

BASELINE DATA FROM THE EDCAT RESEARCH PROGRAMME ON OESTROGENS AND FISH POPULATIONS

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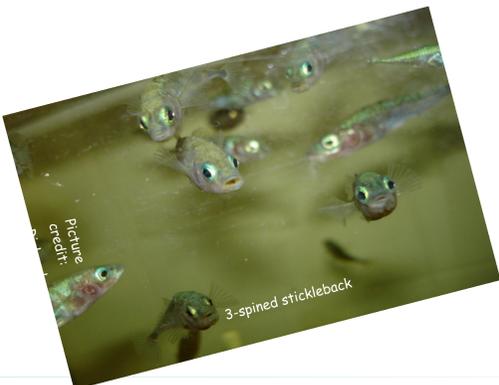
ABSTRACT

- EDCAT is studying whether fish populations are at risk from endocrine disruptors (EDs) in sewage effluent.
- We have gathered 2 year's-worth of baseline data from the River Ray, UK, prior to the upgrading of a major sewage effluent at Rodbourne with granular activated carbon (GAC) to remove oestrogens.
- Chemical analysis and oestrogen activity assays show that the river is less contaminated with EDs than expected, and stickleback (*Gasterosteus aculeatus*) populations in the river are not experiencing major oestrogenic effects. Exposure modelling based on predicted oestrogen contamination of the Rodbourne effluent also suggests that assumptions about inputs from the discharge may be incorrect.
- However, complex changes in the sticklebacks suggest that their endocrine systems may be responding to anti-androgens, to interference with steroid endocrinology leading to reduced steroid titres, and/or to slight temperature differences between the rivers. Sticklebacks are nevertheless reproducing in the Ray, and are significantly larger than in the reference River Ock (probably due to organic matter in the sewage).
- Breeding experiments with wild roach (*Rutilus rutilus*) have shown that mildly intersex males are able to compete successfully with normal males in terms of offspring produced per male. Experiments are currently in progress with fish which are predicted to be more severely intersex.
- EDCAT is now studying how remediation of the Rodbourne sewage effluent with GAC starting in February 2008 is affecting fish populations in the River Ray.



River Ray, Wiltshire, UK

Rodbourne sewage treatment plant, Swindon



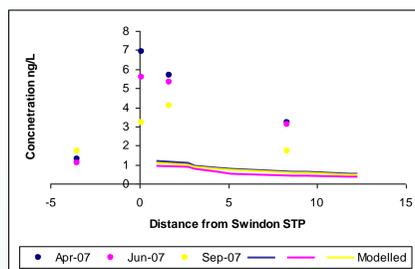
INTRODUCTION

- KEY QUESTION:** Are fish populations at risk from endocrine disruptors in sewage effluent?
- EDCAT now has 2 years of baseline chemical and ecological data from the River Ray collected before the Rodbourne Sewage Treatment Plant (STP) was upgraded in February 2008 to remove oestrogens (and other contaminants) with granular activated carbon (GAC)
- A major purpose of EDCAT is to monitor and model contamination with endocrine disruptors in the R. Ray before and after the STP is remediated, and to track the effects on the stickleback *Gasterosteus aculeatus* population
- The other main purpose is to conduct breeding experiments with wild roach *Rutilus rutilus*, using microsatellite markers to measure whether intersex males produce fewer offspring than normal fish

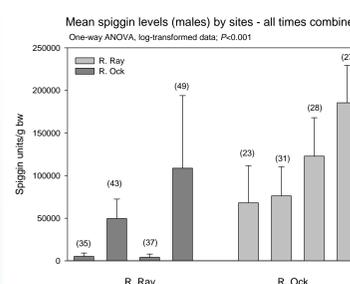
RIVER RAY MONITORING

- Oestrogen concentrations and activity in the R. Ray prior to remediation of the STP were lower than expected, and not easily predictable by modelling, perhaps because of the earlier introduction of nutrient stripping in 2005. Male sticklebacks from the Ray do not show elevated vitellogenin titres.
- A complex of other effects is observed in sticklebacks, including increased EROD activity compared with fish from the R. Ock reference sites; reduced vitellogenin titres in females; and reduced spiggin titres in males. This suggests the possibility of anti-androgenic effects from unknown substances, alterations in steroid metabolism caused by the induced cytochrome P450 system, or asynchronous reproductive cycles.
- However, trophic conditions in the Ray seem better than the Ock, because Ray fish are larger, and show elevated whole-body glucose levels.

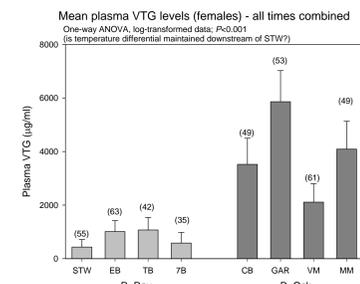
Measured and predicted oestradiol in the R. Ray



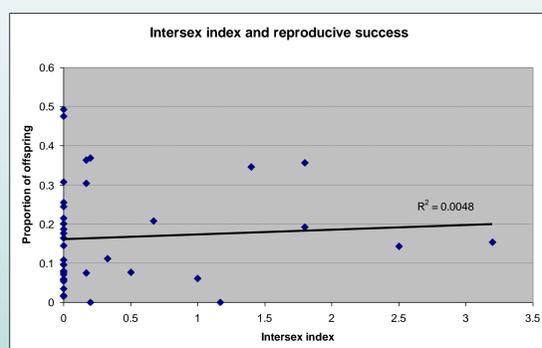
Spiggin in male sticklebacks



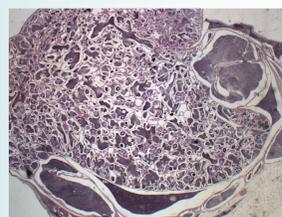
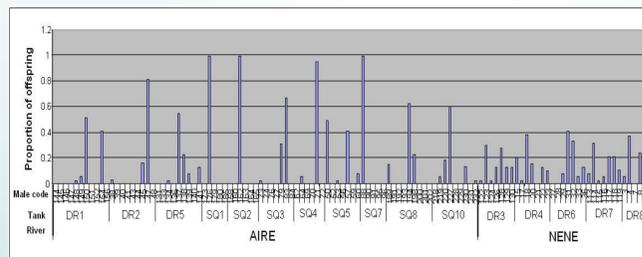
Vitellogenin in female sticklebacks



Relationship of male roach intersex index with reproductive success in the first breeding experiment



Reproductive success of male roach in the second breeding experiment



Section of testis from the severely intersex male (intersex index = 5.0) which was able to reproduce in Experiment 2.

ROACH BREEDING EXPERIMENTS

- Microsatellite markers have been used in 2 roach breeding experiments to measure the reproductive success of intersex males in competition with normal males.
- In the first experiment, mildly intersex males (intersex index ≤ 3.2) produced as many offspring as normal males.
- The second experiment used fish from rivers (Aire and Nene) expected to contain more severely intersex fish, but only 2 males proved to have high intersex indices (>5). Of these 2, one reproduced and one failed to reproduce. Again, the more mildly intersex males were also able to compete against normal males.
- A third breeding experiment is now in progress, hopefully with more severely intersex roach.
- Until larger numbers of severely intersex male roach have been subjected to competitive breeding with normal males, it will not be possible to say whether intersex causes a reproductive disadvantage.

DISCUSSION AND CONCLUSIONS

- Oestrogen contamination in the River Ray is lower than expected, perhaps due to the introduction of nutrient stripping in 2005. Oestrogenic effects in Ray sticklebacks are relatively mild.
- Nevertheless, Ray sticklebacks show a complex of effects which are possibly attributable to anti-androgenic action or interference with normal steroid metabolism, although some differences may be caused by slight temperature disparities. However, the Ray population appears to be growing better than that in the River Ock reference sites, and there is no evidence as yet that the Ray population has been disadvantaged.
- Roach breeding experiments to date have shown that mildly intersex males can compete reproductively with normal fish. Of 2 severely intersex males, one reproduced and one did not. A third experiment is in progress.
- Monitoring of the Ray is continuing after GAC plant was installed at Rodbourne STP in February 2008. It is too early to say whether exposure to EDs is able to cause damage at the fish population level.

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