



Olfactory responses of male and female Mozambique Tilapia (*Oreochromis mossambicus* Peters 1952) to a putative urinary pheromone that signals male dominance



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Introduction

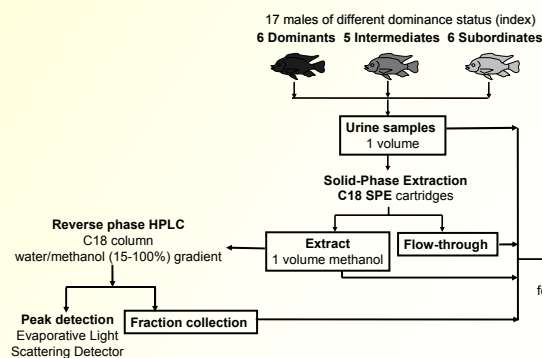
- Tilapia males establish dominance hierarchies; females mate preferentially with dominant territorial males
- Dominant males increase urination frequency during courtship and aggression (Barata et al., 2008. *JCE*)
- A sulphated amino-sterol urinary odorant for females has been suggested to act as pheromonal signal of dominance

➤ **Do both sexes detect the same urinary odorants from males of different social rank?**

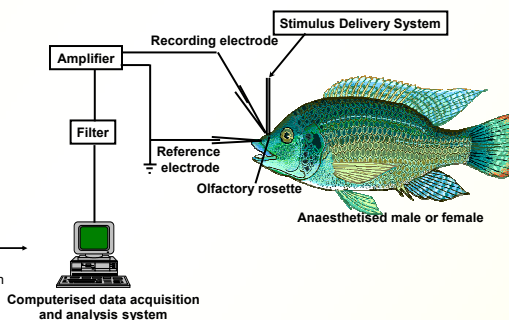
Methods

Assessment of the olfactory activity of male urine, and corresponding SPE and HPLC fractions in males and females

Sample collection and processing

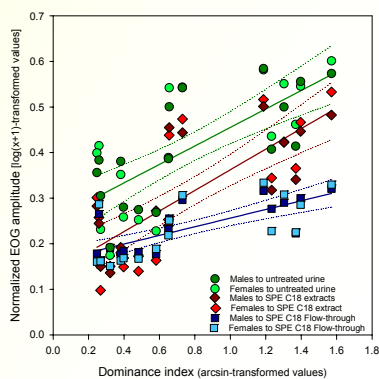


Recording of the Electro-Olfactogram



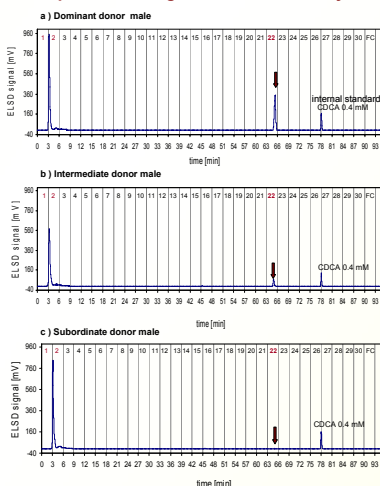
Results

EOG responses of both sexes to urine and SPE fractions from males of different social rank



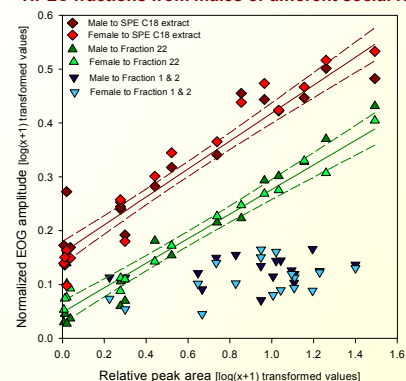
- EOG responses of males and females were similar.
- The olfactory potency of the three stimulus types increased with the social rank of male donor (regression lines and dashed 95% CL lines).
- The raw urine was the most potent stimulus. The extract had a regression line with similar slope, but lower elevation ($P < 0.001$).
- The flow-through was the least potent stimulus with a regression line of lower slope ($P < 0.001$) and elevation ($P < 0.001$) than raw urine and extract.

Example chromatograms of male urinary extracts



- HPLC of urine extracts revealed a well retained peak (RT = 65 min; fraction 22).
- The area of this peak was larger in dominant males than in subordinates.
- The total area of non-retained peaks in fractions 1 & 2 did *not* correlated with males' social rank.

EOG responses of both sexes to urine extracts and its HPLC fractions from males of different social rank



- The urine extract and corresponding HPLC fractions 22 containing this peak (putative amino-sterol) evoked concentration-dependent EOG responses in both sexes (regression lines and dashed 95% CL lines).
- EOG responses to fraction 22 had a similar regression line slope to that of the urine extract, but a lower elevation ($P < 0.001$).
- In contrast, the EOG amplitudes elicited by another HPLC fraction (fractions 1 & 2) containing several non-retained peaks were *not* correlated with its total peak area.

Conclusion

➤ **Both males and females detect the same dominance pheromone!**

- Most likely, increased release of the amino-sterol signals dominance to both sexes, but in a blend comprising other unidentified urinary odorants.

Next steps

- Mass (MS)- and structure (NMR) analysis of the amino-sterol like dominance pheromone
- Bioassay guided separation and fractionation of further (more hydrophilic) urinary odorants