THE THREE-SPINED STICKLEBACK (*Gasterosteus aculeatus*); A UNIVERSAL INDICATOR SPECIES FOR ENDOCRINE DISRUPTION

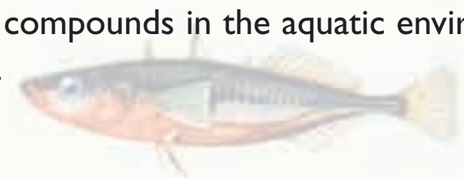
by Ioanna Katsiadaki[1], Sandy Scott[1] and Ian Mayer[2]

CEFAS The Centre for Environment, Fisheries & Aquaculture Science

http://www.cefas.co.uk

[1]CEFAS Weymouth Laboratory, Barrack Road, Weymouth, Dorset DT4 8UB, UK.
[2]Department of Zoology, Stockholm University, 106 91 Stockholm, Sweden.

An increasing number of studies have linked the appearance of reproductive disorders in wildlife species to exposure to environmental contaminants that are capable of eliciting responses typically induced by sex steroids. These compounds have been termed endocrine-disrupting chemicals (EDCs). We report that the three-spined stickleback, *Gasterosteus aculeatus*, is an ideal indicator species for androgenic and anti-androgenic compounds in the aquatic environment. We are presently developing a vitellogenin ELISA so that we can simultaneously assess oestrogens and anti-oestrogens.



Description of the unique bioassay for (anti)-androgens

During breeding and in response to androgens, the kidney of the male stickleback increases considerably in size and produces a novel structural, glue protein, which is used as a cementing substance for the building of a nest (Borg *et al*, 1993). This protein has been characterised as a 203kDa glucoprotein and termed spiggin (Jakobsson *et al*, 1999) from the Swedish name of the stickleback, the spigg. The development of an ELISA for spiggin, the only known so far, androgen-induced protein in teleosts, has been reported before (Katsiadaki *et al*; 2000). The validation of the immunoassay was accomplished by comparing the Kidney Epithelium Height (KEH) of female sticklebacks and the spiggin units induced by methyltestosterone treatment at increasing concentrations.

Measurement of spiggin with the ELISA method is as accurate as, has a higher resolution than, and is considerably quicker than the histological method employed previously, involving measurements of KEH. Upon regression analysis of the data obtained from the two assays, an excellent coefficient of correlation (r^2=0.93) was revealed.

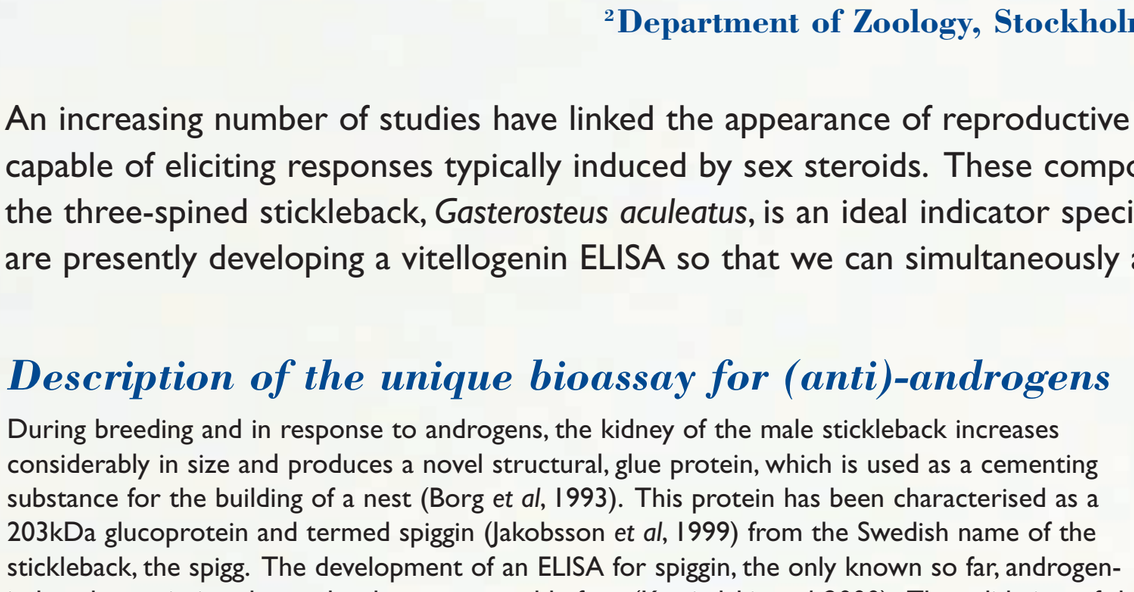


Figure 1: Dose-response curve for MT (Semi-static flow system)

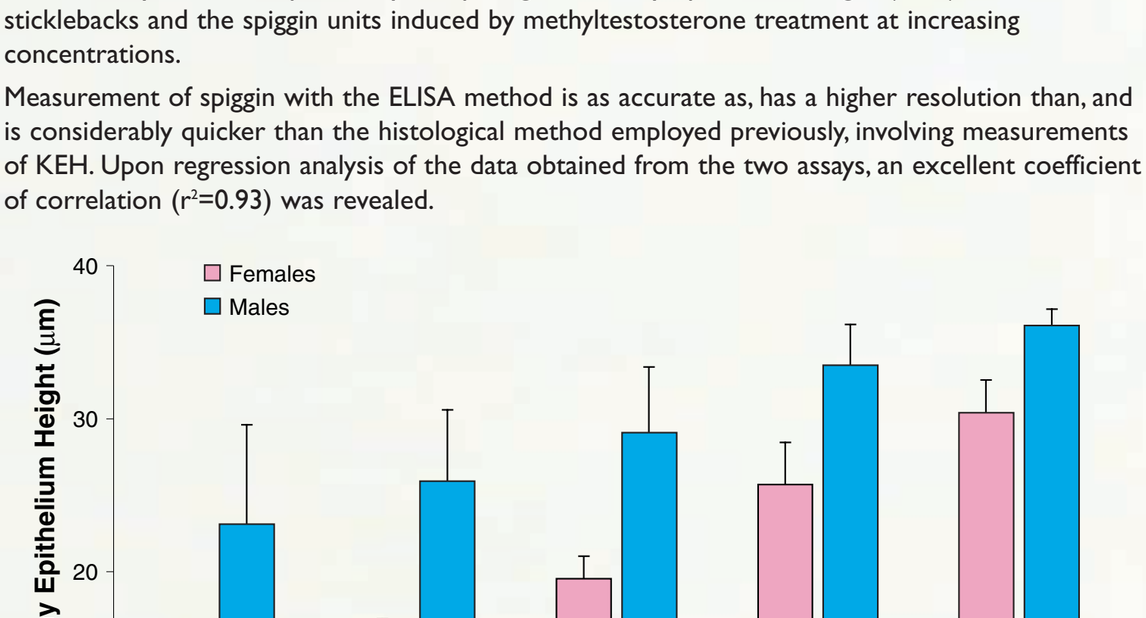


Figure 2: ELISA Validation

Kidney stimulation and spiggin production was demonstrated in intact female sticklebacks when androgens such as methyltestosterone (MT) and dihydrotestosterone (DHT) were added to the water at lower concentrations than the plasma concentration of the endogenous androgen, 11-Ketotestosterone, in males.

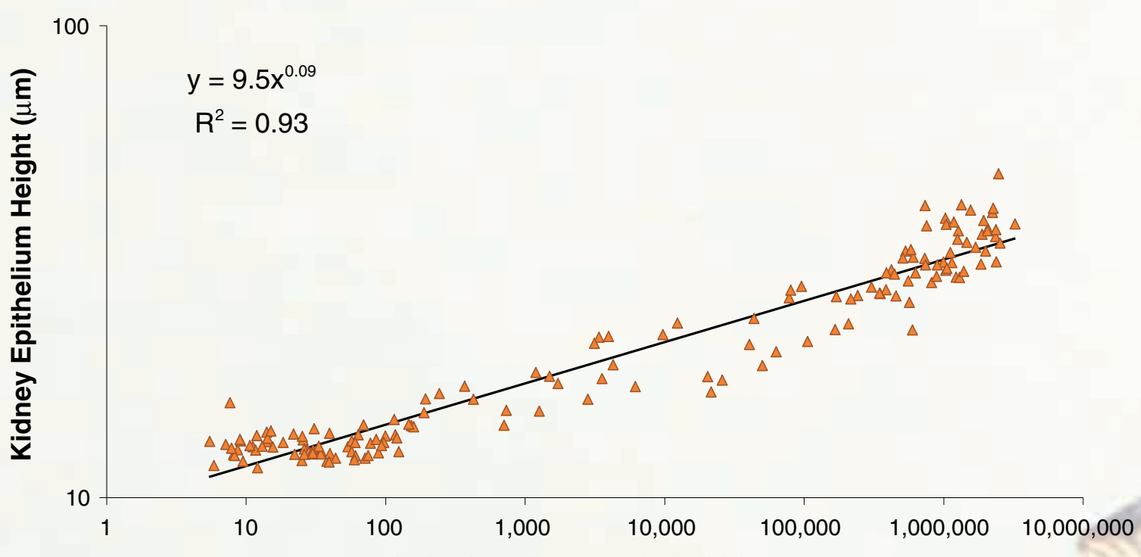


Figure 3: Dose response curve for DHT (females only) (pooled from 3 sets of experiments)

Flutamide (FL), a model anti-androgen completely inhibited spiggin induction in DHT-treated female sticklebacks. Treatment with ethinyl-oestradiol (EE2), had no stimulatory effect on the stickleback kidney

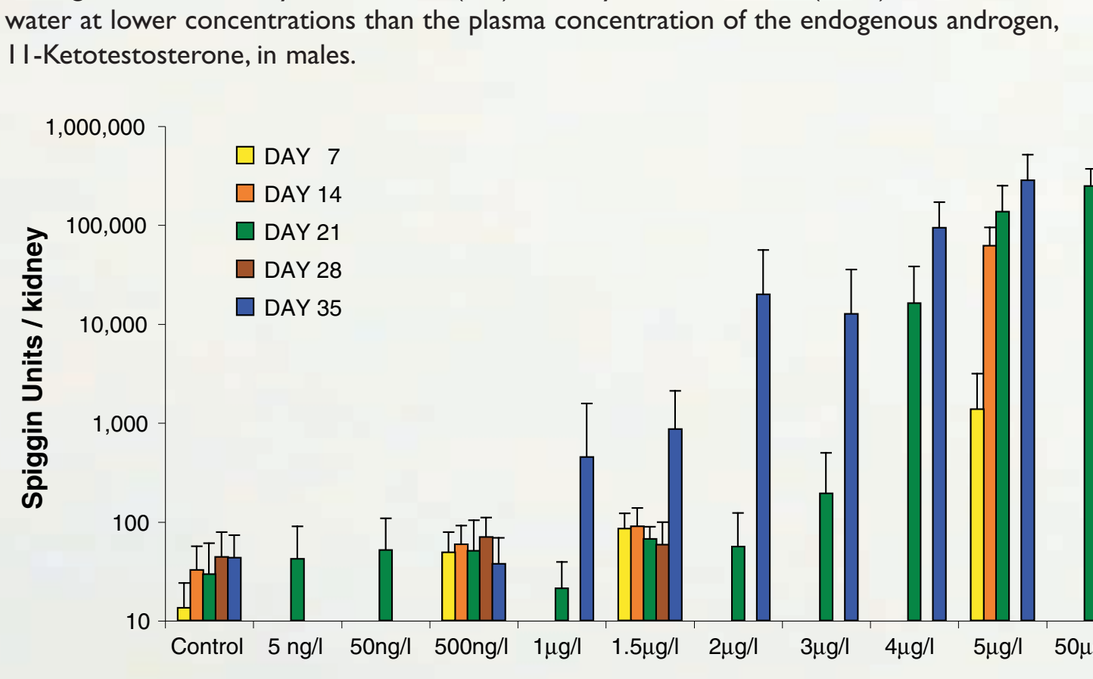


Figure 4: Effect of flutamide and EE2 on spiggin production

Female sticklebacks, exposed to Pulp Mill Effluent (PME) demonstrated significant kidney stimulation, confirming the androgenicity of this effluent. This finding was established by the application of both the ELISA and the histological assay. Again, regression analysis showed an excellent coefficient of correlation (r^2=0.96).

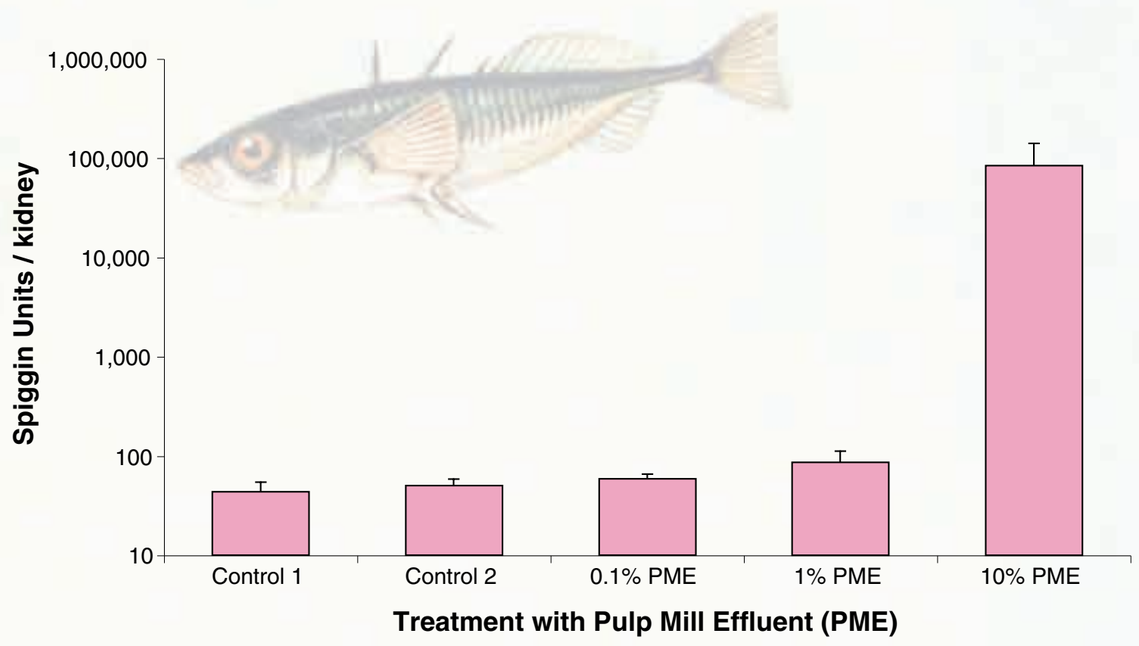


Figure 5: Female sticklebacks treated with PME

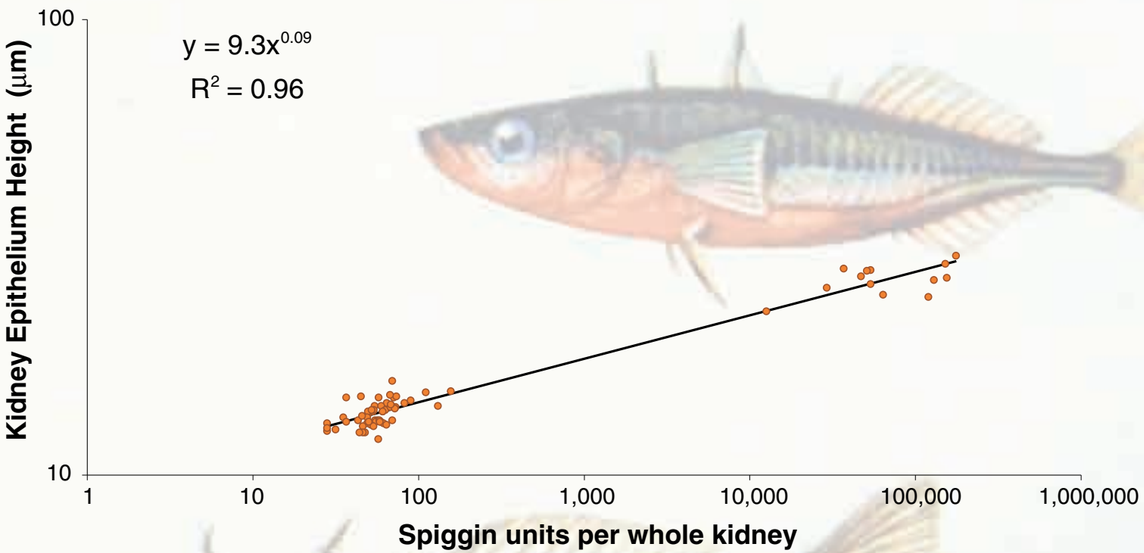


Figure 6: Kidney epithelium height and spiggin induction by PME in female sticklebacks

Why use the stickleback as a test-species for EDCs

- it is the only teleosts with an androgen-specific end-point, readily quantifiable
- the spiggin assay can be adapted to detect anti-androgens
- a vitellogenin assay is under development and will soon be available
- the simultaneous testing on androgen and oestrogen end-points will dramatically reduce the number of fish and the costs used for testing
- the stickleback is endemic in Europe, unlike other fish used as test-species
- it is an ubiquitous species found in all aquatic habitats from full seawater to freshwater
- it can be readily sampled in the field in large numbers, allowing for *in situ* biomonitoring
- its reproductive endocrinology is well-documented
- it is easy to maintain in laboratory aquaria
- it has a short life cycle
- very high egg/fry survival rates (close to 100%) can routinely be obtained
- it can be readily induced into breeding condition all-year-round by photothermal manipulation- making it particularly good species for 'partial life-history' tests
- the female has a relatively low fecundity
- the eggs are easy to count since they are laid in nests built by the males
- other quantifiable reproductive endpoints include reproductive behaviour (courtship and fanning) and sperm motility

Conclusion

The stickleback's clear-cut androgen/anti-androgen end-point gives it undoubtedly an advantage over other proposed EDC test-species. No problems are foreseen in developing an oestrogen/anti-oestrogen end-point, exploiting the great potential of simultaneous assessment of androgens and oestrogens. Androgenicity has already been established using the spiggin ELISA for pulp mill effluent.

Acknowledgements

This work was been supported by the Ministry of Agriculture, Fisheries and Food (MAFF), the Environment Agency (EA), The Scotland and Northern Ireland Forum for Environmental Research (SNIFFER), the European Chemical Industry Association (CEFIC) and the department of the Environment, Transport and the Regions (DETR), UK and the Swedish Natural Science Research Council.

References

Borg B., Antonopoulou E., Andersson E., Carlberg T., Mayer I. (1993). Effectiveness of several androgens in stimulating kidney hypertrophy, a secondary sexual character, in castrated male three-spined sticklebacks, *Gasterosteus aculeatus*. Can. J. Zoo., 71,1993, pp 2327 (Notes).

Jakobsson S., Borg B., Haux C., Hyllner S. J. (1999). An 11-ketotestosterone induced kidney-secreted protein: the nest building glue from male three-spined stickleback, *Gasterosteus aculeatus*. Fish Physiology and Biochemistry, 20:79-85, 1999.

Katsiadaki I., Scot A. P., Matthiessen P. (2000). The use of the three-spined stickleback as a potential biomarker for androgenic xenobiotics. In the Proceedings of the 6th International Symposium on the Reproductive Physiology of Fish, Bergen, July 1999. Edited by B. Norberg, O. S. Kjesbu, G. L. Taranger, E. Andersson and S. O. Stefansson, pp 359-361.

© Crown Copyright 2001