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Visual communication stimulates reproduction in Nile tilapia, *Oreochromis niloticus* (L.)

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Reproductive fish behavior is affected by male-female interactions that stimulate physiological responses such as hormonal release and gonad development. During male-female interactions, visual and chemical communication can modulate fish reproduction. The aim of the present study was to test the effect of visual and chemical male-female interaction on the gonad development and reproductive behavior of the cichlid fish Nile tilapia, *Oreochromis niloticus* (L.). Fifty-six pairs were studied after being maintained for 5 days under one of the four conditions (N = 14 for each condition): 1) visual contact (V); 2) chemical contact (Ch); 3) chemical and visual contact (Ch+V); 4) no sensory contact (Iso) - males and females isolated. We compared the reproductive behavior (nesting, courtship and spawning) and gonadosomatic index (GSI) of pairs of fish under all four conditions. Visual communication enhanced the frequency of courtship in males (mean ± SEM; V: 24.79 ± 3.30, Ch+V: 20.74 ± 3.09, Ch: 0.1 ± 0.07, Iso: 4.68 ± 1.26 events/30 min; $P < 0.05$, two-way ANOVA with LSD *post hoc* test), induced spawning in females (3 spawning in V and also 3 in Ch+V condition), and increased GSI in males (mean ± SEM; V: 1.39 ± 0.08, Ch+V: 1.21 ± 0.08, Ch: 1.04 ± 0.07, Iso: 0.82 ± 0.07%; $P < 0.05$, two-way ANOVA with LSD *post hoc* test). Chemical communication did not affect the reproductive behavior of pairs nor did it enhance the effects of visual contact. Therefore, male-female visual communication is an effective cue, which stimulates reproduction among pairs of Nile tilapia.

Key words**:** Courtship; Cichlid; Gonadosomatic index; Visual cues; Chemical cues

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Introduction

The reproductive behavior of many fish species involves a complex courtship that facilitates sexual recognition, supplies information about reproductive conditions of the mate, stimulates reproduction, and synchronizes spawning (1,2). During male-female interactions, pairs use different sensorial cues, which affect their reproductive behavior and gonadal state. In *Oncorhynchus* species, for example, visual and mechanical cues from females induce spermiation in males (3). The sound produced by some species facilitates intraspecific recognition and accelerates spawning (4,5), whereas sighting a male increases ovulation frequency in female *Oreochromis mossambicus*

(6), *Trichogaster trichopterus* (7), and *Danio rerio* (8).

Pheromones often are sensorial ways for modulating reproduction in fish (9). In cyprinids, the pheromone 4-pregnen-17α,20β-diol-3-one (17,20βP) delivered by female urine synchronizes spawning and enhances sperm production (10).

Vision is well developed in the cichlid fish species (11) and visual communication is an important part of their reproductive interaction (e.g., 12). However, chemical communication has also been reported for cichlids as an important way for reproductive communication (e.g., 13,14). In the cichlid Nile tilapia, *Oreochromis niloticus*, the dominant male increases chasing and courtship of females injected with 17,20βP, but in anosmic males these behaviors are not demonstrable (15). However, the background color of a substrate modulates the frequency of spawning in Nile tilapia (16) and the species shows elaborated courtship movements (17), suggesting that visual communication is part of its reproductive behavior.

The presence of females in a group of Nile tilapia stimulates males to build nests (18). Although this indicates the effect of the opposite sex on reproductive behaviors, the specific stimuli taking part in male-female interaction (chemical, visual, or both) are unknown. Because such effects are probably genetically determined, understanding the relative importance of sensorial modalities in Nile tilapia reproduction may help control the reproduction of this species for research and aquaculture.

The objective of the present study was to test the effect of visual and chemical cues on the reproductive behavior of Nile tilapia. This species establishes a social hierarchy in which dominant males take priority for mating. They build circular nests (spawning site) by mouth digging, defend the nests against intruders, court females, and mate inside the nests (17). Nile tilapias are mouthbrooders, a female behavior that includes caring for eggs and larvae by holding them inside her mouth (19). We analyzed the effect of visual and chemical cues on courtship, nest building, and spawning. Since the male-female interaction can also affect the gonad state of the fish (1), we also investigated the effect of visual and chemical cues on male and female gonad development.

Material and Methods

We tested the effects of male and female visual and chemical communication on the reproduction of Nile tilapia under four sensorial conditions: visual, chemical, chemical and visual, and no sensorial contact (males completely isolated from females). The reproductive behavior and gonadosomatic index (GSI = 100 x gonad weight / body weight) were determined to assess the effect of visual stimuli on the reproductive behavior of the species.

Holding conditions and general procedures

Adult Nile tilapias were purchased from a commercial supplier and held in outdoor ponds under natural conditions of temperature and photoperiod. Before the experiments, the fish were acclimated to laboratory conditions in 500-L asbestos tanks (1 fish/5 L; 27°C; 12-h light/dark cycles, starting at 7:00 am) for at least 20 days. The fish were overfed with a tropical fish ration (commercial pellets, 28% protein) twice a day.

The fish were removed from the holding tanks, anesthetized with benzocaine (128 mg/L), weighed, sized, and sexed by staining the genital papillae with methylene blue, which shows the opening of the female oviduct (20).

Little et al. (21) reported that 5 days of social interaction are sufficient to induce spawning in Nile tilapia. However, a control group of completely isolated fish was also required because isolation is known to induce gonad regression in another cichlid species (22). Thus, we conducted a preliminary study to test the effect of isolation on GSI. This experiment was necessary to ensure that the effects of sensory treatments could be attributed to the stimulatory effects of visual and chemical cues, rather than to inhibitory effects of social isolation.

Table 1. Standard length, body weight and gonadosomatic index (GSI) of male and female Nile tilapia after 5-day isolation (Experiment 1).

	Male			Female		
	Length (cm)	Body weight (g)	GSI (%)	Length (cm)	Body weight (g)	GSI (%)
Isolated	10.60 ± 0.28	42.25 ± 3.16	0.69 ± 0.07	10.42 ± 0.22	40.26 ± 3.16	3.07 ± 0.33
Non-isolated (from tanks)	10.69 ± 0.17	44.81 ± 2.18	0.61 ± 0.09	10.48 ± 0.23	44.29 ± 2.99	3.34 ± 0.35

Data are reported as mean ± SEM for 8 pairs of fish in each group. There were no statistical differences between groups (independent *t*-test).

Experiment 1: isolation and gonad development

Isolated and non-isolated fish (from 500 L-holding tanks) were compared to test the effect of isolation on gonad regression. Eight males and eight females (Table 1) were taken randomly and gonads were obtained immediately, to calculate GSI, a reliable indicator of gonad development for male and female Nile tilapia (23). At the same time, 8 males and 8 females were caught and maintained completely isolated in glass aquaria (40 x 30 x 40 cm; 27°C; 12-h light/dark cycles, starting at 7:00 am) for 5 days. After this period, GSI was measured.

GSI was not affected by the 5-day isolation (Table 1). Thus, the isolated treatment was used as the control condition in Experiment 2.

Experiment 2: sensorial cues and stimulation of reproductive condition

This experiment was conducted immediately after Experiment 1 to assess the role of visual and/or chemical communication on stimulation of reproductive condition in Nile tilapia. The fish were maintained under one of the four treatments designed for behavioral observations for 5 days. Each treatment included 14 pairs of fish and is described below. Fish in all treatments were similar in size and weight (Table 2).

Visual communication (V): 1 male and 1 female of two contiguous aquaria could see each other, no other contact was allowed. Each compartment of the experimental aquaria measured 30 x 40 x 40 cm (and contained about 48 L).

Chemical communication (Ch): a larger aquarium was divided into two compartments of the same size, following the criterion of equivalent compartment sizes of the other treatments. An opaque partition divided this aquarium and prevented visual male-female communication. Chemical communication was allowed by water circulation between compartments, through three holes (1.5 cm in diameter) near the bottom. Small pipes placed in the holes inhibited visual contact. Water circulated continuously by an aeration system (~3 L/min) whose efficiency was demonstrated by transferring water mixed with methylene blue from one compartment to the other.

Chemical + visual communication (Ch+V): this and the previous treatment were similar, except that here a transparent partition divided the aquarium into two compartments.

Isolation (Iso): aquaria receiving male or female fish isolated from any communication with conspecifics.

Lateral and posterior walls of the aquaria were covered with an opaque blue plastic to prevent undesired visual contact with the fish in adjacent aquaria. Blue is known to reduce stress (24) and improve reproduction (16) in Nile tilapia. Water temperature was maintained at 27 ± 1°C by individual thermostats and the photoperiod was 12-h light/dark cycles, starting at 7:00 am. Aquaria bottoms were covered with a 3.0-cm layer of gravel to allow nesting. The fish were fed an amount equivalent to 2% of their body weight twice a day, 1 h after lights were turned on and 1 h before lights were turned off. A biological filter preserved water quality in each aquarium during the experiment, with ammonia and nitrite kept lower than 0.25 ppm and 0.025 ppm, respectively.

Analysis of behavior

The fish were video-recorded for the analysis of behavior (15 min/day) on the 2nd and 4th days, from 1-5 pm because the highest reproductive activity in this species occurs in the afternoon (17). The frequency of undulation was recorded because this is a known courtship-related behavior among Nile tilapia (17) and other related species (12). Undulation occurs when the fish beats its tail sideways, thus undulating its body.

The aquaria were examined daily, at the beginning of the light period and just before its end, to record spawning (revealed by female mouthbrooding) and nest occurrence. Male tilapias excavate the gravel from the bottom with their mouth, making circular nests (17,19). At the end of the experiment, the diameter and length of nests we measured and elliptical areas of nests were calculated. When two nests were found in the same aquarium, their areas were summed to indicate fish investment in nesting. This measurement permitted us to test the association between sensorial condition and nesting behavior.

Gonads

After 5 days of pairing, the fish received a lethal dose of

Table 2. Standard length and body weight of male and female Nile tilapia used in different sensorial contacts (Experiment 2).

Sensorial cues	Male		Female	
	Length (cm)	Weight (g)	Length (cm)	Weight (g)
Visual	10.14 ± 0.07	36.09 ± 1.01	9.90 ± 0.11	34.27 ± 1.38
Chemical + visual	10.25 ± 0.07	37.57 ± 0.87	9.95 ± 0.14	33.92 ± 1.40
Chemical	10.32 ± 0.11	37.57 ± 1.28	9.94 ± 0.13	34.53 ± 1.33
Isolated	10.30 ± 0.10	37.28 ± 1.18	9.75 ± 0.12	32.96 ± 1.34
One-way ANOVA	$F_{(3,52)} = 0.78$	$F_{(3,52)} = 0.93$	$F_{(3,52)} = 0.54$	$F_{(3,52)} = 0.25$

Data are reported as mean ± SEM for 14 pairs of fish for each sensorial cue. There were no statistical differences between groups (one-way ANOVA).

the anesthetic benzocaine and fish were then sized and weighed, and their gonads collected for GSI calculation (as in Experiment 1). Testes and ovaries were fixed in Bouin solution and preserved in 70% alcohol for histology. Eight pairs from each experiment were randomly selected for gonad histology (gonads embedded in paraplast to make permanent slides). We made 3 and 5 µm cuts in eggs and 3 µm cuts in testes, and used hematoxylin-eosin staining. The aim of this analysis was to compare gonad maturation stages among experiments.

Statistical analysis

Data were tested for normality by Shapiro Wilk's test (25). Treatments were compared separately for males and females. Nest areas were compared among treatments by one-way ANOVA completed with the LSD *post hoc* test, and nest frequency was assessed by the chi-square test (25). The frequency of undulation and GSI were analyzed by two-way ANOVA completed with the LSD *post hoc* test (25), considering all four sensorial conditions and the males that built nests and those that did not.

Taxonomy and ethical considerations

Five samples of fish used in this experiment were identified as *Oreochromis niloticus* and deposited in the Zoological Collection of Department of Zoology and Botany, IBILCE, UNESP, São José do Rio Preto, under the supervision of Dr. Francisco Langeani Neto. This study was conducted in agreement with the precepts of the Brazilian College for Animal Experimentation (COBEA, Colégio Brasileiro de Experimentação Animal; www.cobea.org.br).

Results

Effect of sensorial cues on nesting and spawning

Nesting occurred in all treatments, and nests were similar in area and frequency (Table 3). Despite literature reports that only males dig nests, both males and females built nests. Spawning occurred only in treatments with visual communication between males and females (V and Ch+V; 3 spawnings each). In all 6 spawns both males and females built a nest. In three cases, males and females built a half-nest in each compartment. The halves were near one another at the compartment partition, forming a complete circular nest.

Table 3. Nesting behavior of Nile tilapia as a function of sensorial cues.

Sensorial conditions	Male		Female	
	Nesting relative frequency^a	Nest area^b (cm^2)	Nesting relative frequency^a	Nest area^b (cm^2)
Visual	0.43	158.7 ± 24.29	0.57	85.3 ± 12.94
Chemical + visual	0.43	269.3 ± 52.97	0.29	159.7 ± 34.18
Chemical	0.57	310.9 ± 48.67	0.29	107.5 ± 26.59
Isolated	0.43	139.7 ± 18.44	0.29	139.3 ± 18.39

Data are reported as mean ± SEM for 14 pairs of fish for each condition. There were no statistical differences among sensorial conditions (^{a}chi-square test; ^{b}one-way ANOVA).

Effect of sensorial cues on courtship behavior

Because nests were not built in some of the experiments, we compared the effect of treatment on undulation behavior among replicates where males built and those where they did not build nests, using a two-way ANOVA.

There was no statistical interaction between male nest building and treatment ($F_{(3,48)}$ = 0.73, P = 0.53) and no effect of investment on nesting ($F_{(1,48)}$ = 2.71, P = 0.11). However, the sensory treatments significantly affected the frequency of male undulations, a component of courtship behavior ($F_{(3,48)}$ = 23.61, P = 0.0001). Undulation frequencies of male V and V+Ch fish were similar (P = 0.21, LSD test) but significantly larger than Ch (P < 0.0001) and Iso fish (P < 0.0001).

The same procedure was used for females, with similar results. There was no statistical interaction between female nest building and treatment ($F_{(3,48)}$ = 0.24, P = 0.87) and no nesting effect ($F_{(1,48)}$ = 0.55, P = 0.46). The sensory treatment significantly affected the frequency of female undulations ($F_{(3,48)}$ = 8.69, P = 0.0001). Undulation frequencies of female V and V+Ch fish were similar (P = 0.84, LSD test), but significantly larger than Ch (P < 0.001) and Iso fish (P < 0.001). Figure 1 shows undulation data grouped by treatments because there was no effect of nest building on this variable.

Effect of sensorial cues on gonads

Fish that spawned were not included in the GSI analysis because gonad weight is reduced after spawning (23). We determined if spawning had occurred by the presence of eggs in the female's mouth, but we could not observe spermiation by males. However, we excluded from GSI analysis three male-female pairs from the V treatment and three pairs from the Ch+V treatment, with 11 pairs remaining in each of these treatments, in order to avoid an effect of reduced GSI on them.

Differences between the GSI of males that built or did not build nests were detected. Of the four Ch+V females that built nests, three also spawned. After excluding spawned females, we had only one pair, which did not allow us to include it in the statistical analysis, but the GSI values are shown in Figure 2B.

Two-way ANOVA indicated the effects of treatment ($F_{(3,42)} = 4.05$, $P = 0.01$) and nest building ($F_{(1,42)} = 13.25$, $P = 0.0007$) on male GSI, and no interaction effect ($F_{(3,42)} = 2.38$, $P = 0.08$; Figure 2A). According to the LSD *post hoc* test, GSI was higher for males that built nests ($P = 0.0006$, LSD test). However, the GSI of males that built nests was lower in Iso and Ch conditions than in V and Ch+V ($P < 0.02$; Figure 2A). No significant GSI differences were found for females in any of the treatments ($F_{(2,32)} = 1.06$, $P = 0.35$; Figure 2B).

According to the histological analyses of gonads, all fish were mature and ready for reproduction, independent of gender or experimental treatment. Testes showed spermatic cells in different stages and forming a line in the seminiferous tube, which contained a large number of free spermatozoids. Ovaries showed oocytes in different stages of development and several large and mature oocytes in full vitellogenesis activity.

Discussion

This study showed that visual communication between sexual partners modulates reproductive behavior of Nile tilapia, both stimulating courtship and gonad development in males and spawning in females. This is supported by the spawning frequency, courtship behavior, and GSI of males that build nests, which are documented here.

Male and female nest digging was observed irrespective of treatment. According to Lowe-McConnell (19), only males of Nile tilapia build nests in natural environments. However, Gonçalves-de-Freitas and Nishida (17) observed that after females were driven to the nests by males, females worked to increase nest size. In the present study, isolated females also

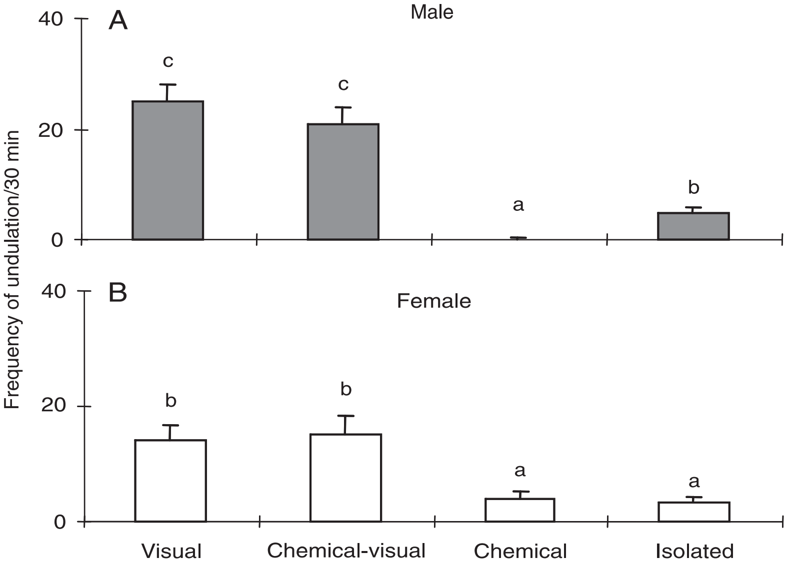

Figure 1. Undulation frequency of males and females under four sensorial conditions (N = 14 pairs in each condition). Data are reported as means ± SEM. Different letters indicate statistically significant differences compared with other groups ($P < 0.05$, two-way ANOVA followed by the LSD test).

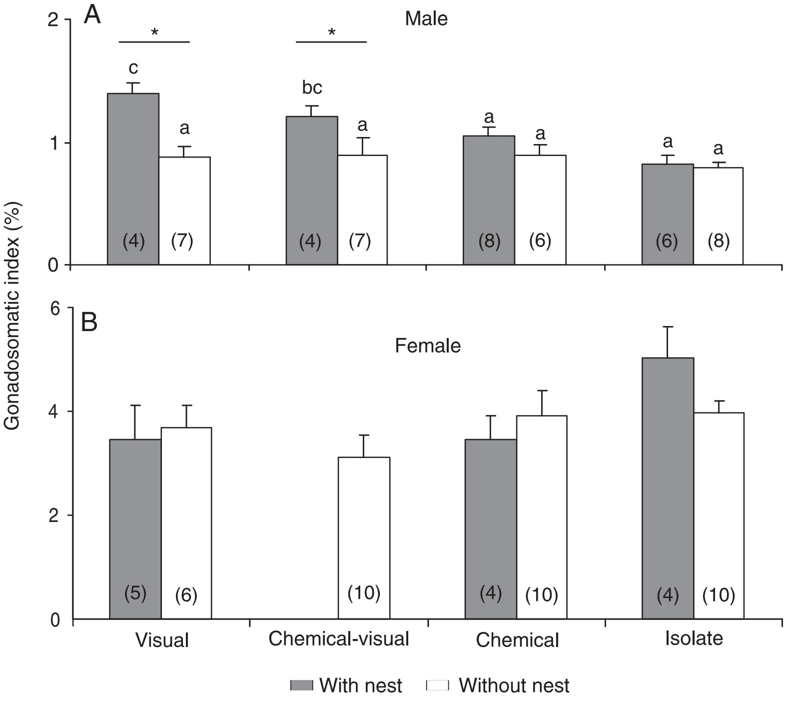

Figure 2. Gonadosomatic index (GSI) of Nile tilapia for each sensorial condition, with and without nests. Data are reported as means ± SEM. Numbers of replicates are indicated within parentheses inside the respective bar. Lines over conditions compare GSI between fish with and without nesting in the same treatment: $^*P < 0.05$ (LSD test). Letters compare means among sensorial groups for the same condition of nest building. Different letters indicate statistically significant differences ($^*P < 0.05$, two-way ANOVA followed by the LSD test).

built nest, with no male stimulation. This may occur when some structural components are absent in the environment, as reported for other cichlids (6,26).

Nile tilapia reproductive behavior is plastic and adjustable to artificial environment. Reproduction can take place even in the absence of nest (27). Thus, the environmental structure of the present study might have stimulated females to build nest.

Nile tilapia courtship is a complex behavior (17), but in the present study the complete repertoire was not exhibited because males and females were physically separated. In fact, only undulation predominated and could be clearly observed, so that it was considered for the courtship analysis. Moreover, this behavior has already been considered for other fish species (e.g., 12,28).

Presence of a reproductive mate enhanced undulation in the V and Ch+V treatments. Chemical communication alone was not sufficient to enhance the frequency of undulation, which was similar to the frequency among isolated animals. Visual cues, however, were more effective in stimulating courtship. In fact, female courtship behavior is elicited only by visual traits of the male and not by a multicomponent signal from different sensory modalities (29).

Visual contact with females increased male GSI. Males were in the same stage of maturation and, considering that spermatogenesis is continuous, increased GSI reflects an increase in semen production and volume (30). GSI enhancement is correlated positively with androgen hormones (31) and modulation of such hormones by visual stimulation was reported for other cichlid species (32,33). Thus, visual pathways should be linked to hormonal control mechanisms in the Nile tilapia.

Chemical communication enhanced milt volume (sperm and seminal fluid) in *Carassius auratus* and *Cyprinus carpio* (10,34,35). Pinheiro et al. (36) have shown that Nile tilapia males exposed to water with 17,20βP (5 x 10^{-9} M) increased milt volume, spermatozoa motility and durability. However, in the present study, chemical communication alone failed to affect male GSI. Such disagreement might be due to differences in 17,20βP concentration in the water and/or time of exposition.

Spawning occurred at a low frequency in V and Ch+V, but not in Ch and Iso treatments, which demonstrated that watching males stimulates spawning in females. In *Oreochromis* genera, male-female contact occurs close to the final oocyte maturation period (6), and in other cichlids spawning occurs a few hours after ovulation (to *Tilapia macrocephala*) (37). Thus, visual contact with males can be one of the factors triggering ovulation followed by spawning in Nile tilapia, probably by hormonal stimulation (e.g., 33).

Although chemical communication did not affect Nile tilapia's reproductive behavior, rank signaling has been reported to be mediated by chemical cues in this species (38,39). This suggests that cichlid visual and chemical information seems to be important at different moments of fish life. In fact, multimodal signs provide more reliable information for receiver fish, and could be an advantage for males and females to obtain more precise information about quality of the partners during mate choice (2). This multimodal role for vision and chemicals in Nile tilapia reproduction, however, needs further investigations.

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