

## Urine as a Social Signal in the Mozambique Tilapia (*Oreochromis mossambicus*)

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### Introduction

Despite the high degree of social organization and complex behaviour of many cichlids, little is known about the role of chemical communication in these fish. The Mozambique tilapia is a maternal mouth-brooding cichlid; territorial males congregate in leks and are actively sought by ripe females. When approached by a female, males display a range of well-characterized behaviours such as nest-digging, trembling and circling the female (Baerends and Baerends van Roon, 1950). The female then chooses a mate, spawns and carries the fertilized eggs away in her mouth to incubate them in solitude. The extent to which olfactory cues participate in this process is unknown.

Several species of freshwater fish have been shown to release pheromonal messages via the urine (Stacey and Sorensen, 2002); we have previously shown that female tilapia have high olfactory sensitivity to the urine of male conspecifics (Frade *et al.*, 2002). In order to test whether this species is actively signalling to conspecifics or the olfactory sensitivity evolved as a form of chemical spying (Sorensen and Stacey, 1999), the current study assessed changes in urination rates of both sexes in different social contexts.

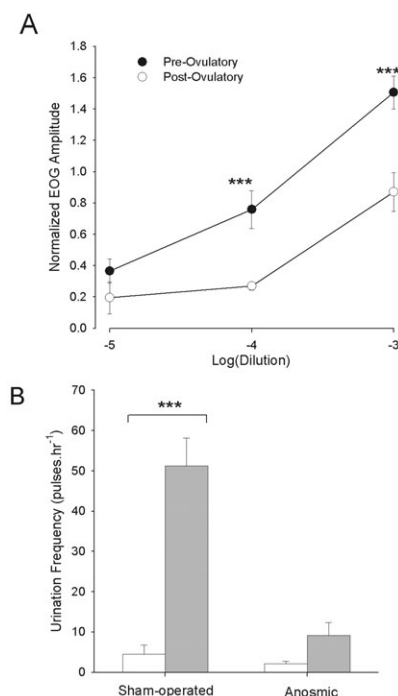
### Materials and methods

Urination was visualized by i.m. injection of 2.4 mg of isosulfan blue (Sigma) in 20  $\mu$ l 0.9% saline (Appelt and Sorensen, 1999). In behavioural experiments, fish were isolated 24 h prior to the experiment. Females or male intruders were then transferred to the male's tank (or a control tank). Only one fish was injected in each experiment and the number of independent trials ranged from 6 to 14. Experiments lasted 90 min: 45 min control followed by 45 min in the presence of a conspecific. Fish were videotaped for behavioural analysis and the frequency and duration of each urine pulse were recorded. Anosmia was produced by removing the cartilage rings surrounding the nostrils and cauterizing the olfactory epithelia (under anaesthetic); sham-operated males were used as controls. Fish urination frequency was compared between control and presence of a conspecific by Student's *t*-test for paired samples. Collection of urine and electro-olfactogram (EOG) recording were carried out as previously described (Frade *et al.*, 2002). Repeated measures ANOVA followed by *post hoc* LSD testing were used to compare EOG responses of males to increased concentration of urine from pre- or post-ovulatory females. Results are given as mean  $\pm$  SEM.

### Results

In the presence of a pre-ovulatory female, territorial males dramatically increased their urination frequency (control,  $4.5 \pm 1.5$  pulses/h; with female,  $54.4 \pm 6.6$  pulses/h;  $n = 13$ ,  $P < 0.001$ ). The duration of each pulse did not change, implying that the total volume released also increased. Although males also increased urination frequency in the presence of post-ovulatory females, this was less marked (control,  $7.0 \pm 2.3$  pulses/h; with female  $16.7 \pm 4.5$  pulses/h;  $n = 14$ ,  $P = 0.05$ ). This apparent discrimination of female reproductive

status is mediated by olfaction; based on EOGs, urine from pre-ovulatory females smells quantitatively different from that of post-ovulatory females (Figure 1A) and anosmic males fail to increase urination frequency in the presence of pre-ovulatory females (Figure 1B), although they show courtship behaviours (data not shown). In the presence of territorial males, both pre- and post-ovulatory females reduced their urination frequency (pre-ovulatory—control,  $36.2 \pm 11.1$  pulses/h; with male,  $16.0 \pm 4.5$  pulses/h;  $n = 12$ ,  $P < 0.05$ ; post-ovulatory—control,  $30.8 \pm 5.4$  pulses/h; with male,  $9.2 \pm 2.7$  pulses/h;  $n = 12$ ,  $P < 0.001$ ). However, urination rate was also decreased to a similar extent when females were simply transferred to another tank indicating that it was caused by manipulation (pre-ovulatory—control,  $21.3 \pm 4.6$  pulses/h; tank change,  $13.6 \pm 3.2$  pulses/h;  $n = 10$ ,  $P < 0.05$ ). Pulse duration was unchanged.



**Figure 1** Olfactory discrimination of female reproductive status by male tilapia (*Oreochromis mossambicus*). **(A)** Semi-logarithmic plot of normalized EOG amplitudes recorded from male tilapia in response to pooled urine from pre-ovulatory (filled circles) and post-ovulatory (open circles) females ( $n = 6$ ). **(B)** Histogram of urination frequencies of sham-operated and anosmic male tilapia under control conditions (open bars,  $n = 6$ ) and in the presence of a pre-ovulatory female (shaded bars,  $n = 6$ ). Data are shown as mean  $\pm$  SEM; \*\*\* $P < 0.001$ .

In male–male interactions, resident males immediately increased their urination rate upon introduction of an intruder (control,  $5.2 \pm 2.2$  pulses/h; with intruder,  $29.3 \pm 6.0$  pulses/h;  $n = 9$ ,  $P < 0.005$ ), while the intruder stopped urinating almost completely, especially during the first 5 min. Control experiments showed that this was not due to handling stress. Again, the duration of the pulses remained unchanged. In the ensuing fight, urination was reduced in both to near zero.

## Discussion

The present study shows that male tilapia modulate their rate of urine release depending on the social context. Together with the fact that conspecific urine contains potent odorants, this strongly suggests that the urine is being used as a chemical signal. Furthermore, territorial males may be signalling their status to potential rivals via their urine as well as via changes in coloration and behaviour (Baerends and Baerends van Roon, 1950).

Normally, males store urine; the bladder of a 100 g fish may contain up to 2 ml of urine. This ability is much less apparent in females and is reflected by their higher urination frequency under control conditions. In the presence of a female, territorial males were able to dramatically increase their urine release. During the courtship ritual the male's urination rate remained elevated, being significantly higher in the presence of pre-ovulatory females. This discrimination of female reproductive status is based on olfactory cues; anosmic males did not increase urination rate in the presence of pre-ovulatory females, but sham-operated controls did. However, anosmic males did exhibit other normal courtship behaviours. This suggests that olfactory cues are processed differently by the CNS from visual and/or other cues in the context of mate choice.

Unlike males, females did not change their urination rate in the presence of males. As it is the female that makes the ultimate choice with which male to mate, she has no advantage in advertising her reproductive condition. Nevertheless, the male olfactory system has developed the capacity to discriminate the reproductive status of females through chemical cues present in urine and/or faeces, which can be classified as chemical spying (Sorensen and Stacey, 1999).

In male–male interactions, the resident may be signalling to potential rivals entering his territory that they are unwelcome. As the intruder was unable to flee, the encounter usually culminated in a fight. Nevertheless, it must be borne in mind that these interactions

took place in aquaria; in the wild, intruders may flee from the resident's territory before any fighting occurs. It is possible, therefore, that the chemical signal may lower the aggressiveness of the intruder, and that inhibition of urination is one manifestation of this process. How the intruder recognizes that the signal comes from his opponent and not himself or, conversely, how the resident is not inhibited by the substances in his own urine is not clear. The closely-related Nile tilapia, *Oreochromis niloticus*, is capable of some degree of individual recognition (Giaquinto and Volpato, 1997) and this is mediated, at least in part, by chemical cues; certainly the faeces and bile also contain powerful odorants (Frade *et al.*, 2002).

In conclusion, males may actively advertise their dominant status via substances released in the urine, whilst females do not actively advertise their reproductive status. The identity, origins and functional roles of the compounds involved remain to be established.

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