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Male Blue Gourami (*Trichogaster trichopterus*) Nest-Building Behavior Is Affected by Other Males and Females

Gad Degani[1,2*], Michael Bar Ziv[1]

[1]Faculty of Science and Technology, Tel-Hai Academic College, Tel Hai, Israel
[2]MIGAL-Galilee Technology Center, Kiryat Shmona, Israel
Email: *gad@migal.org.il, mb_z_c@hotmail.com

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Abstract

In this study, we examine male blue gourami (*Trichogaster trichopterus*) nest-building behavior that is affected by other males and interactions under experimental conditions. Males found in the area control the territory and sexual behavior, and are influenced by the behavior of other males. The results of this study show that the sexual behavior (nest-building) of male blue gourami is affected both by the behavior and pheromones of other males. We suggest that males must defend their territories in order to prevent these two factors from interfering in nest-building.

Keywords

Male Blue Gourami, Blue Gourami, Sexual Behavior and Pheromones

1. Introduction

Blue gourami belonging to the Labyrinthici suborder, which belongs to the Perciformes order, is characterized by the presence of a chamber, or labyrinth, above the gills for the retention of air for breathing [1]. The 16 known genera contain about 80 species distributed throughout most of southern Asia, India and central Africa [2]. In Labyrinthici, an air-filled breathing cavity known as the labyrinth is located above the gills under the operculum on top of the head behind the eyes. The blue gourami (*Trichogaster trichopterus*) or three-spot gourami is native to Malaysia and Thailand. The blue gourami gets its name from its basic blue coloring. The gourami has a touch of silver on its belly and even a slash of green on the tips of its gill covers and fins. This native

*Corresponding author.

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blue gourami also has two large black spots on its sides, with the eye making up the third spot (hence the name three-spot) [1]. The labyrinth is a circular spot of very wrinkled tissue that offers more surface area for oxygen intake. This cavity supplements the breathing function, since it is well suited to gaseous interchange [1]. Because of these accessory organs, anabantidae fishes can even survive in water with very little oxygen content. The males often become territorial and very protective of the nest. As the female prepares to lay her eggs, the male wraps himself around her, catches the eggs in his mouth and spits out the eggs into the nest. The bubbles give the fry the oxygen they need for their first stages of development. Common habitats include rice fields. Forselius [3] reviewed the behavior, systematics, distribution and biology of the anabantidae species.

Many aspects of growth reproduction and commercial production of the blue gourami are described in detail [4].

The blue gourami in particular exhibits a very complex social and spawning behavior, and because of this, it has become a common subject for ethological studies. Miller [5] described in detail the social and spawning behavior of the blue gourami. The literature contains other studies on the same subject [6]-[9]. The aggressive behavior that enhances the ability of male blue gourami to defend their territories in signaled contests is studied in detail [10]. In blue gourami, as in many other vertebrate species that exhibit resource defense polygyny, males establish their territories well in advance of the arrival of females and, thus, are able to defend them over a relatively long period of time [10].

In addition to the aggressive behavior of males found in the same area as the females, many other parameters affect the nest-building of male blue gourami. Temperature affects males in building nests under different conditions, and brain and pituitary gene expression related to reproduction and growth is described [11]. Males that are maintained under non-reproductive conditions do not build nests, and the gonado-somatic index (% GSI) is significantly higher in fish maintained at 27˚C compared to fish maintained at 23˚C. The relative mRNA levels of brain gonadotropin-releasing hormone 3 (GnRH3), pituitary adenylate cyclase-activating polypeptide (PACAP), insulin-like growth factor-1 (IGF-1), pituitary b-luteinizing hormone (bLH) and prolactin are significantly higher when the fish are maintained at 27˚C than at 23˚C or 31˚C. β-Follicle-stimulating hormone (βFSH) mRNA levels were significantly lower when maintained at 31˚C than at the other temperatures. Nests were observed only in males under reproductive conditions. In these fish, higher mRNA levels of GnRH3, PACAP, βFSH, βLH and prolactin were detected at 27˚C, and higher mRNA levels of IGF-1 were detected at 23˚C, when compared to other maintenance temperatures or with fish that did not build nests.

In conclusion, we propose that temperature has a greater effect on the transcription of genes associated with reproduction than on those pertaining to growth. The purpose of the present study was to examine the effect of males and females on nest-building blue gourami under experimental conditions [11]. Water of different salinities affected hormones involved in reproduction and male nest-building [12]. In non-reproductively active males, mRNA levels of brain gonadotropin releasing hormone 1 (GnRH1), pituitary βFSH and prolactin (PRL) were significantly higher in males maintained in underground water. In reproductively active males, mRNA levels of brain GnRH1, GnRH3 and pituitary PRL were significantly higher than in males maintained in saline water. Thus, it is suggested that underground water having high levels of salinity and conductivity affects the gene expression of reproduction-related hormones; in reproductively active males, it shortened the duration of nest-building by blue gourami males [12]. Another very important parameter of interaction between males and females is that of pheromones [13]-[16].

A number of studies concerning chemical communication in *Trichogaster trichopterus* from the Belontiidae family indicate that this species may serve as a model for the whole systematic group [6] [8] [15]. An analysis by gas chromatography-mass spectrometry was performed to identify steroids and steroid glucuronides in gonads of t blue gourami and in water in which the fish were maintained. Full mass spectra of estradiol-17fl (E2), testosterone, 17αt-hydroxyprogesterone, cholesterol, stigmasterol, 4fl-methylcholesterol, estrone, 17αt, 20fl-hydroxy-4-pregnen-3-one (17, 20-P) and sitosterol were obtained. The above-mentioned steroids were detected in both female and male gonads, with the exception of estrone, which was detected only in the male, and 17, 20-P, which was detected only in the female. All steroids except for 17, 20-P were detected in the water in which the fish were maintained [13].

It is well known that for blue gourami maintained in a group at high densities in different combinations (males, females and both sexes), no sexual behavior and nest-building occur.

The purpose of this study was to examine in more detail the effect of males and females on the nest-building of blue gourami male under experimental conditions.

2. Materials and Methods

2.1. Fish and Experimental Design

Blue gourami fish (*T. trichopterus*, *Perciformes*, *Osphronemidae*), which were maintained and bred at MIGAL laboratories in northern Israel, were used in this study. The fish were kept in an aquarium (50 × 50 × 30 cm) at a temperature of 27˚C from November to April under a light regime of 12 h light:12 h darkness [11] until the beginning of the experiments, and were fed an artificial diet (45% protein, 7% fat), supplemented with frozen live food (Artemiasalina). All research involving the fish was approved by the committee in MIGAL dealing with animals and conforms to NIH guidelines. After an acclimation period of 10 days, the experiment was carried out on the fish for seven days. All the mature fish were weighed and measured before the experiments commenced. In each experiment, eight aquariums were used for the experiment and eight aquariums for control (number of replications) (**Figure 1**). Only the experiments for which the males built nests in the control groups were measured and are presented in the results.

Experiment 1. One blue gourami male was maintained in the aquarium for control, and two males (**Figure 1(c)**) were used in the experiments. The number of nest-building males (**Figure 1(e)** and **Figure 1(f)**) in the experimental and control aquariums was monitored.

Figure 1. The experimental aquarium systems. (a) and (b) illustrate the aquarium systems; (c) shows two males in experiment 1; (d) shows two males in the cage in experiment 3; (e) and (f) illustrate male nest-building in the aquariums.

Experiment 2. Three reproductively active blue gourami males were maintained in each experimental aquarium and the control group was the same as described in experiment 1 (one male maintained in the aquarium).

Experiment 3. One male was maintained in the experimental aquarium and two other males were maintained in the same aquarium in a small cage (10 × 15 × 10 cm) (**Figure 1(d)**).

2.2. Statistical Analysis

A t-test was used in order to examine whether significant differences exist between the measurements (weight and length) of fish in the experimental and control groups. To compare the number of nest-building males in the experimental and control groups, we compared the results to random distributions using X^2-test.

3. Results

No significant differences were found between the experimental and control groups using the t-test of weight and length measurements of fish in the three experiments in the present study (**Table 1**).

The two mature males maintained in the aquarium (50 × 50 × 30 cm) and the aquariums where nest-building occurred were monitored. There were no significant differences among the percentage of nest-building males in the experimental group (two males in one aquarium, 50% of males in the aquarium built nests) and the control group (one male in the aquarium, 62% of males in the aquarium built nests) (**Figure 2**).

Table 1. Comparison between the measurements of fish in the experimental and control groups in the present study.

Experiment Number	Number of Replications	Mean Weight (g) ± SD	Mean Length (cm) ± SD
Experiment 1	8	8.0 ± 2.6	7.85 ± 0.4
Control 1	8	7.5 ± 2.5	7.8 ± 0.4
P (Test)		P = 0.9 > 0.05	P = 0.7 > 0.05
Experiment 2	8	10.3 ± 3.5	7.9 ± 0.4
Control 2	8	10.0 ± 2.6	7.8 ± 0.5
P (Test)		P = 0.8 > 0.05	0.8 > 0.05
Experiment 3	8	10.2 ± 2.4	8.4 ± 0.5
Control 3	8	10.6 ± 4.1	8.4 ± 0.6
P (Test)		P = 0.5 > 0.05	P = 0.5 > 0.05

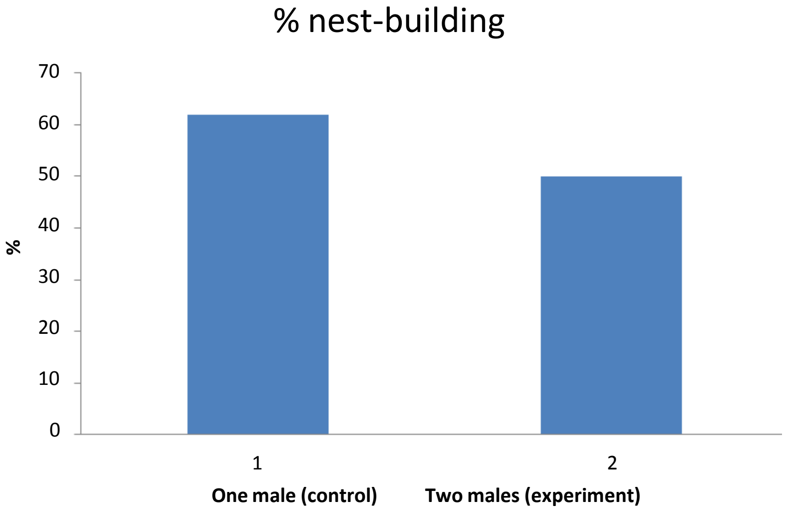

Figure 2. Comparison between the % of nest-building in the aquariums in the experimental group where two males were held in the aquarium (number of aquarium = 8) and the control group where one male was held in the aquarium (number of aquarium = 8). No significant differences were found (X^2-Test, P > 0.05).

On the other hand, nest-building was significantly reduced to 25% where three males were maintained in one aquarium in the experimental group compared to 50% in the control. Significant differences exist between the control group (one male maintained in one aquarium) compared to the experimental group (three males maintained in one aquarium) (**Figure 3**).

Similar results were found in the next experiment where three males were maintained in the aquarium but two were held in the cage (**Figure 4** and **Figure 5**) or three females were maintained with one male. The percentage of nest-building was significantly less (25%) compared to the control group (75%) in the aquariums where nest-build activity took place (**Figure 4**).

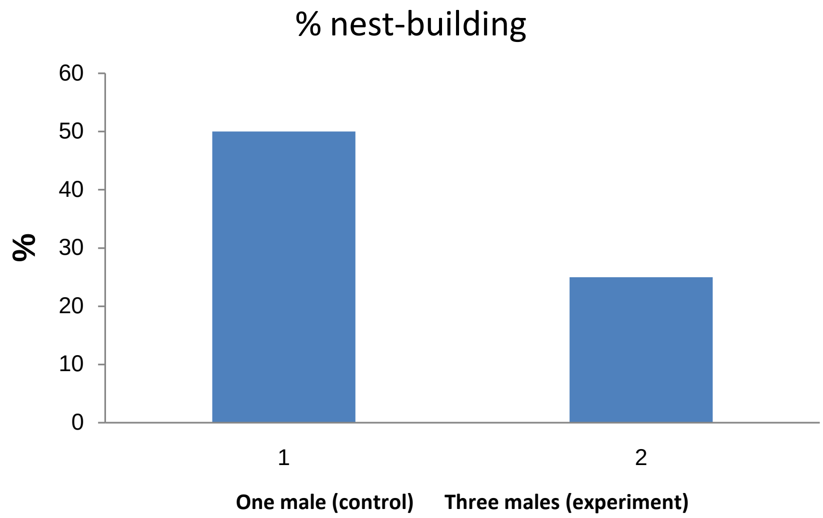

Figure 3. Comparison between the % of nest-building in the experimental group where three males were held in the aquarium (number of aquarium = 8) and the control group where one male was held in the aquarium (number of aquarium = 8). Significant differences were found (X^2-Test, $P < 0.05$) between the experimental group (three males in the aquarium) and the control group (one male in the aquarium).

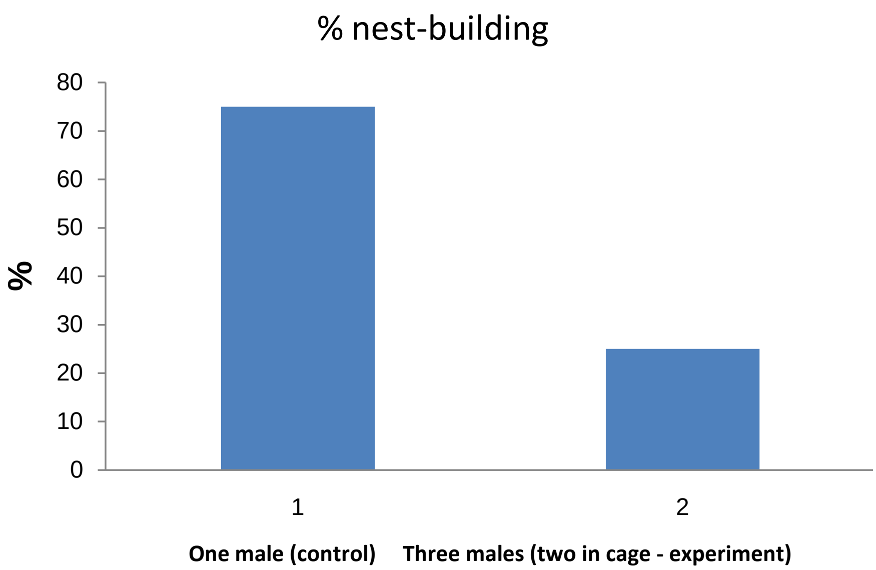

Figure 4. Comparison between the % of nest-building in the experimental group where three males were held in the aquarium (number of aquarium = 8) and two of them in the cage (**Figure 1(d)**) and the control group where one male was held in the aquarium (number of aquarium = 8). Significant differences were found (X^2-Test) between the experimental (three male) and control groups.

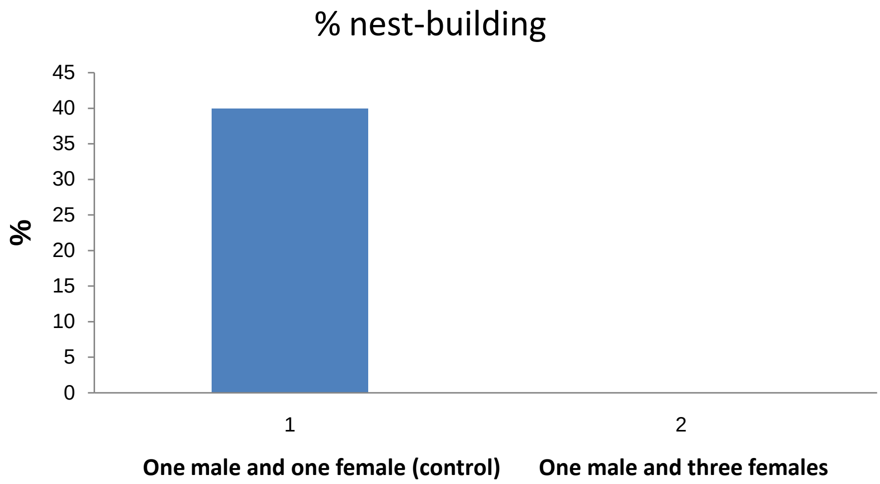

Figure 5. Comparison between the % of nest-building in the experimental group where three females were held in the aquarium (number of aquarium = 8) and one male and the control group where one male and one female were held in the aquarium (number of aquarium = 8).

4. Discussion

In this study, we support the well-known hypothesis that blue gourami requires territory in order to build nests [1], and that males found in the area of the territory are affected by other males as is sexual nest-building behavior [3] [5]. The contribution of the present study supports the hypothesis that not only the behavior of males or females in relatively high densities prevents nest-building but also that the pheromones of other males [13] reduces the number of nests built. Our suggestion for explaining this result is that the two males maintained in the cage in the same aquarium are affected only by the pheromones of one male in reducing the number of nests built, as is found in the present study. This result is supported by previous studies on the sexual behavior of blue gourami [6]-[9]. The aggressive male behavior enhancing the ability of male blue gourami to defend its territories was studied in detail [10]. The present study supports the hypothesis that the territories also prevent the effect of pheromones of other males on sexual behavior and nest-building. During reproduction, only the dominant male that has a territory builds nests. The territory sends away the other males and only the pheromones of the dominant male affect the female. The pheromones of male blue gourami affect female gametogensis and gonadotropin cells in the pituitary [13]-[16].

In summary, the present study and previous studies suggest that both aggressive behavior and pheromones affect interactions among males, and only the males that have territories preventing both factors are able to build nests.

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