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The potential of the three-spined stickleback (*Gasterosteus aculeatus* L.) as a combined biomarker for oestrogens and androgens in European waters

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Abstract

The majority of endocrine disruption studies in Europe have been on non-indigenous species (some of them tropical!)—and none of which has traits that make them suitable for the detection of androgenic compounds. To overcome these problems, we have been developing the stickleback as a model biomarker for testing the effect of endocrine disrupters in European waters. Its advantages are: 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 compound; 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; it displays a variety of pronounced reproductive behaviours; it has a simple and short life cycle, low fecundity and high egg/fry survival rates.

Keywords: Stickleback; Biomarker; Androgens; Anti-androgens; Oestrogens

In response to androgens, the male stickleback kidney produces a unique protein—spiggin (Jakobsson, Borg, Haux, & Hyllner, 1999)—which is used as a glue in the building of the nest. A consequence of spiggin production by the male stickleback kidney is a dramatic increase in the cell height of the secondary proximal kidney segment from about 13 μm in non-breeding males and females to 35 μm or more in nest building males and in castrated males which have been injected with androgens (Borg, Antonopoulou, Andersson, Carlberg, & Mayer, 1993). This ability of

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the kidney of the stickleback to respond to androgens makes it a potentially useful test-species for endocrine disrupter research. This potential has been increased by the development of an ELISA for spiggin (Katsiadaki, Scott, & Matthiessen, 1999) which is simpler and much quicker than the procedures involved with kidney epithelium cell height (KEH) measurement.

Intact adult female three-spined sticklebacks were purchased from a trout farm in Kent. The potent synthetic androgen methyltestosterone (MT) was administered via the water in a semi-static system where solutions were renewed every 48 h. Dihydrotestosterone (DHT) was administered in a continuous flow system. MT was replaced by DHT as the 'standard' androgen when concerns were expressed that, at high concentrations, MT has oestrogenic activity (Ankley, Jensen, Kahl, Korte, & Makynen, 2001; Mori, Matsumoto, & Yokota, 1998). Fish (30–50; >0.4 g) were placed in 40-l glass aquaria containing seawater, which was filtered and UV sterilised before being added to the tanks. The photoperiod was 12 h light and 12 h dark and the temperature 15 °C. The fish were fed daily and throughout the exposure period (3 weeks) with a combination of dried flakes and live daphnia.

The spiggin ELISA has been validated by exposing female sticklebacks to different concentrations of MT, dividing the kidney into two equal parts and measuring KEH in one part and spiggin in the other. Regression analysis of the data obtained from the two assays yielded an excellent coefficient of correlation ($r^2=0.93$; $n=160$). Results from the ELISA were available within 3 days as opposed to 30 days for KEH measurements. There was a 100,000-fold difference between the highest and the lowest ELISA value and only a four-fold difference for KEH measurements.

Simultaneous treatment of female sticklebacks with the anti-androgen Flutamide (FL) and the androgens DHT and MT totally inhibited or severely reduced spiggin production by the kidneys, respectively (Fig. 1). This antiandrogenic effect of FL

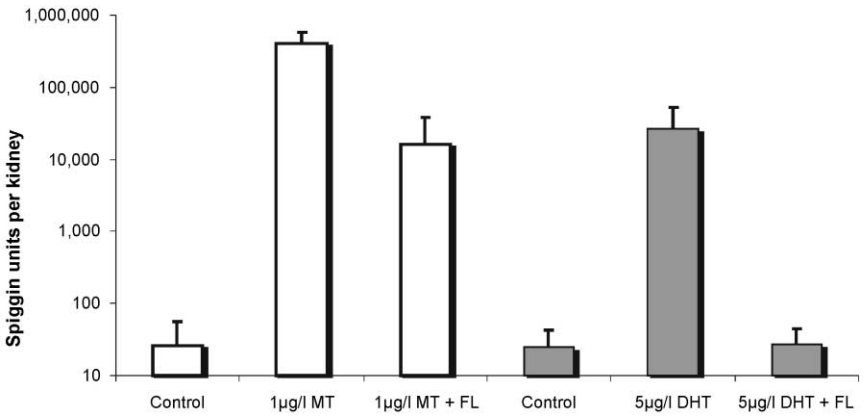

Fig. 1. Induction of spiggin in kidneys of female sticklebacks which had been exposed to methyltestosterone (MT) and dihydrotestosterone (DHT) via the water—and inhibition/reduction of the same response by concurrent addition of flutamide, added at 500 µg l^{-1}. MT was tested under semi-static conditions and DHT under continuous flow conditions. Induction of spiggin by the androgens and inhibition/reduction by flutamide were both statistically significant ($P<0.05$).

(used at 500 μg l^{-1}) depended on the androgen used and its concentration and suggests that an androgen receptor of the classic mammalian type may be present in the stickleback kidney. MT was more potent than DHT in inducing spiggin production by female kidneys (Fig. 1).

The masculinising effect of pulp mill effluent is well-known and has been confirmed by placing female sticklebacks in a 10% dilution of effluent for a period of 6 weeks. Both KEH and spiggin ELISA detected significant kidney stimulation (Fig. 2). Regression analysis of the data obtained with the two methods again yielded a high coefficient of correlation ($r^2 = 0.96$).

A polyclonal antiserum has now been raised to stickleback vitellogenin. For this, female sticklebacks were treated with oestradiol added to the aquarium water to meet a concentration of 10 ng l^{-1}. Blood was collected after a treatment period of 6 weeks. Plasma was pooled and stored at −80 °C until purified by direct injection of whole plasma through a Sephadex 200HR column linked to a Pharmacia FPLC system. The purity of peak fractions was checked by electrophoresis before it was injected into a rabbit. The antisera collected after a boost immunisation was efficient at 1/500,000 dilution. Optimisation of the ELISA for stickleback vitellogenin is currently taking place.

The two most important characters that make the stickleback an ideal biomarker for ED studies in Europe are: (1) it is an indigenous species and (2) it is the only species with a quantifiable androgen (and anti-androgen) endpoint. Upon validation of the vitellogenin assay, it is hoped that simultaneous assessment of xenoandrogenic and xenoestrogenic effects will result in a dramatic reduction in the number of fish used for testing (a very important factor from an ethical point of view and also for minimising costs).

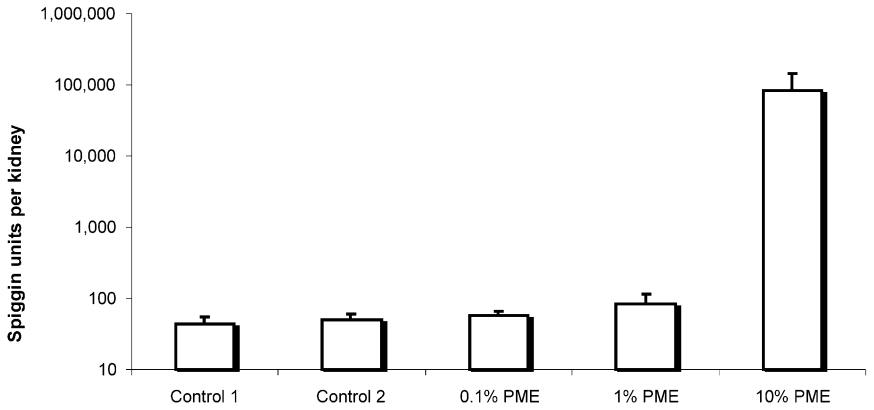

Fig. 2. Amounts of spiggin induced in the kidneys of female sticklebacks ($n = 15$) which had been exposed to different dilutions of Pulp Mill Effluent. The highest concentration of effluent (10%) was statistically significantly different from the other groups ($P < 0.01$).

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References

Ankley, G. T., Jensen, K. M., Kahl, M. D., Korte, J. J., & Makynen, E. A. (2001). *Environmental Toxicology and Chemistry*, *20*, 1276–1290.

Borg, B., Antonopoulou, E., Andersson, E., Carlberg, T., & Mayer, I. (1993). *Canadian Journal of Zoology*, *71*(Notes), 2327–2329.

Jakobsson, S., Borg, B., Haux, C., & Hyllner, S. J. (1999). *Fish Physiology and Biochemistry*, *20*, 79–85.

Katsiadaki, I., Scott, A. P., & Matthiessen, P. (1999). The use of three-spined stickleback as a potential biomarker for androgenic xenobiotics. In B. Norberg, O. S. Kjesbu, G. L. Taranges, E. Andersson, & S. O. Stefansson (Eds.), *Proceedings*, *6th International Symposium on the Reproductive Physiology of Fish*, Bergen, Norway, 4–9 July 1999 (pp. 359–361). Bergen, Norway: Bergen 2000.

Mori, T., Matsumoto, H., & Yokota, H. (1998). *Journal of Steroid Biochemistry and Molecular Biology*, *67*, 133–141.