of fry from hatched eggs. Average seed production per female that spawned was also determined by dividing the total number of fry by the number of females that spawned. Dissolved oxygen and water temperature were monitored every other day between 0800 h and 0900 h and between 1400 h and 1500 h.

2.4 Statistical analysis

All data were statistically analyzed using SPSS software version 16.0. Percentage data were arcsine transformed prior to statistical analysis. Test for normality of data was done using the Shapiro-Wilk Test, and Test for Homogeneity of Variance was also performed. Since both assumptions were met, a parametric test was used. T-test was used to compare the spawning rate, total seed production, and seed production per female spawner.

3. RESULTS

The spawning rate, hatching rate, total seed production, and seed production per female spawner during the first and second seed production cycles are presented in Table 1. On the spawning rate, PB obtained 38.89±14.70% and 33.33±8.87% during the first and second seed production cycles, respectively.

The RB, on the other hand, got 33.33±9.62% and 22.22±2.48% in the two cycles. However, statistical analysis showed no significant differences between the two groups' mean spawning rates in the two seed production cycles. On the hatching rate, PB obtained 81.06±1.03% compared to 78.41±0.00% in the RB during the first seed production cycle, while RB obtained 84.48±2.48% compared to 70.66±8.77% in PB during the second seed production cycle. Likewise, no significant difference was detected in both cycles.

On total seed production, PB produced 1,906.22±733.72 and 1,681.19±1070.48 fry in both seed production cycles compared to 996.35±218.11 and 461.39±151.37 fry in the RB group. However, the two social groups were not statistically different in terms of total seed production. On seed production per female that spawned, however, significantly ($P<0.05$) higher means (796.33±77.68 and 726.33±124.08 fry) were observed in the PB group in the two seed production cycles compared to those in the RB group with means of 522.73±54.68 and 335.84±44.98 fry.

During the breeding period, the water temperature ranged from 29.40 to 30.50 °C in the morning and from 31.50 to 34.60 °C in the afternoon. On dissolved oxygen, the value ranged from 4.28 to 5.16 mg L⁻¹ in the morning and 5.90 to 6.72 mg L⁻¹ in the afternoon. These values were within the desirable range of 20°C to 35°C for temperature and greater than 3 mg L⁻¹ for dissolved oxygen required for normal development, reproduction, growth, and survival of tilapia (El-Sayed 2006).

Table 1. Reproductive performance of the two social groups during the two breeding trials under the hapa-in-pond seed production system

Treatment	Mean ± SE			
	Spawning Rate (%)	Hatching Rate (%)	Total Seed Production (number of fry)	Seed Production per Female (number of fry)
First Seed Production Cycle				
PB	38.89±14.70 ^a	81.06±1.03 ^a	1,906.22±733.72 ^a	796.33±77.68 ^a
RB	33.33±9.62 ^a	78.41±0.00 ^a	996.35±218.11 ^a	522.73±54.68 ^b
Second Seed Production Cycle				
PB	33.33±8.87 ^a	70.66±8.77 ^a	1,681.19±1,070.48 ^a	726.33±124.08 ^a
RB	22.22±2.48 ^a	84.48±2.48 ^a	461.39±151.37 ^a	335.84±44.98 ^b

Note: Means ($n = 3$) having different letter superscripts within the cell in a column are significantly different from each other at the 5% probability level by T-test.

4. DISCUSSION

The proactive female breeders produced a significantly higher number of fry than reactive female breeders. Proactive female breeders had a numerically higher spawning rate and a total number of fry compared to the reactive female breeders in both seed production cycles.

During the isolation period, fish exposed to a novel environment exhibited varying ECPs. Therefore, the fish's ECP after transfer to the new environment most likely reflects some aspects of the fish's behavioral response to confinement stress (Bero 2008; Vera Cruz and Brown 2007). According to Vera Cruz and Tauli (2015), ECP indicates behavioral stress response in *O. niloticus*. In the present study, fish with low mean ECP value (2 or less) during isolation were classified under the PB group and may be termed as the "bold" or proactive individuals. Those with high mean ECP value (6 and above) were classified under the RB group and may be termed as the "shy" or reactive individuals. Breeders with mean ECP of 3 can also be classified as PB and fish with mean ECP of 5 as RB. These breeders were not selected in the present study. There may only be a small difference in the reproductive capacity between these breeders that might influence the statistical significance of the reproductive parameters.

Proactive breeders produced a numerically greater total number of fry in both seed production cycles than the RB group; however, the differences were not statistically different. The absence of a significant difference may be attributed to the wide variation of the total number of seed production among replications within treatment. This, in turn, was due to the variation of the number of female breeders spawning per treatment-replicate, especially in the second cycle. One possible explanation may be that the breeding period was short (14 days) and that some may require a more extended period to spawn.

On spawning rate, although no significant difference between social groups was observed, the PB group obtained numerically higher values ($38.89 \pm 14.70\%$ and $33.33 \pm 8.87\%$) in both seed production cycles as compared to those ($33.33 \pm 9.62\%$ and $22.22 \pm 2.48\%$) in the RB group. According to Schreck et al. (2001), it was found that stress experienced at various periods during gonad maturation has an effect on the reproductive traits of *O. niloticus*. Delayed ovulation was observed when stressors (agitation and disturbance) were encountered during early ovarian development.

Successful and fast spawning was observed when stress was experienced during late vitellogenesis (Contreras-Sanchez, unpublished data cited by Schreck et al. 2001). Therefore, *O. niloticus* breeders respond by acceleration or complete reproduction inhibition, depending on the maturational stage when the stressor is experienced. This may be what had happened in some of the female breeders in both PB and RB groups. However, since reactive individuals had lesser ability to cope in a particular condition than proactive individuals, reactive females were more prone to stress and, therefore, spawned less than proactive females. Furthermore, in fish that experienced stress, trade-offs between reproductive efforts, somatic growth, and survival may occur (Schreck et al. 2001). According to Chichinadze et al. (2014), differences between dominant (proactive) and subordinates (reactive) on reproductive system functioning are a consequence of the following: (a) different levels of stress among animals of different social ranking; (b) preferences achieved by individuals of particular social standing; and (c) the physiological or biochemical traits characterizing individuals before reaching a certain social status.

Behavioral stress can prevent animals from achieving normal reproductive success (Moberg 1991). The present study showed that the behavioral stress response or ability to cope with certain conditions influenced fish reproductive performance. Proactive females produced a significantly higher number of fry than reactive females. This finding supports Abella's (2011) study that proactive females (based on feeding response) produced more eggs per spawning than reactive females. Moreover, in previous studies, it has been pointed out that social status or stress can influence an organism's reproductive state. For some species, subordinate males or females have their reproduction inhibited (Goncalves-de-Freitas and Ferreira, 2004). In the study by Tauli (2013), reactive *O. niloticus* during isolation (based on ECP and ventilation rate) are most likely to become subordinate individuals during dyad social interaction. Since reactive individuals have lesser ability to cope in a particular condition than proactive individuals, reactive females are more prone to stress and are expected to produce fewer eggs and thus a lesser number of fry. In the present study, it was observed that seed production was influenced by social grouping the breeders belonged based on their manifested ECP during isolation. Although proactive breeders were expected to be more aggressive than reactive breeders, social interaction or fighting of male

breeders (which may be stressful to the fish) may not be as intense as predicted since there were only two males and also there were more females (6 females: 2 males) present in the breeding unit. Aggression occurs when the fish feels being threatened by others. During breeding, each male breeder established a territory. And since the breeding unit was large enough (2 m x 1 m x 1 m), the two male breeders might have been able to establish their territories. The female entered the territory and laid the eggs. The male then fertilized the eggs.

In cichlid fishes, it is believed that dominant status gives priority for reproduction (Goncalves-de-Freitas and Ferreira 2004). On the other hand, environmental stressors, mainly nutrition, can adversely affect reproduction (Tilbrook et al. 2000), specifically fecundity (Schreck et al. 2001). Environmental stress, such as pollution, in a broader sense, appears to have the same capacity to inhibit reproduction as acute or chronic stresses associated with capture and handling procedures (Pankhurst and Van Der Kraak 1997).

5. CONCLUSION

Potential social status or the ability to cope in certain conditions, as determined by their behavioral stress response (i.e., ECP), can influence the reproductive performance of *O. niloticus*. Higher seed production is expected in proactive individuals than in reactive individuals. The higher seed production in proactive females than reactive females demonstrated that seed production in *O. niloticus* could be increased by selecting breeders with a low-stress response by evaluating their ECP during isolation.

Since proactive males were paired to proactive females and reactive males to reactive females, succeeding studies should evaluate the effect of the pairing of proactive males and reactive females and vice versa on seed production. The possible effect of the ratio of proactive and reactive breeders in a breeding population on seed production should also be assessed.

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