

EMSG55: Evaluating Plant Leaf Extracts for Endocrine Activity & Reproductive Toxicity in Fish

Brian, J.V.¹, Beresford, N.¹, Smith, A.², White, S.², Bean, T.³, Katsiadaki, I.³, Sebire, M.³ & Hutchinson, T.H.³. (contact tom.hutchinson@cefas.co.uk)

¹Institute for the Environment, Brunel University, Middlesex, UK; ²Centre for Environment, Fisheries & Aquaculture Science (CEFAS) Lowestoft Laboratory, Suffolk, UK & ³CEFAS Weymouth Laboratory, Dorset, UK.

HIGHLIGHTS

- The goal of this research is to characterize the *in vitro* and *in vivo* activities of plant-derived endocrine active compounds (EACs) and to evaluate potential effects of relevance to fish reproductive health
- Fallen leaves (beech, oak & reed) were collected from south west England and processed for screening using a battery of (anti)estrogenic, (anti)androgenic & (anti)gestagenic *in vitro* screens (the YAS, YES and YPS assays, respectively). This initial work addressed the impact of temperature and solvent on the extraction of dissolved organic carbon (DOC) and consequent responses in the YAS, YES and YPS assays (as both agonism and antagonism)
- Aqueous extracts (10 µl volumes) were directly screened in the YAS, YES and YPS assays. Aqueous extract subsamples (10 ml) were concentrated by solid phase extraction (SPE) onto a Sep-Pak C18 cartridge & the concentrated ethanol extracts were then also screened in the three yeast assays.
- Extraction temperature had a marked proportionate effect on aqueous DOC values. For the reed aqueous extracts, there was a DOC-related response in YES assay responses, while oak aqueous extracts gave DOC-related anti-YAS activity. Impacts of leaf extracts on fish reproduction will be addressed.

1. INTRODUCTION & OBJECTIVES

There is a growing interest in understanding the cumulative effects of both natural and synthetic endocrine disruptors on aquatic organisms. Recent publications have demonstrated the presence of endocrine active chemicals in leaf extracts from European ecosystems, with oak leaf extracts showing anti-androgenic effects in amphibians¹. Little is known, however, about the impacts of European plant leaf extracts on the reproductive systems of native fish species.

This CEFIC-LRI funded project has been started in order to address two key questions:

- ➔ (a) the relative endocrine activity of plant leaves collected from wetland and woodland ecosystems *in vitro*;
- ➔ (b) to address the endocrine activity of the most potent leaf aqueous extracts *in vivo* for freshwater fish.



Fig 1. Sampling sites in south England for beech & oak (a) & reed (b)

2. MATERIALS & METHODS

For each plant species sampled, beech (*Fagus sylvatica*), oak (*Quercus robur*) and reed (*Phragmites australis*), ca. 30L of dried leaves were collected from April to June 2011. Leaves were air dried (7d) then stored @ -80°C before homogenization in aqueous media for 24 h at 20, 15 or 4°C. The aqueous extracts (approx 2L) were stored at -80°C and subsamples used to measure DOC. Aqueous extracts (10 µl volumes) were directly screened in the YAS, YES and YPS assays. In addition, aqueous extract subsamples (10 ml) were concentrated by solid phase extraction (SPE) onto a Sep-Pak C18 cartridge which was then eluted with methanol. The samples were dried under vacuum before being resuspended in ethanol. The concentrated extracts were then also screened in three recombinant yeast assays incorporating the human androgen, oestrogen and progesterone receptors (YAS, YES & YPS assays, respectively)^{2,3,4}. Representative examples of the results are shown here (full details in the technical report by Brian et al., 2012⁵).

3. RESULTS & DISCUSSION

Extraction temperature had a marked proportionate effect on aqueous DOC values; ranging from 406 – 665 mg/L (BEECH-2011-1), 365 – 1030 mg/L (OAK-2011-1) and 650-984 mg/L (REED-2011-1)

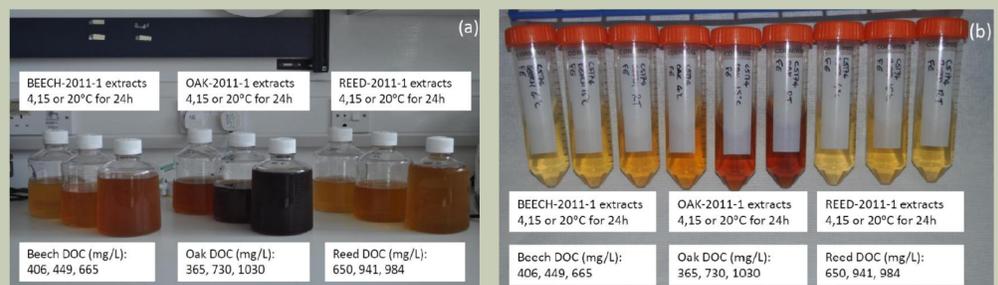


Fig 2. Aqueous extracts of fallen leaves of beech, oak or reed.

Aqueous & ethanol extracts of the leaf extracts were run in the YAS, YES and YPS assays for agonist activity (Figures 3a-c).

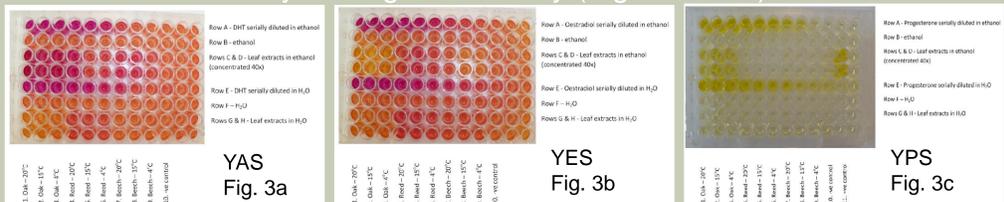


Fig 3. Examples of YAS, YES and YPS assay plates

Aqueous & ethanol extracts of the leaf extracts gave differing responses in the yeast assays (Figure 4)

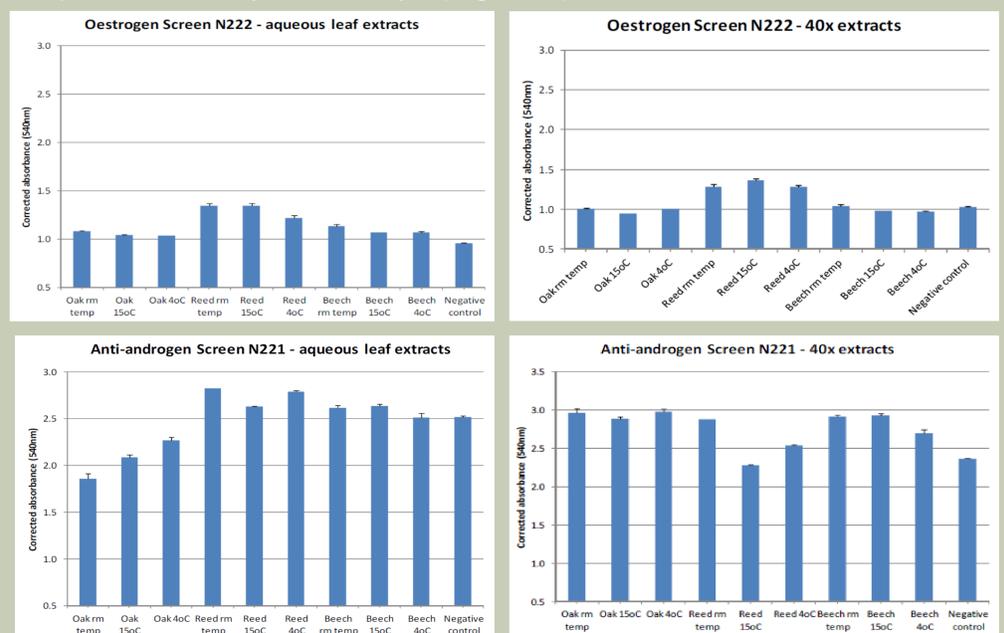


Fig 4. Examples of key results for YES and anti-YAS results

More samples have been screened since 2011, confirming the endocrine activity of aqueous extracts of oak and reed leaves.

REFERENCES

- Hermelink B. et al., 2010. Aqueous leaf extracts display endocrine activities *in vitro* and disrupt sexual differentiation of male *Xenopus laevis* tadpoles *in vivo*. *General and Comparative Endocrinology* 168, 245-255
- Sohoni, P., Sumpter, J.P., 1998. Several environmental oestrogens are also anti-androgens. *J Endocrinology* 158, 327-329
- Routledge, E.J., Sumpter, J.P. 1996. Estrogenic activity of surfactants and some of their degradation products assessed using a recombinant yeast screen. *Environmental Toxicology Chemistry* 13, 241-248
- Gaido K.W. et al., 1997. Evaluation of chemicals with endocrine modulating activity in a yeast-based steroid hormone receptor gene transcription assay. *Toxicology Applied Pharmacology* 143, 205-212
- Brian J et al., 2012. Cefas report reference C5174, January 2012, 36pp

ACKNOWLEDGEMENTS

This work is funded by the CEFIC Long-range Research Initiative (LRI) project reference CEFIC RfP LRI EMSG55. We are grateful to the CEFIC and ECETOC project monitoring team for their valuable advice and to Professor John Sumpter & Kevin Gaido for generously providing the yeast cell lines used in the YAS, YES and YPS assays. Our thanks also to Andy Pratt for granting permission for reed sampling from Slapton Ley, UK and staff at the National Laboratory Service, Leeds for conducting the DOC analyses.