Assessment of the Role of Chemoreception in the Mate Choice in Barbs of the *Barbus intermedius* Complex from Lake Tana, Ethiopia

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Abstract—The role of chemoreception in mate choice was studied in barbs of the complex *Barbus intermedius* from Lake Tana. It was found that chemical communication may be used in reproductive interactions of the barbs, and that males are responsible for the choice. Mature males tend to prefer ripe females and avoid both immature females and mature males, or are indifferent to them. It was shown that selectivity in mate choice by certain barb morphotypes could reduce the probability of interbreeding between different morphotypes, but not provide for reproductive isolation between them. It was hypothesized that the mate groups are finally formed as a result of direct interactions between potential partners, mediated by tactile reception or specific behavioral patterns.

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Lake Tana, the largest lake in Ethiopia, is situated at the altitude of 1786 m above sea level and has an area of 3156 km^2 and a maximum depth of 14 m (Chorowicz et al., 1998). As in other African lakes (Mina et al., 1998), the "large African Barbus" (sensu Banister, 1973) are represented by numerous forms, some of which differ significantly in their external morphology. As a result of many years of studies, Nagelkerke and Sibbing (1997, 2000) concluded that Lake Tana is inhabited by 15 barb species, 14 of which these authors considered as morphotypes (forms of uncertain status, Nagelkerke et al., 1994). Earlier, in a revision of large barbs from Eastern and Central Africa, Banister (1973) identified all species of Lake Tana barbs as a single species *Barbus intermedius* Rüppell, 1836. Even earlier, Brunelli (1940) suggested that some forms are true species whereas others are morphs of a single species.

The hypothesis that Lake Tana barbs represent different biological species, that is, boundless species sensu Mayr (1974), presupposes that they are reproductively isolated. Studies of the major hystocompatibility complex suggest that there may be reproductive isolation between some of them (Dixon et al., 1996; Kruiswijk et al., 2005), because individuals of different morphotypes may have no common alleles of the same gene.

Nonetheless, the mechanisms of reproductive isolation between morphotypes, especially spawning in the same river, require specific studies. Nagelkerke and Sibbing (1996) suggested that reproductive isolation between morphotypes depends on differences in spawning time; Palstra et al. (2004) thought that individuals of different morphotypes spawn at different times and in different places. However, Dgebuadze

et al. (1999) have shown that individuals of different morphotypes ready for spawning may be present in the spawning grounds at the same time. They suggested that even though temporal and spatial segregation of barb morphotypes from Lake Tana could facilitate their reproductive isolation, this segmentation could hardly prevent their interbreeding (p. 420), and the results of experimental spawnings (Alekseyev et al., 1996) revealed no postzygotic isolation between them. It was concluded that if there is reproductive isolation, it should be based mainly on differences in spawning behavior (Dgebuadze et al., 1999, p. 417). The same authors particularly noted that visual discrimination is very difficult because water in the spawning river is extremely turbid, the fish spawn mostly at the dark time, and the morphotypes have no specific visual discrimination signs (stripes, spots, etc.)

Thus, it is reasonable to suppose that if reproductive isolation does exist, it should be based on prezygotic mechanisms and involve chemoreception. The significant role of chemical signals in mate choice is well documented in fish (Partridge et al., 1976; Ryan, 1990; Andersson, 1994; Sorensen and Stacey, 1999). Even in cichlids (fam. Cichlidae), in which mate choice is primarily based on vision, chemoreception plays an important role (Crapon de Caprona, 1974; Jordan et al., 2003). Moreover, chemoreception helps fish of many species to distinguish mature conspecifics of the opposite sex from mature individuals of closely related species (McLennan, 2004; Wong et al., 2005).

In this study, we assessed the role of chemical signalization in the reproductive behavior Lake Tana barbs. We tried to determine whether the barbs use
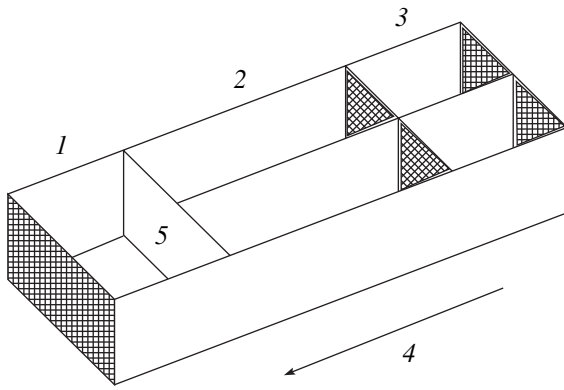
Fig. 1. Experimental apparatus. Length 1.9 m, width 0.8 m, height 0.6 m. *1*—Start compartment, *2*—corridors, *3*—chambers with potentially attractive stimulus fish, *4*—direction of the water flow, *5*—guillotine door.

chemical signals in mate choice; to determine the role of males and females in this process; to determine the differences in the behavior of different morphotypes; and to determine possible mate preferences for the same or other morphotypes.

MATERIALS AND METHODS

The experiments were conducted from November 29 to December 12, 2001 (dry season), and from September 15 to November 2, 2003 (the end of rain season, beginning of dry season), in the proximity of the village Wanzaye, 11°46′ N 37°43′ E, located close to the place where a small river Dukalit falls into the Gumara River, one of the basic tributaries of Lake Tana. Barbs, both constantly living in the Gumara River (Dukalit almost dries out during the dry season) and migrating from the lake for spawning, spawn in both rivers.

In 2001, the study was conducted at the end of the spawning period, when, with rare exception, spawned only barbs representing the groups that Alekseyev et al. (1996) designated as "precocious intermedius" and "normal intermedius," and considered age groups of the same populations. Individuals of the former group and a part of individuals of the latter group corresponded to the description of the "shorthead" morphotype (Nagelkerke et al., 1995), later distinguished into a true species *Barbus brevicephalus* Nagelkerke et Sibbing, 1997. Most of the fish, however, did not correspond to this description. According to Nagelkerke and Sibbing (1997, 2000), they should be considered as the "near-shore complex," an aggregation with uncertain status. In our experiments, we used individuals of the first and the second groups, which are designated, respectively, as "inter I" and "inter II." In experiments conducted in 2001, we did not distinguish individuals of the "intermedius" group into "inter I" and "inter II."

In 2003, we were able to investigate the spawning of several forms in addition to "inter I" and "inter II." Our study involved fish belonging, according to the classification of Nagelkerke et al. (1994), to the morphotypes "bigmouth big-eye," "bigmouth small-eye" and "acute," at the species level identified as, respectively, *B. macrophtalmus* Bini, 1940, *B. megastoma* Nagelkerke et Sibbing, 1997, and *B. acutirostris* Bini, 1940.

The fish were caught with fixed nets, cast nets, large landing nets, and hook and line in the Gumara River in the proximity of the experimental station. The fish were kept in cylindrical fishboxes made of net, which were installed in the river downstream of the experimental apparatus. The diameter and the height of the fishboxes was approximately 1 m. The fish were acclimated in these fishboxes from several hours to two days. The fish were not fed during the whole experimental period. We determined the sex and the ripeness stage in all individuals participating in the experiments. The ripeness and the sex of ripe fish were determined by drawing off the genital products at the moment the fish were transferred to the start compartment of the experimental apparatus. This procedure did not cause any significant stress in the fish affecting further behavior. In addition, there was an acclimation period after the fish was transferred into the experimental apparatus, as explained below.

After testing, we measured the standard length of the fish SL (up to the bases of the middle rays of the caudal fin), and dissected them, to check whether the sex and ripeness had been determined correctly. The length of male barbs ranged from 133 to 367 mm (mean 235.15 mm), and females, from 163 to 416 mm (mean 253.24 mm).

The experimental apparatus (Fig. 1) represented a traditional construction used in such studies, consisting of parallel corridors, connecting the start compartment with two compartments for the potentially attractive stimulus fish, the source of the smell (Newcombe and Hartman, 1973; Honda, 1982; Keefe and Winn, 1991). The apparatus was constructed of a metal frame with walls made of thin plastic film, and the partitions were made of plastic net. The guillotine door was metal.

The tested fish was transferred into the start compartment, separated from two parallel corridors by a guillotine door. The corridors led to two identical chambers, which contained stimulus fish potentially attractive for the fish. In some experimental series, one chamber was left empty. The chambers were separated from the parallel corridors by a small net and darkened (covered by an opaque film). The apparatus was located in the bed of the Dukalit River so that the water depth in the apparatus was 25–35 cm and the current was directed from the chambers to the start compartment. Thus, the tested fish could not see the individuals in the chambers. The smell from each chamber was distributed by the natural water flow along the respective corridor up to the start compartment.

The water temperature in the Gumara River changed during the day over the whole working period from 17.6 to 26.5°C (the average temperature was 22.1°C);

daily fluctuations were, on average, 3.2°C. Water temperature in the Dukalit and the experimental apparatus ranged from 19.6 to 29.0°C (average 23.3°C); average daily fluctuations, 7.1°C.

The water current speed in the corridors was maintained at 0.2–0.3 m/s. To determine the current speed, we simultaneously poured a small quantity of concentrated manganese solution into the chambers (100 ml in each) and recorded the speed of the colored cloud. If the water current speed in the chambers was not equal, the flow was adjusted by stones put down to the river bottom upstream of the apparatus.

The tested fish was placed into the start compartment and kept there at least 15 min, after which the guillotine door was opened. Orienting to chemical stimuli, the fish could freely move in the corridors from 30 to 60 min (in different series of experiments) and choose the corridor leading to the chamber with the more attractive stimulus. The behavior of the fish was recorded using a Sony DCR-TR17E video recorder, fixed above the experimental apparatus. The results of testing were assessed from the position of the tested fish, recorded by the video recorder for 2 s each 5 min.

When the fish was presented with its own and an alien morphotype, the selection of its own morphotype was recorded as "positive" response whereas selection of the different morphotype was a "negative" response. In cases of the choice between the stimulus fish and an empty chamber, the selection of the fish was a positive response, while empty chamber was a negative response. When the tested fish remained in the start compartment, "no response" was recorded. In some experiments, we assessed the percentage of the time spent by the fish in a particular corridor. Each fish was tested only once. The stimulus fish, providing the smell, were used from one to three times with different tested fish. The time from the introduction of a new stimulus fish to the beginning of the experiment was at least 15 min.

To characterize the choice of potentially attractive objects by a particular group of fish, we determined the distribution of these individuals over time (percentage of the total duration of the experiment), spent in one of the two corridors. We did not take into account the fish that stayed all the time in the start compartment. The statistical analysis included analysis of contingency tables and correlation analysis (gamma correlation). Additionally, we used the sign test.

RESULTS AND DISCUSSION

In the data collected in 2001, we assessed the time during which the fish stabilized the choice of a particular corridor. We assessed the correlation between the response of the fish during 60 min after the beginning of the experiment with responses after 15, 30, and 45 min. It was found that the choice made 15 min after the beginning of the experiment significantly correlated with the choice made 60 min after the beginning of the experiment even though the magnitude of the correlation was not too high (gamma correlation coefficient = 0.64, $n = 61$, $p < 0.009$). When we compared the fish responses 30 and 60 min after the beginning of the experiment, the correlation was quite high ($r = 0.85$, $n = 61$, $p < 0.001$), and it was even higher when we compared the choice 45 and 60 min after the beginning of the experiment ($r = 0.97$, $n = 61$, $p < 0.001$).

The results obtained in 2001 indicate that the choice of the fish was consistent, not random, and reflected real preferences. This is well documented in different fish species (Newcombe and Hartman, 1973; Honda, 1982; McLennan, 2004), but was not noted in Lake Tana barbs. The choice was developed quite rapidly, a 30-min session was enough for its reliable detection. Thus, the duration of the recording in 2003 was 30 min.

It should be noted, however, that the consistency of the choice observed in 2003 and assessed in the same way, by correlation between the responses of the tested fish at different times, was significantly lower and nonsignificant ($p > 0.05$); therefore, we assessed the time conducted by the tested fish in a particular corridor with respect to the overall duration of the experiment rather than the fixed response.

The differences between males and females were most pronounced in locomotor activity, which corresponds to the data obtained in some other species (see Budaev et al., 1999). In 2001, this measure was assessed by counting positive and negative responses in contrast to the absence of any response in the fish without respect to the ripeness. Thus, 93.33% of the tested males ($n = 30$) chose one corridor whereas 70.97% of females ($n = 31$) remained in the start compartment not making any choice. This sex difference in the behavior was highly significant ($\chi^2 = 26.41$, df = 1, $p < 0.001$).

Another characteristic associated with sex was noted in the response to representatives of the same or the opposite sex. As noted above, females in most experiments remained in the start compartment and did not reveal significant preferences ($\chi^2 = 1.88$, df = 2, $p = 0.390$). At the same time, the responses to individuals of the same or the opposite sex in males were significantly different ($\chi^2 = 8.18$, df = 2, $p = 0.017$). In all, 72.2% of males responded positively to the stimulus female and only 16.6% negatively. Males more often responded negatively to male stimulus fish (64.3% of cases), and a positive response was noted only in 35.7% of cases.

In addition, both ripe and immature males responded differently to individuals with ripe and immature (not reaching stage V) gonads. The corridor leading to the chamber with ripe male or immature female was chosen by males with the same probability as the corridor leading to an empty chamber. The corridor leading to the chamber with an immature male was chosen two times more rarely, while with mature female, it was chosen four times more frequently, than

Table 1. Responses of "intermedius" males to ripe and immature males and females of the same group

Stimulus fish		Response		Number of tested males
sex	ripeness stage	positive	negative	
Males	Ripe	2	2	4
Males	Immature	3	6	9
Females	Ripe	8	2	10
Males	Immature	4	3	7

Note: Positive response: the number of tested males choosing the chamber with the fish during 60 min; negative response: choosing empty chamber.

that leading to an empty chamber (Table 1). In other words, the tested fish responded indifferently or avoided males and immature females, but tended to prefer ripe females. The number of tests was too small to compute the significance levels in all tests, but the tendency was obvious. It is worth noting that the significance of the preference in the experiment with the maximum number of tests (the choice of ripe female), assessed using the sign test, was significant ($p = 0.05$). These results agree with the results obtained in other fish species (Partridge et al., 1976; Honda, 1982).

All this suggests that the observed interactions are associated with the development of reproductive relationships between individuals, in particular, the mate choice, and that the choice is made by males.

Taking into account the above results, in 2003 we tested only ripe males. The fish were presented with the choice between females of the same morphotype or a different one. It was found that males of "bigmouth bigeye," "bigmouth small-eye," and "acute" conducted more time in the start compartment (more than 50%) than males of "inter I" and "inter II" ($t_{157} = -3.89$, $p < 0.001$).

Table 2 presents the results of the experiments in which we tested at least five males of the same morpho-

type, choosing between females of the same or a different morphotype; Fig. 2 has histograms characterizing the variants. Additionally, the same figure shows the histogram (Fig. 2a) based on the data from 2001. In this variant of the experiment, seven ripe "intermedius" males were given the choice between a ripe female and empty chamber.

"Inter I" males demonstrated some preference of the same-morphotype females, when given a choice between "inter I," "inter II," and "bigmouth small-eye" females (Table 2). "Acute" males demonstrated females of the same morphotype when presented with it and "inter I" females.

The average time conducted by males of the same morphotype in the corridor leading to the chamber containing a female of the same morphotype depends on the distribution of this measure in individuals and the distribution of individuals with different preference profiles. For example, 50% overall preference could be observed in a situation when each of the tested males spends 50% of time with a female of the same morphotype and 50% with an alternative morphotype. The same overall preference would be recorded when 50% of males spend 100% of time with female of the same morphotype and another 50%, 100% of time with alternative female. But in the first case, we see no preference, whereas in the second case, we see choice with equal preference of females. The absence of any choice is revealed by a unimodal distribution with the mode close to 50%. A shift of the mode towards the values exceeding 50% would point to preference of female of the same morphotype, and a shift towards values less than 50% would indicate a preference for an alternative female. Finally, bimodal distribution with a minimum at 50% reflects a situation when most males prefer one of the females, but some males prefer females of the same morphotype, while other males prefer an alien morphotype.

Analyzing the histograms presented in Fig. 2, we must conclude that a tendency to the choice of a female (the alternative is an empty chamber) is observed in situation 2a, even though it is impossible

Table 2. The percentage of the time spent by barbs of four morphotypes in the corridor leading to the chamber with a female of the same morphotype, in contrast to the morphotype of alternative female

Morphotype of alternative female	Morphotype of the tested male			
	inter I	inter II	acute	bigmouth big-eye
Inter I		40.0 (0–66.6), $n = 9$	66.6 (25–75), $n = 10$	50 (33.3–100), $n = 21$
Inter II	75.0 (50–80), $n = 23$			
Bigmouth small-eye	66.6 (0–66.6), $n = 5$			
Bigmouth big-eye	50.0 (0–100), $n = 17$			
Acute	50.0 (0–66.6), $n = 7$			

Note: The duration of the experiment is presented excluding the time spent in the start compartment. Medians, 25 and 75% quartiles (in parentheses) are presented, n is the number of males tested in particular experiments.
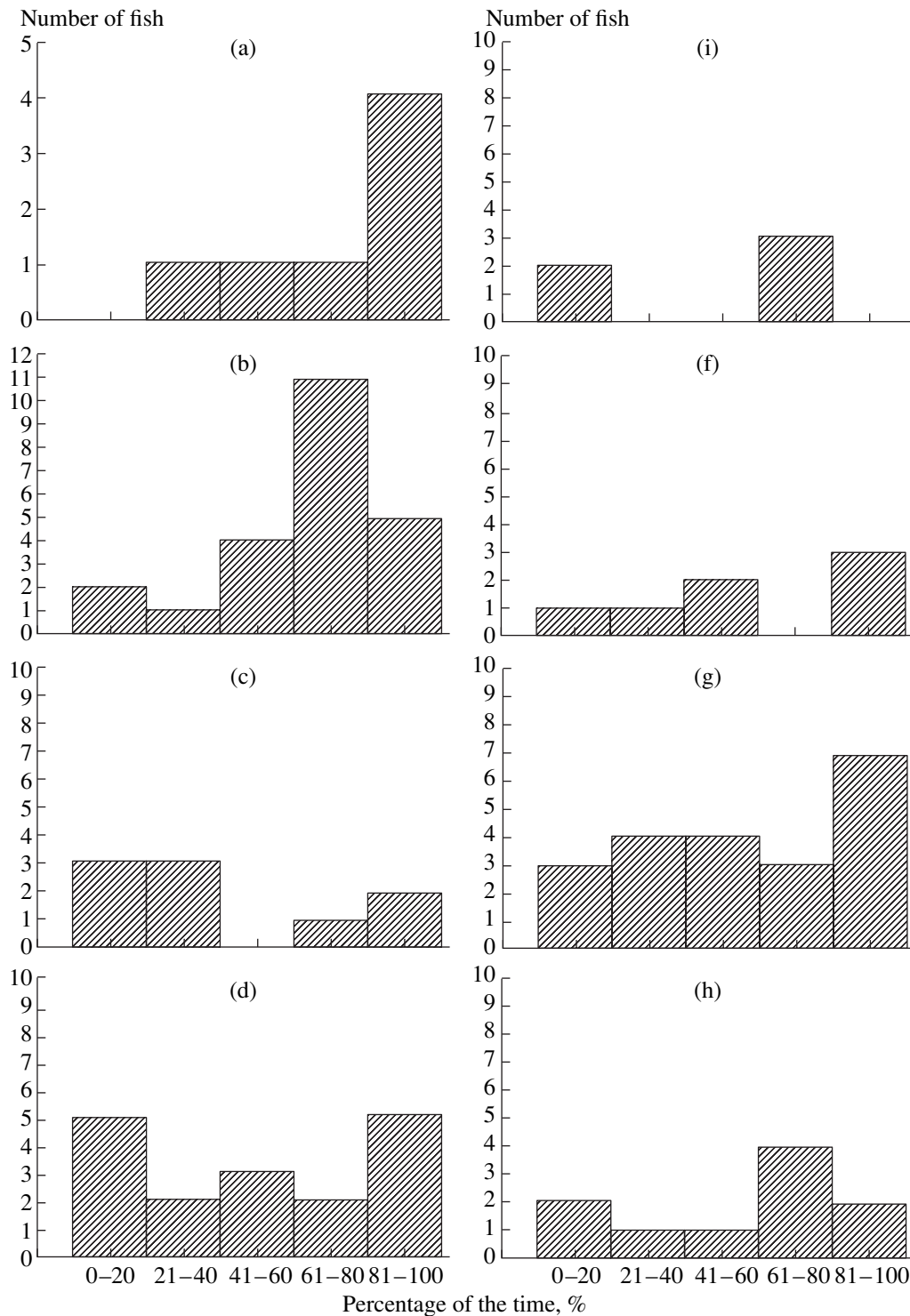
Fig. 2. Distribution of the time spent in the corridor leading to the chamber with the same-morphotype female (% of the total duration of experiment) in the tested males of the *Barbus intermedius* complex: (a) "intermedius" males, alternative, empty chamber; (b) "intermedius" males, alternative, female "inter II"; (c) "inter II" males, alternative "inter I" female; (d) "inter I" males, alternative "bigmouth big-eye" female; (e) "inter I" males, alternative "bigmouth small-eye" female; (f) "inter I" males, alternative "acute" female; (g) "bigmouth big-eye" males, alternative "inter I" female; (h) "acute" males, alternative "inter I" female.

to assess the significance of this tendency because of the small sample size. Nonetheless, this result may be considered as supporting the hypothesis that barbs could use chemical signals in interindividual interactions.

The shift of the mode, pointing to the reference by "inter I" males of the same morphotype females (with the alternative "inter II"), is observed in Fig. 2b. If we consider the males choosing females of the same mor-

photype as those which spent more than 60% of the time in the respective compartment, and those choosing alternative female, who spent not more than 40% with the same-morphotype female, the difference between the number of individuals in these groups (16 and 3) is significant at $p < 0.01$ (sign test, null hypothesis is that the number of males in the first and the second groups is equal). If individuals of the "inter I" and "inter II" groups belong to the same population and differ only in age or size (SL of males in the former group is at least 180 mm, females, less than 240 mm) (Alekseyev et al., 1996), the result obtained can be considered as evidence for mate choice by size or age.

At the same time, "inter II" males, presented with the choice between females of the same morphotype and "inter I" females (Fig. 2c) did choose, but did not reveal any particular preference. This is also true of the "inter I" males, choosing between females of the same morphotype, "bigmouth big-eye" (Fig. 2d) or "bigmouth small-eye" (Fig. 2e), or "acute" (Fig. 2f). Some insignificant preference for females of the same morphotype was found in "bigmouth big-eye" males when the alternative female was "inter I" (Fig. 2g), and in "acute" males when the alternative was "inter I" female (Fig. 2h). In all experimental series, except 2b, we did not find preferences based on the fish size.

Of course, we cannot exclude the possibility that the males barbs (at least some of them) used in our experiments were not ready for spawning. Most fish were caught during the period prior to spawning, and some were captured during the spawning migration. It is possible that the motivation that should determine active choice of the spawning partner was still too low. We also cannot exclude the possibility that the motivation of the experimental fish could decrease as a result of the experimental manipulations.

CONCLUSIONS

Our study revealed that chemical signalization may be used by Lake Tana barbs in reproductive interactions. The preference for ripe females by ripe males and avoidance of immature females and any males (or indifferent responses to them) suggest that the patterns found in our experiments are associated with mate choice and it is the males who choose.

On the whole, selectivity in the choice of certain morphotypes as mating partners could reduce the probability of interbreeding, which agrees with the hypothesis of Dgebuadze et al. (1999), but cannot provide for reproductive isolation between the morphotypes, suggested by several authors (Nagelkerke and Sibbing, 1996; Palstra et al., 2004).

It is possible that the mating groups are composed after direct contact of the potential partners, mediated by tactile reception and/or specificity of mating behavior. Such patterns have been documented in other fish species, however, usually in monogamous species (see Budaev et al., 1999). The reproductive system of Lake Tana barbs probably represents a kind of polyandry, as follows from the observations by Alekseyev et al. (1996) and Dgebuadze et al. (1999). We cannot also exclude the role of acoustic signalization in mate choice.

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