HOW AND WHY THE STICKLEBACK WILL BE USED AS A BIOMARKER FOR STUDYING THE EFFECTS OF MIXTURES OF OESTROGENS AND (ANTI)-ANDROGENS

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CEFAS The Centre for Environment, Fisheries & Aquaculture Science

The problem

The majority of Endocrine Disruption studies in Europe have been on non-indigenous fish species and almost exclusively on xenoestrogens. Europe not only needs its own test-species but one which also enables the detection of xenoandrogenic compounds.

So why use the stickleback?

- It is the only fish with a quantifiable *in vivo* **androgen and anti-androgen endpoint** (the production of the glue protein, spiggin, by the kidney).
- It is the only fish in which it is possible to simultaneously test oestrogenic and androgenic properties of compounds - **thereby halving the number of fish which need to be used in experiments**.
- It has a **genetic sex marker**.
- It is **EUROPEAN**.
- It survives **and breeds** in both **seawater and freshwater**.
- It is **extremely robust** and can be **readily deployed** *in-situ* **in cages**.
- It displays a variety of **pronounced reproductive behaviours**.
- It has a simple and short life cycle, **low fecundity** and **high egg/fry survival rates**.

What have we done to develop the stickleback as a test-species?

Over the past four years, we have developed a method that allows the detection of androgens and anti-androgens present in the water. This involves exposing male and female sticklebacks to test compounds over a 3 to 5 weeks period. They are then sacrificed, their kidneys removed, extracted and assayed (by ELISA) for the kidney glue protein, **spiggin**. The development of the ELISA for spiggin, the only known so far, specifically androgen-induced protein in teleosts has been reported by Katsiadaki *et al* (2002). Validation of the ELISA has involved a comparison between kidney spiggin content and kidney epithelium cell heights (KEHs) in Methyltestosterone (MT)-treated female sticklebacks. We have also: established dose-response curves for MT (Figures 1 & 2) and 5α-Dihydrotestosterone (DHT); have shown that the effect of both androgens is much reduced, or even abolished, by the addition of the anti-androgen, Flutamide (FL; Figure 3) to the water; confirmed the androgenicity of Pulp Mill Effluent; and the anti-androgenicity of diazinon (Figure 4).

More recently, we have developed an ELISA for **vitellogenin** (VTG) in the stickleback. This assay can be applied to either blood plasma, whole body extracts, liver extracts or heart extracts with equal validity. VTG concentrations have been shown to increase in response to Ethinyl Estradiol (EE2) exposure (Figure 5).

Our results

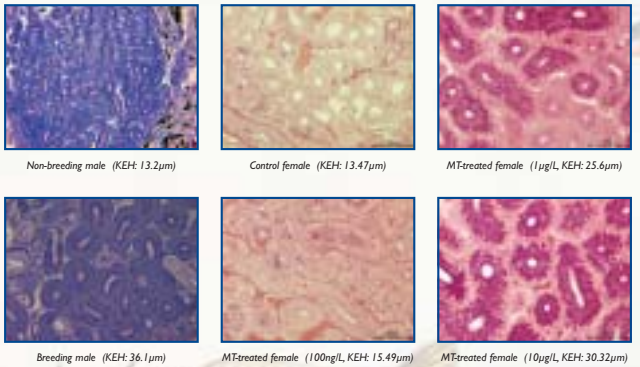

Figure 1: Kidney histology of control and androgen-treated (methyl-testosterone) sticklebacks (KEH: kidney epithelium height).

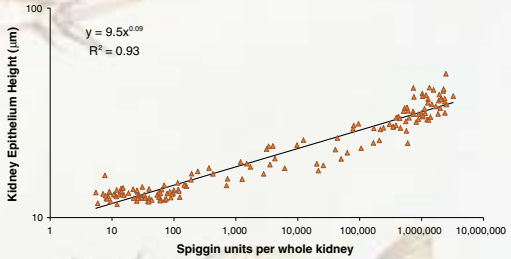

Figure 2: ELISA Validation. Comparison between kidney spiggin content and kidney epithelial cell height in female sticklebacks exposed to MT.

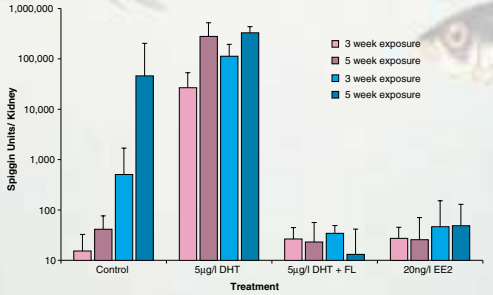

Figure 3: Data showing that FL, a model anti-androgen, inhibits spiggin induction in DHT-treated male and female sticklebacks. Data also shows that ethinyl-oestradiol (EE2), has no stimulatory effect on spiggin production

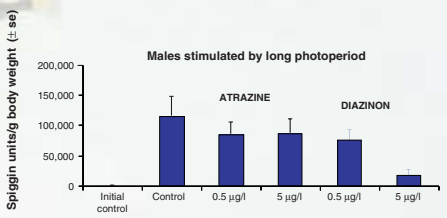

Figure 4: Preliminary evidence that diazinon has an anti-androgenic effect on male sticklebacks.

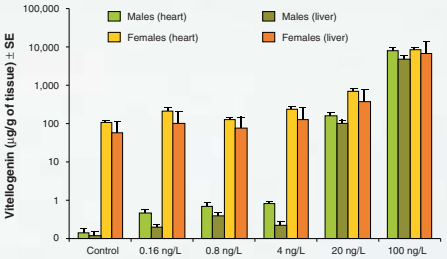

Figure 5: Dose response curve for VTG induction by EE2 in male and female sticklebacks

Conclusions

- The stickleback's clear-cut androgen/anti-androgen end-point gives it an undoubted advantage over other proposed EDC test-species.
- The development of the vitellogenin ELISA means that we can now investigate the effects of mixtures of (anti)-oestrogenic and (anti)-androgenic compounds
- Taken together with its many other advantages, the stickleback should prove to be the 'fish of choice' for testing of endocrine disruption in European waters.

References

Katsiadaki, I., Scott, A. P., Hurst, M. R., Matthiessen, P. and Mayer, I. 2002. Detection of environmental androgens: a novel method based on enzyme-linked immunosorbent assay of spiggin, the stickleback (*Gasterosteus aculeatus*) glue protein. Environmental Toxicology and Chemistry 21, 1946-1954.

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