PROMOTING THE STICKLEBACK AS A COMBINED BIOMARKER FOR OESTROGENS AND ANDROGENS IN EUROPEAN WATERS

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The problem

The majority of Endocrine Disruption studies in Europe have been on non-indigenous species and almost exclusively on xenoestrogens. Europe not only needs its own test-species but one which 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 will be 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 found in **all EU countries**
- 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 three years, we have developed a method that allows the detection of androgens and anti-androgens 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 (Jakobsson *et al.*, 1999) has already been reported by Katsiadaki *et al* (2000). Validation of the ELISA has involved a comparison between kidney spiggin content and kidney epithelium cell heights (KEHs) (Borg *et al.*, 1993) in Methyltestosterone (MT)-treated female sticklebacks. We have also: established dose-response curves for MT 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) to the water; and confirmed the androgenicity of Pulp Mill Effluent.

The other ecotoxicology test-species: *fathead minnow (USA); zebrafish (tropical); rainbow trout (USA); medaka (Japanese).*

Our results

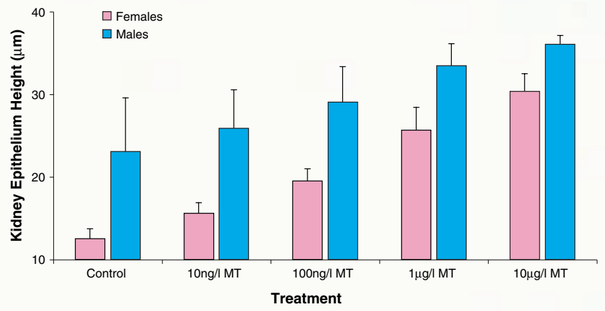

Figure 1: Dose-response curve for MT (Semi-static flow system)

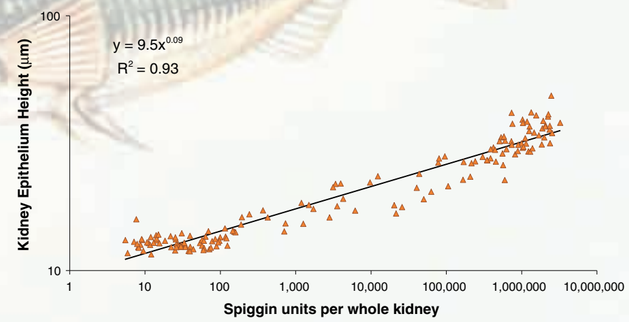

Figure 2: ELISA Validation.
Comparison between kidney spiggin content and kidney epithelial cell height in female sticklebacks exposed to MT.

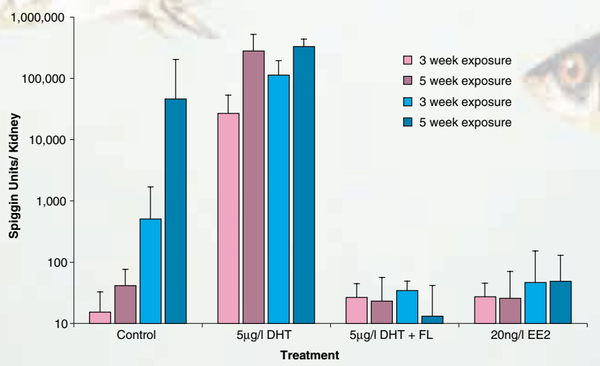

Figure 3: Data showing that FL, a model anti-androgen, inhibits spiggin induction in DHT-treated male and female sticklebacks. Data also shows that ethinyl-oestradiol (EE2), has no stimulatory effect on spiggin production.

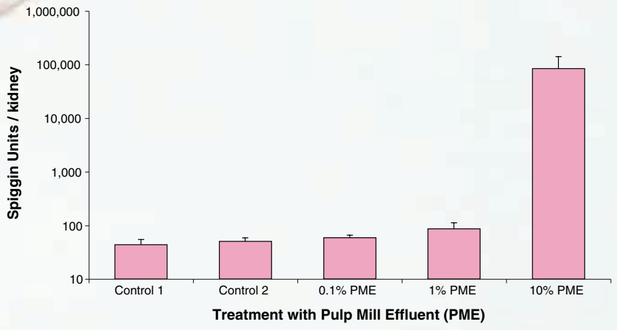

Figure 4: Female sticklebacks, exposed to Pulp Mill Effluent (PME) demonstrate significant kidney stimulation, confirming the androgenicity of this type of effluent.

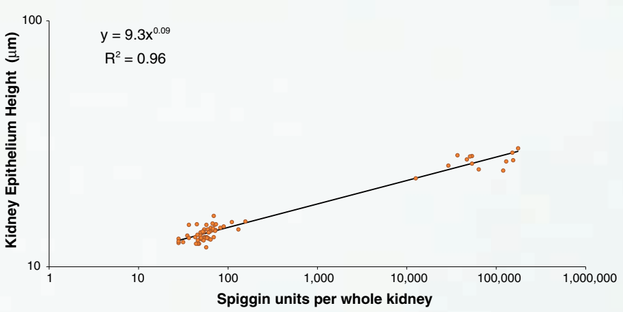

Figure 5: Kidney epithelium height and spiggin induction by PME in female sticklebacks

Conclusions

- The stickleback's clear-cut androgen/anti-androgen end-point gives it an undoubted advantage over other proposed EDC test-species.
- A vitellogenin ELISA is presently being developed as an oestrogen/anti-oestrogen end-point - yielding the potential for simultaneous assessment of androgens and oestrogens.
- Taken together with its many other advantages, the stickleback should be the fish of choice for testing of endocrine disruption in European waters.

References

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