Sexual Behavior: Social and Ecological Influences in the Anabantoid Fish, *Trichogaster trichopterus*

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The influence of behavioral and environmental variables on the initiation of spawning in *Trichogaster trichopterus* was investigated. The behavioral responses of isolated males and females to conspecific visual and chemical stimuli were also examined. Fish in divided aquaria were administered visual cues of a conspecific through a glass partition, chemical cues by an exchange of water between the two chambers, or both visual and chemical cues. Males and females differed in their response to stimuli from the opposite sex. In addition, responses differed with the sex of the stimulus fish. Chemical cues elicited nest building in males but not females. In the spawning experiments, when one member of a pair of fish was exchanged daily, the male spawned the first day without a nest. The female spawned only after several days. Two manipulations shortened spawning latency: introducing a new male daily and isolating the female for 3 days in the spawning aquarium prior to union with a male. Spawning was delayed, or did not occur, when the female was exchanged each day during the mating period or when a second, smaller female was kept in the spawning aquarium for 3 days prior to introduction of a male. These results suggest that the induction of spawning readiness in females is more susceptible to social and environmental variables than it is in males.

Trichogaster trichopterus sumatranus, a teleost fish of the suborder Anabantoidae and the family Belontiidae, spawn as the male and female clasp so that the urogenital pores are juxtapositioned. The pair roll upside down, simultaneously emitting eggs and sperm, which float to the bubble nest at the surface of the water. Courtship and spawning behavior have been described in detail by Forselius (1957) and Miller (1964). Little is known, however, about the possible role of premating social interactions and ecological factors in regulating spawning latency. Johns and Liley (1969) and Kramer (1972) suggested that spawning is facilitated in *T. trichopterus* by establishing pairs

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on opposite sides of a loose fitting partition for a few days before uniting them. The limited visual, chemical, and tactile contact around the partition was postulated to stimulate the pair. Hall (1966) suggested that courtship acts to reduce the aggressivity of males which otherwise interferes with spawning. Whether male stimulation influences spawning readiness in the female has apparently not been systematically examined. One aim of the present experiments was to elucidate the effects of social and environmental variables on spawning latency in males and females.

Male *T. trichopterus* are reported to discriminate the sex of a conspecific on the basis of the length of the dorsal fin and the extent of swelling of the abdomen (Picciolo, 1964). Although Picciolo found no evidence for discrimination due to chemical cues, sounds, or stimulation of the lateral line, he suggested the possibility of summation of visual and chemical cues. Rossi (1969) demonstrated that a female pheromone could elicit male nest building in two anabantoids of the genus *Colisa*. Chemical cues produced reproductive or aggressive changes in males where visual cues did not. The effect of male stimuli was not tested. Another aim of the present experiments was to investigate the responses of isolated male and female *T. trichopterus* to conspecific chemical and visual stimuli.

METHOD

Subjects

Mature male and female *T. trichopterus*, obtained from local tropical fish distributors and ranging in weight from 5 to 26 g, were maintained on a diet of frozen brine shrimp, Tetramin Conditioning Food, and live mosquito larvae. They included both the blue gourami, with black ocelli, and the opaline gourami, a color variant with an irregular pattern of black marks. Blue and opaline gouramis exhibit similar behavioral patterns and spawn interchangeably (Picciolo, 1964). The fish storage rooms and the experimental aquaria were illuminated with cool white fluorescent lamps on a cycle of 14 hr light and 10 hr dark. Fish were isolated in individual aquaria for at least 10 days prior to the first, and between successive experiments. Fish that spawned were isolated at least 28 days between experiments. Individuals were sometimes reused as no training was involved and they all had prior spawning experience. Within each experiment, size of animals was counterbalanced between the conditions, and the male of a pair was approximately the same size or somewhat larger than the female.

Apparatus

The experimental aquaria (20 X 60 X 30 cm deep) had gravel bottoms, aquatic plants, and heaters which maintained the water at 26-28°C. Behavioral

responses were observed directly and recorded on prepared data sheets in Expt. 1 and on an Esterline-Angus Operation Recorder in Expt. 2. The size of a nest was recorded as none, 1-3 cm in diameter, 3-8 cm, and greater than 8 cm.

EXPERIMENT 1

This experiment investigated the influence of conspecific chemical and visual stimuli, separately and together, on nestbuilding and various social responses in males and females.

Procedure

A male and a female *T. trichopterus* were introduced into an experimental aquarium, one on each side of a sealed, glass partition which presumably attenuated fish-produced vibration. Sixteen and female pairs were rotated in a counterbalanced manner through four successive treatments, one pair in each condition at a time. In the first (None), the glass partition was draped with opaque plastic so that no visual stimuli from the opposite sex conspecific was allowed. Water in each chamber was circulated independently by an air-drive water lift tube from an ordinary, plastic aquarium filter. In the second (Visual), the glass partition was clear and water was circulated within each chamber as described above. In the third (Chemical), the glass partition was opaque and the water lift tubes were turned across the partition to exchange water between the two chambers. In the fourth (Visual-Chemical), water was circulated between the two chambers and the partition was clear. Eight of the 16 original pairs were used for a fifth treatment (Visual-Chemical-Same) which differed from the fourth only in that the pair were of the same sex.

The behavior of each subject was observed for 4 min each morning and afternoon for 4 days. Preliminary tests indicated that relative response frequency did not change with longer observations. The occcurrence of four different responses was recorded: Approach, Bite-butt, Display, and Nestbuild. Approach was recorded as movement of the fish to within 5 cm of the glass partition. Bite-butt was defined as a contact of the fish's mouth with the partition. Display consisted of full extension of the dorsal, anal, and caudal fins as in the lateral spread display described by Miller (1964). Nestbuild denoted blowing bubbles at the surface of the water.

Results

In the four opposite-sex conditions, males and females showed the most Approach, Bite-butt, and Display when visual cues were present (Table 1A).

TABLE 1

Means/4-min Observation and F Values for Behavioral Responses
in Sensory-Mode Experiment

A. Response to Visual and Chemical Stimuli of the Opposite Sex

| | Males $n = 16$ | | | | | Females $n = 16$ | | | | |
	None	Visual	Chem-ical	Visual chemical	F^a	None	Visual	Chem-ical	Visual chemical	F^a
Approach	2.06	6.54	1.46	6.51	54.97^b	2.06	5.99	1.45	5.96	25.42^b
Bite-butt	0.07	28.62	0.03	20.72	48.08^b	0	4.11	0	4.87	$6.67^{c,d}$
Display	0.75	3.45	0.88	3.61	34.65^b	0.63	1.33	0.31	1.41	8.42^b
Nestbuild	0.12	0.84	0.45	1.80	10.99^b	—	—	—	—	—
Color	1.97	2.84	2.12	3.10	9.28^b	1.38	1.68	1.40	1.83	NS

B. Response to Opposite and Same Sex Stimuli

| | Males $n = 8$ | | | Females $n = 8$ | | |
	Opposite	Same	F^a	Opposite	Same	F^a
Approach	7.04	6.04	NS	6.78	6.06	NS
Bite-butt	26.71	11.48	31.12^b	4.88	11.98	5.78^e
Display	4.41	9.41	15.19^c	1.21	1.99	NS
Nestbuild	1.35	0.45	NS	—	—	—
Color	2.70	3.81	7.29^e	1.21	1.80	5.77^e

[a] Repeated measures analysis of variance, all probabilities two-tailed tests.
[b] $P < 0.001$.
[c] $P < 0.01$.
[d] $n = 8$/group.
[e] $P < 0.05$.

Chemical cues had no effect on the frequency of these responses. There were significant differences in Nestbuild of the males for each condition. Males showed more Nestbuild in Chemical than in None (two-tailed, matched pair t test, $t = 2.54$, $P < 0.05$) and more in Visual-Chemical than in Visual ($t = 2.29$, $P < 0.05$). Females did not show Nestbuild, nor did their color vary with the different stimulus conditions. Males, however, were darker when visual cues were present whether or not chemical cues were exchanged.

The same-sex condition revealed that the frequency of Approach in males and in females was not significantly influenced by the sex of the stimulus fish (Table 1B). Males and females showed equal Bite-butt to partners of the same sex; but males responded more to the opposite sex, whereas females responded less. Males showed more Display to males than to females, while females responded similarly to both sexes. Nestbuild did not

vary with the sex of the stimulus fish. Skin color was darker in both sexes when the partner was of the same sex.

EXPERIMENT 2

This experiment was to investigate whether a premating period of contact with the opposite sex facilitates spawning. As previously indicated, it was not clear whether the male or the female or both respond to such stimulation.

Procedure

A male and female were separated by a partition of white plastic 1.5-cm mesh lattice for a 3-day premating period in each of six experimental aquaria. One member of the pair and the partition were removed at the end of the premating period and a second, previously isolated fish of the same sex, the Exchanged fish, was introduced. The other member of the pair, the Nonexchanged fish, remained in the aquarium until spawning or for 7 days. Exchanged fish remained in the aquarium for 24 hr and a different fish was introduced each morning. After spawning some eggs were removed from the aquarium in a petri dish. Viability of the embryos was noted 24-48 hr later.

Behavior was observed for 15 min in the morning and afternoon during the mating period. Approach, Bite-butt, Chase, Display, Flee, and Nestbuild were recorded. Approach was recorded when a fish oriented toward and moved slowly to within one body length of the partner. Bite-butt was forceful or gentle touching or tugging of the partner with the mouth. Bite and butt were not distinguished as it was not always possible to tell if the act included the use of teeth (Frey and Miller, 1972). Chase was recorded when a male swam rapidly toward or after a female. Flee was rapid movement of a female away from a male. Display and Nestbuild were defined as in Expt. 1.

Results

Exchanged females did not spawn with the Nonexchanged males during the 7-day period (Fig. 1). Five of the six Nonexchanged females spawned on the second day within 5-6 hr after the second Exchanged male was introduced. One Nonexchanged female did not spawn. All spawnings produced viable eggs.

Exchanged males showed longer Display and less Approach and Chase than Nonexchanged (Table 2). Nonexchanged females, paired with the Exchanged males, showed more Approach and Bite-butt, and less Flee than Exchanged females. Exchanged males and Nonexchanged females were darker than Nonexchanged males and Exchanged females.
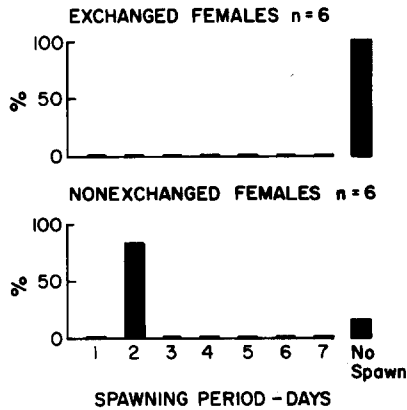
Fig. 1. The effect of exchanging mates on latency to spawning.

TABLE 2

Means/15-min Observation and t Values for First Three Observations of
Behavioral Responses for Exchanged Mates

	Females			Males		
	Non-exchanged	Exchanged	t^a	Non-exchanged	Exchanged	t^a
Approach	21.50	2.30	3.37^b	37.20	11.70	5.43^d
Bite-butt	24.30	0	4.20^b	—	—	—
Chase	—	—	—	8.40	3.10	2.30^c
Flee	3.80	12.10	2.50^c	—	—	—
Display duration	—	—	—	35.80	132.10	2.40^c
Color	2.80	1.77	3.96^b	2.45	3.50	3.50^b

[a]Student t.
[b]$P < 0.01$.
[c]$P < 0.05$.
[d]$P < 0.001$.

The differences in behavior between the morning and afternoon are shown in Table 3. Nonexchanged males showed no changes in responses over days, but Approach, Bite-butt, and Chase occurred more often in the morning than in the afternoon. Exchanged males exhibited longer and more frequent Display in the morning and more Approach and Chase in the afternoon. Nonexchanged females also showed more Approach, Bite-butt, and Display in the morning and more Flee in the afternoon. Exchanged females exhibited more Flee in the morning. No other significant differences in behavior were seen, but Nonexchanged females and Exchanged males were darker in the morning.

TABLE 3

Means/15-min Observation and Statistical Values Comparing Morning and
Afternoon Responses for Exchanged Mates

	Nonexchanged males			Exchanged females		
	AM	PM	F^a	AM	PM	F^a
Approach	36.92	14.06	28.21^b	—	—	—
Bite-butt	86.67	15.39	6.61^c	—	—	—
Chase	12.75	4.25	13.55^c	—	—	—
Flee	—	—	—	14.39	5.00	14.04^c

	Exchanged males			Nonexchanged females		
	AM	PM	t^d	AM	PM	t^d
Approach	6.08	23.00	7.69^c	29.92	4.50	2.93^c
Bite-butt	—	—	—	36.33	0.33	4.19^b
Chase	0	9.17	$0^{c,f}$	—	—	—
Flee	—	—	—	0	11.30	$0^{c,f}$
Display frequency	26.58	6.83	4.52^b	28.58	9.17	$0^{c,f}$
Display duration	179.92	36.50	2.75^c	—	—	—
Color	4.00	2.50	3.33^c	3.40	1.83	5.27^b

[a]Two-way analysis of variance.
[b]$P < 0.01$.
[c]$P < 0.05$.
[d]Matched t test.
[e]$P < 0.001$.
[f]Wilcoxon Signed-Rank T.

EXPERIMENT 3

The foregoing experiment indicates that premating social contacts have a far greater effect on spawning readiness in females than in males. The present study was to further examine the influence of premating social and environmental variables on spawning latency in the female.

Procedure

A female was placed in each of six spawning aquaria with a male, another female, or no fish for 3 days before being united with a male. In the first treatment (Male-Coresident), the female was separated from a male by a partition, as used in Expt. 2, during the 3 days. In the second treatment (Female-Coresident), a smaller female shared the aquarium without a partition,

and was removed just prior to introduction of the male. In the third treatment (No-Coresident), the female was alone in the aquarium without the partition during the premating period. Females tested with the partition in place showed comparable results and these are not discussed. In the fourth treatment (Nonresident), the female was united with a male without a premating period in the aquarium. Spawning latency was tested by keeping the male and female together until spawning or for 7 days. Viability of the embryos was noted as in Expt. 2.

Results

Seven of the 11 Male-Coresident pairs spawned in the 7 days starting as early as the second day; four pairs did not (Fig. 2). Three of the six Female-Coresident pairs spawned, but only after 4 days. Five of the six No-Coresident pairs spawned during the 7 days as did three of the six Nonresident pairs. All spawning produced viable eggs.

Of 51 pairs that were seen to spawn, seven had nests greater than 8 cm, seven had 3–8-cm nests, and six had 1–3-cm nests. Thirty-three of the 51 pairs had no nest. There was no obvious correspondence between nest size at spawning and the latency until spawning.
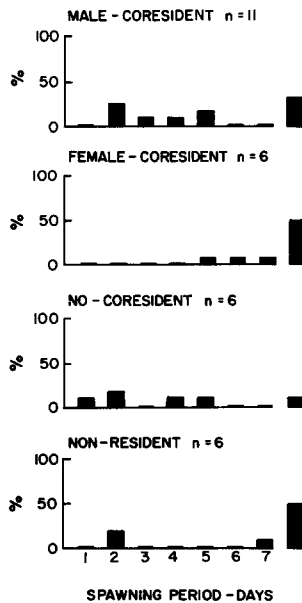

Fig. 2. The effect of premating social variables on latency to spawning.

DISCUSSION

Our application of Liley's procedure did not facilitate spawning. Male and female pairs, separated by the mesh lattice during the premating period, subsequently spawned at various intervals over the 7-day period. Liley and associates (Johns and Liley, 1970; Kramer, 1972) report that pairs separated by a loose fitting partition "generally" built nests during the premating period. Seven out of 10 of their fish spawned on the second day after the partition was removed. In our experiment only one male in 11 pairs built a nest in the premating period and that male did not spawn. Only two out of 11 pairs spawned on the second day. Differences in breeding stock and experimental procedures could be responsible for variation in spawning latency. Our procedure of isolating subjects before experiments, for example, may be a key variable.

Two manipulations that we tried were more effective than Liley's. Spawning occurred sooner when the female was kept alone during the premating period than when a male was in the other chamber. Spawning latency was also shorter when a new male was introduced each day. An analogous facilitatory effect of prior residency is seen in male cats which have a longer latency to mating in unfamiliar surroundings (Green, Clemente, and De Groot, 1957). The "new male" effect resembles the renewal of sexual behavior in satiated male guinea pigs, rats, cats, and other mammals when a novel female is introduced (Fisher, 1962; Hinde, 1970). Two procedures that we tried were less effective than Liley's. Spawning was delayed or inhibited when a female was kept in the other chamber during the premating period or if females were exchanged daily during the mating period.

Johns and Liley (1970) suggested that premating stimulation may induce spawning readiness in both the male and female. Our experiments demonstrate that males need no prolonged social contact with females. If male aggression needs to be reduced, as might be inferred from previous work (Hall, 1966), such changes occur within hours and not days. A male is aggressive when placed with a female, but the motivation is unclear as males are aggressive in both reproductive and nonreproductive situations. Females, on the other hand, seemingly must undergo an internal change before sexual behavior can be elicited. Hall (1966) found that bouts within a sequence were more likely to end in successful spawning when the first social response was made by the female. The onset of the spawning sequence would also appear to be controlled by the female rather than the male.

Our observations suggest that nestbuilding or the presence of a nest is not a prerequisite of spawning even though many investigators have used nestbuilding as an indication of spawning readiness (Braddock and Braddock, 1959; Johns and Liley, 1970; Kramer, 1972). In 51 spawnings in our laboratory only 18 aquaria had nests before eggs were laid. The nest presumably serves

adaptively in nature to protect the young (Forselius, 1957), as well as being the fixed external reference point of the male's territory (Forselius, 1957; Braddock and Braddock, 1959; Miller, 1964). Nestbuilding may also be considered a displacement activity (Hinde, 1970), as nests are built not only when a male is spatially separated from, but receiving visual and chemical cues of another male or female, but also by males in isolation and at other seemingly inappropriate times (Miller, 1964).

The first experiment indicates that physically isolated males and females can detect the sex of the conspecific when exposed to visual and chemical stimuli. Visual cues, such as the structure of the fins and body or patterns of movement, may be involved. Male *T. trichopterus* have proportionally longer and more pointed dorsal fins than females (Juliano, 1965). The ratio of the dorsal fin length to body length in our animals varied from 89 to 107 in males and from 83 to 93 in females. The overlapping distribution would seem to preclude that fish could detect sex solely by the size of the dorsal fin, although dorsal fin shape combined with fin extension during display may be a cue (Picciolo, 1964). Males also have broader mouths, larger eyes, and thicker anal fins than females (Hisoaka and Firlit, 1962), but these are less readily apparent to the human if not the fish.

Skin color could be a stimulus in sexual recognition. Picciolo (1964) found that, if females were darkened with testosterone proprionate, a male first showed lateral display and darker coloration as normally exhibited in response to a male, but soon began to show investigative behavior and lighter color. Males generally were darker than females in our experiments (Table 1). Female angel fish, *Pterophyllum scalare*, can also discriminate males from females on the basis of visual and chemical cues, as shown by the difference in frequency of egg release in the absence of males (Chien, 1973).

The social responses shown in the second experiment illustrate the interaction of male and behavior. When the male was not changed, a high number of Approach and Chase by males was accompanied by a large number of Flee by females (Table 2). Comparison of morning and afternoon data showed that most male Approach, Bite-butt, and Chase were made in the morning (Table 3). Males made fewer responses in the afternoon and there was a corresponding decrease in female Flee. Upon introduction of a new female, the male typically approached the female who fled, followed by the male. When the male was changed daily, on the other hand, the female did not flee upon introduction of the male. Instead there were a high number of female Approach and Bite-butt, to which the male responded with Display (Table 2). By afternoon females no longer initiated social encounters (Table 3).

Experiment 1 revealed only limited evidence that chemical cues are social stimuli in *T. trichopterus*. An increase in male Nestbuild in response to chemical cues from a female was augmented by the addition of visual stimuli. Male visual and chemical stimuli also induced male Nestbuild. Picciolo (1964)

reported that chemical cues alone were ineffective in eliciting courtship or agonistic responses in *T. trichopterus* and several other anabantoids. He also found that anosmic fish exhibited appropriate courtship, nestbuilding, and spawning behavior. However, a response may be evoked or modified by a particular class of sensory stimuli yet persist after deletion of that input. We found in our study, as Picciolo suggested, that visual and chemical cues acting together stimulated more male nest building than either cue alone.

This research has shown that males will spawn on the same day they are placed with a female, whereas, even with experimental manipulation, we have been unable to obtain first day spawning in females. Additional research in necessary to determine the neural and endocrine processes that underlie behavioral changes prior to spawning.

REFERENCES

Braddock, J. C., and Braddock, Z. I. (1959). The development of nesting behaviour in the siamese fighting fish *Beta splendens*. *Anim. Behav.* 7, 222-232.

Chien, A. K. (1972). Reproductive behaviour of the angelfish *Pterophyllum scalare* (Pisces: Cichilidae) II. Influence of male stimuli upon the spawning rate of females. *Anim. Behav.* 21, 457-463.

Fisher, A. E. (1962). Effects of stimulus variation on sexual satiation in the male rat. *J. Comp. Physiol. Psychol.* 55, 614-620.

Forselius, S. (1957). Studies on anabantid fishes. I. II. III. *Zool. Bidrag. Uppsala.* 32, 93-597.

Frey, D. F., and Miller, R. J. (1972). The establishment of dominance relationships in the blue Gourami, *Trichogaster trichopterus* (Pallas). *Behaviour* 42, 8-62.

Green, J. D., Clemente, C. D., and De Groot, J. (1957). Rhinencephalic lesions and behavior in cats. *J. Comp. Neurol.* 108, 505-546.

Hall, D. D. (1966). An ethological study of three species of anabantoid fishes. Doctoral dissertation, Oklahoma State University. Ann Arbor, Mich. University Microfilms No. 67-7226.

Hinde, R. A. (1970). "Animal Behaviour." New York: McGraw-Hill.

Hisaoka, K. K., and Firlit, C. F. (1962). The embryology of the blue gourami, *Trichogaster trichopterus* (Pallas). *J. Morph.* 111, 239-253.

Johns, L. S., and Liley, N. R. (1970). The effects of gonadectomy and testosterone treatment on the reproductive behavior of the male blue gourami *Trichogaster trichopterus*. *Can. J. Zool.* 48, 977-987.

Juliano, R. O. (1965). On the sexual dimorphism of the three spot gourami, *Trichogaster trichopterus* (Pallas). *Natur. Appl. Sci. Bull.* 19, 97-99.

Kramer, D. L. (1972). The role of androgens in the parental behavior of the blue gourami, *Trichogaster trichopterus* (Pisces, Belontiidae). *Anim. Behav.* 20, 798-807.

Miller, R. J. (1964). Studies on the social behavior of the blue gourami, *Trichogaster trichopterus* (Pisces, Belontiidae). *Copeia*, 469-496.

Picciolo, A. R. (1964). Sexual and nest discrimination in anabantid fishes of the genera *Colisa* and *Trichogaster*. *Ecolog. Mon.* 34, 53-77.

Rossi, A. C. (1969). Chemical signals and nest building in two species *Colisa* (Pisces, Anabantidae). *Monitore Zool. Ital. (N.S.)* 3, 225-237.