



The impact of oestrogenic sewage discharges on fish populations



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This presentation

- Background – what is EDCAT?
- Endocrine disrupters in the River Ray
- Improved oestrogen exposure modelling
- Stickleback population effects
- Breeding experiments with intersex roach
- Interim conclusions

Background

- EDCAT is funded by UK Defra and EA
- 3 year programme 2006-2009
- Multi-institute including CEH, Cefas, PML, and Universities of Brunel, Exeter, and Sussex
- Main objective: to investigate whether estrogenic sewage effluent can cause impacts at the fish population level
- 4 projects: exposure, exposure modelling, stickleback populations, and roach breeding

Rationale

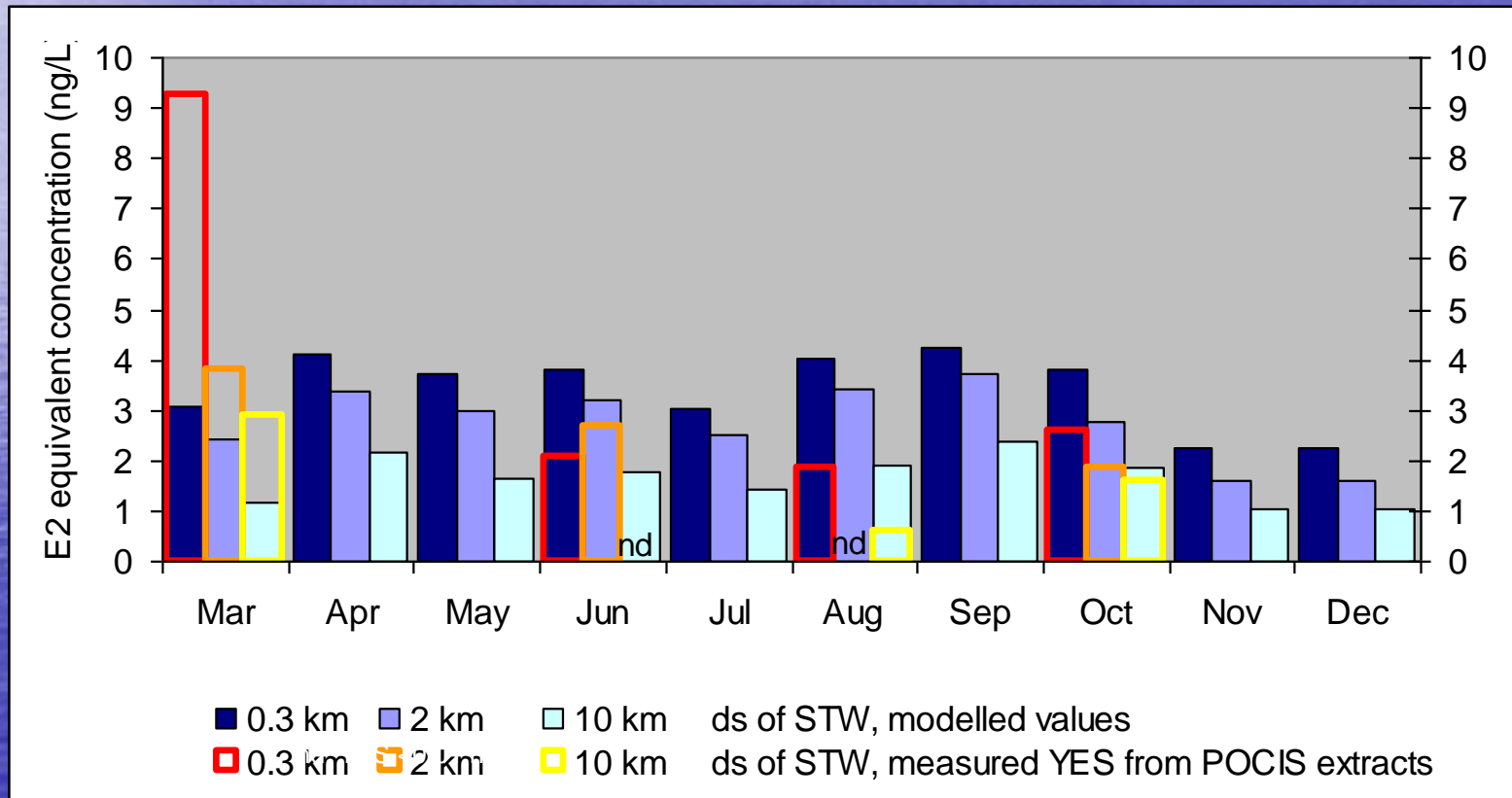
- Take the opportunity to study the River Ray ($1.34 \text{ m}^3/\text{sec}$ at Thames confluence) before and after remediation of the Rodbourne STW effluent with granular activated carbon (GAC)
- Rodbourne STW = $0.51 \text{ m}^3/\text{sec}$ dry weather flow; 135,000 population equivalents; activated sludge plant
- Study chemistry and develop exposure models; measure endocrine activity;
- Study stickleback populations – in R. Ray (receiving river) and R. Ock (reference river);
- Study breeding success of intersex roach in the laboratory

Exposure modelling

Predicted vs measured Swindon STW effluent concentrations

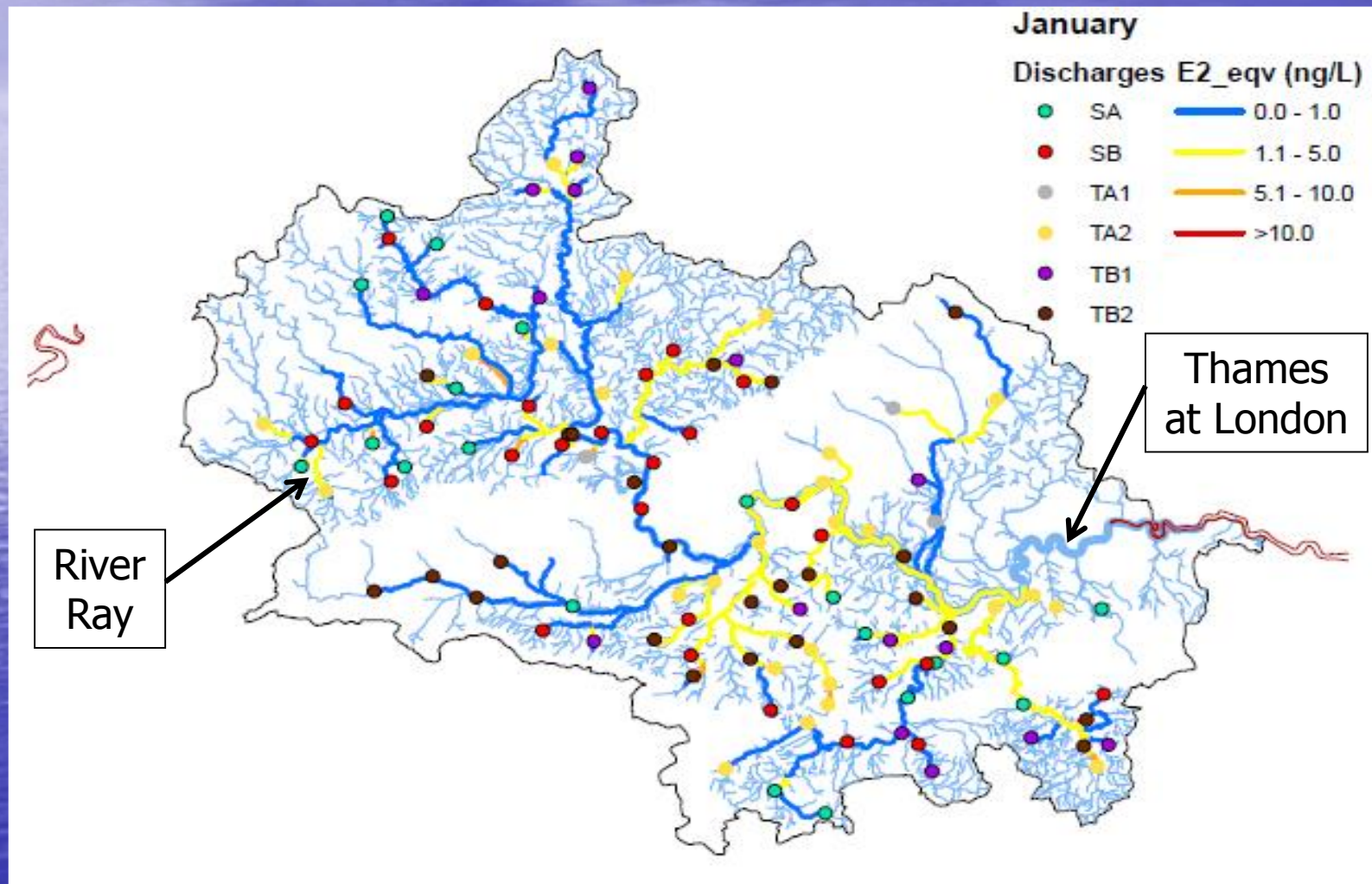
	Predicted (ng/L)	Predicted E2 equiv (ng/L)	EDCAT 3/4 (22/02/08) measured (ng/L)	Predicted E2 equiv (ng/L) based on EDCAT 3/4 (22/02/08) measured (ng/L)
E1	19	16	2.2 (1-3)	7-9
E2	2.5		2.1 (1-5)	
EE2	0.7		0.9 (<0.4- 1.3)	

How do predictions for the **River Ray** compare with the POCIS YES data for 2007?



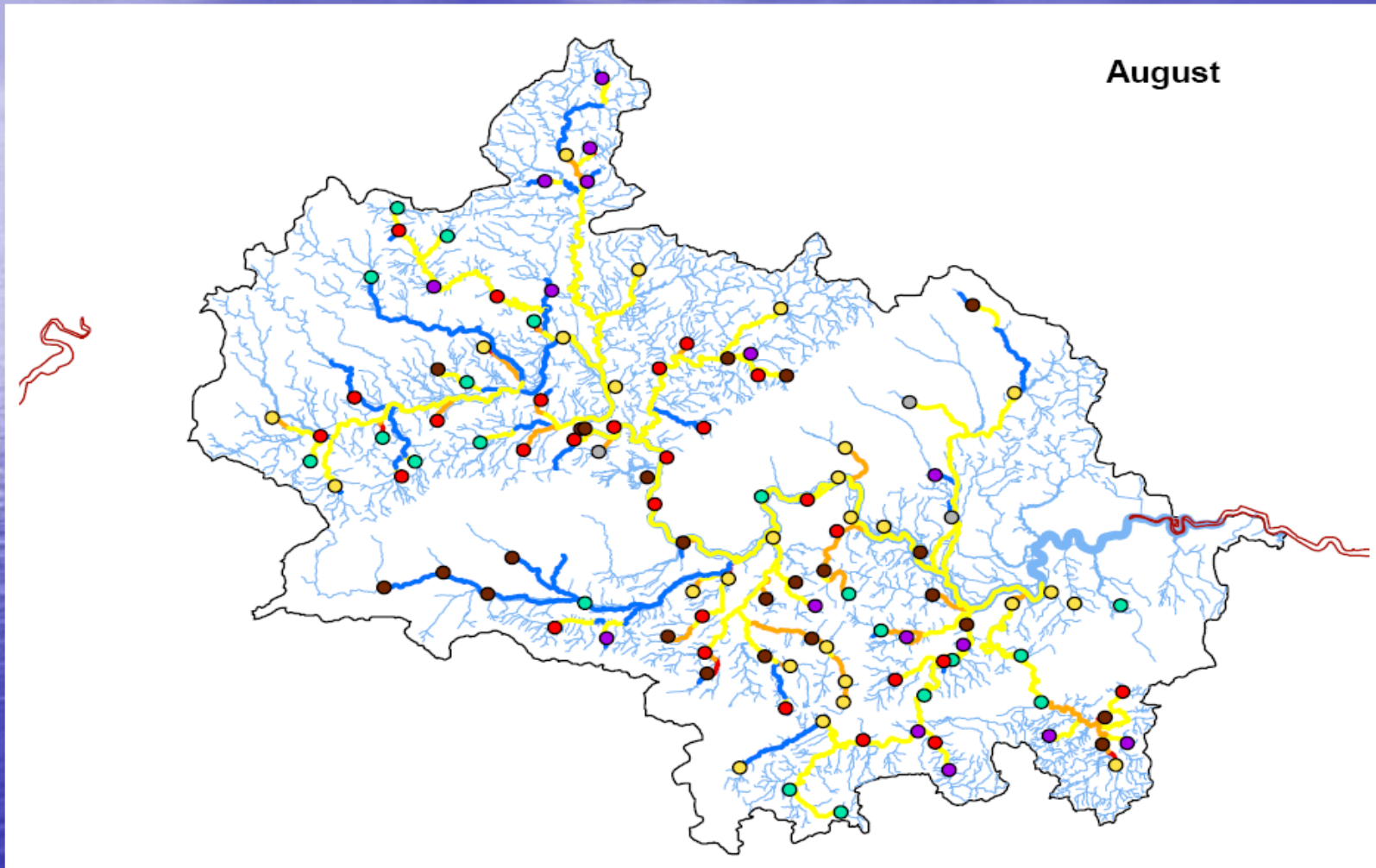
Reasonably similar apart from the March measured values

Thames catchment model - January



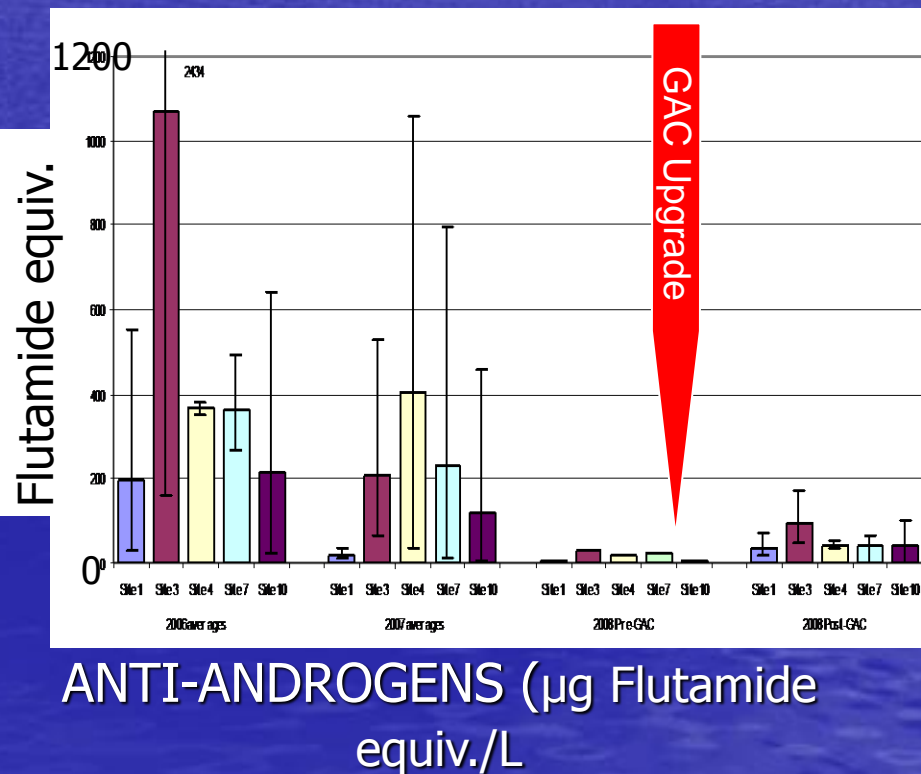
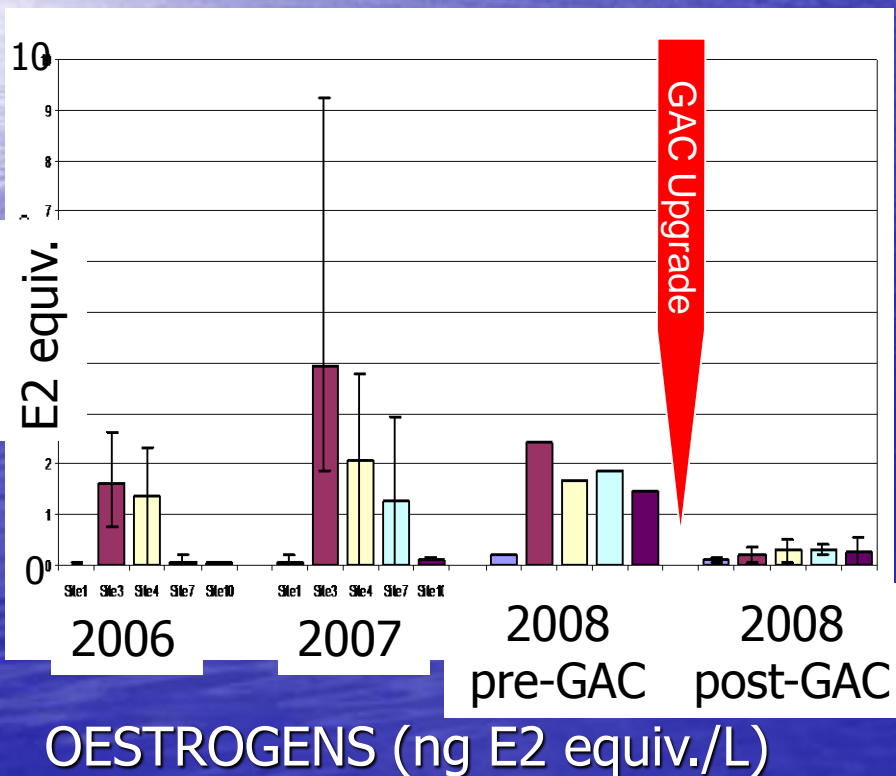
Predicted oestrogen concentrations (E1, E2, EE2) as E2 equiv based on mean flows

Thames catchment model - August

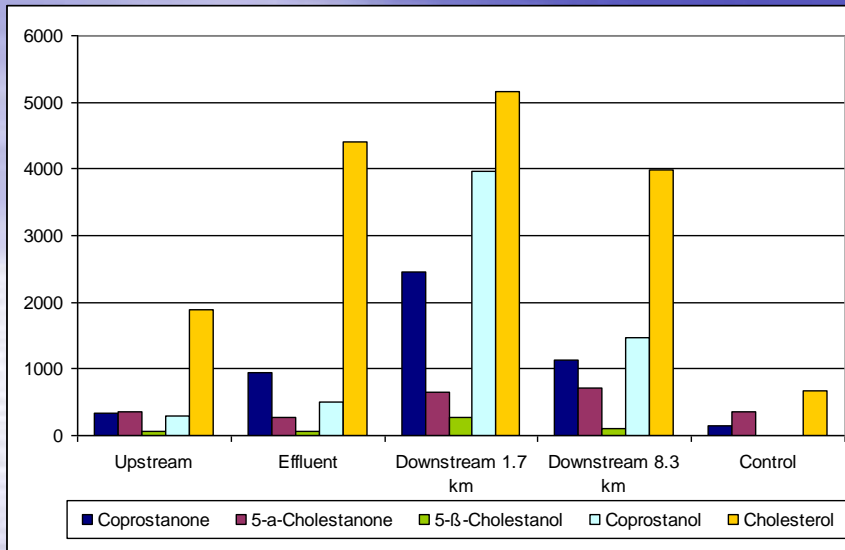


River Ray contaminants

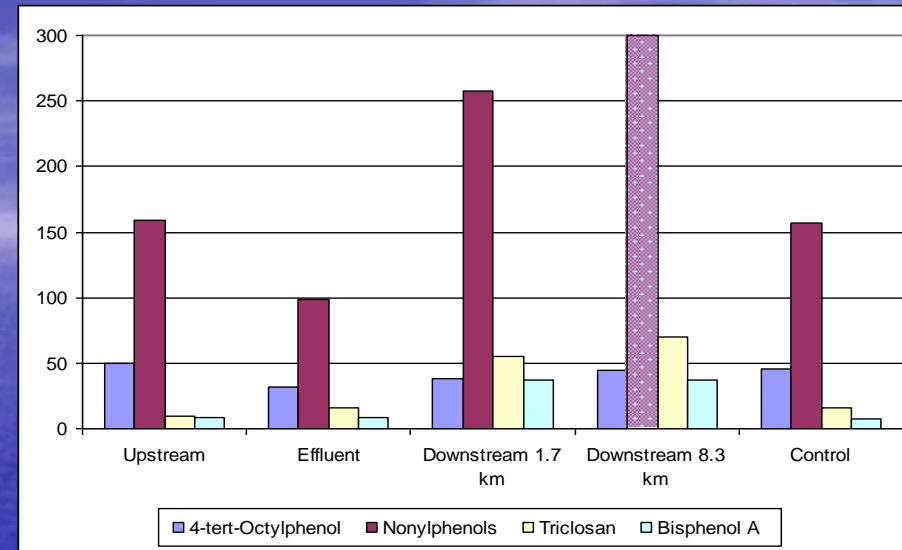
- POCIS passive samplers were placed in the River Ray for periods of 1 month, up- and downstream from Rodbourne STW
- POCIS extracts were analysed by YES and YAS bioassays
- Spot samples (data not shown) were analysed for individual contaminants



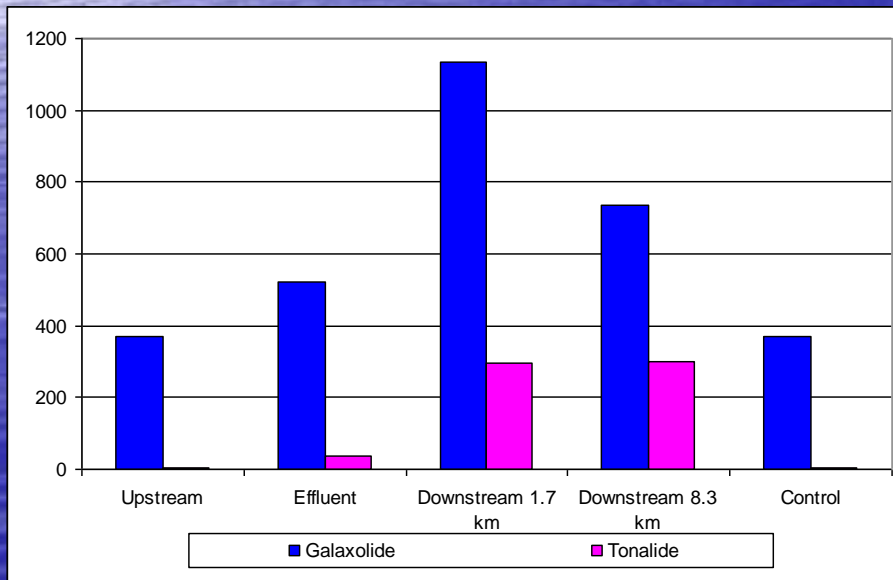
Chemical Concentrations in Sediment (ng/g dry weight)



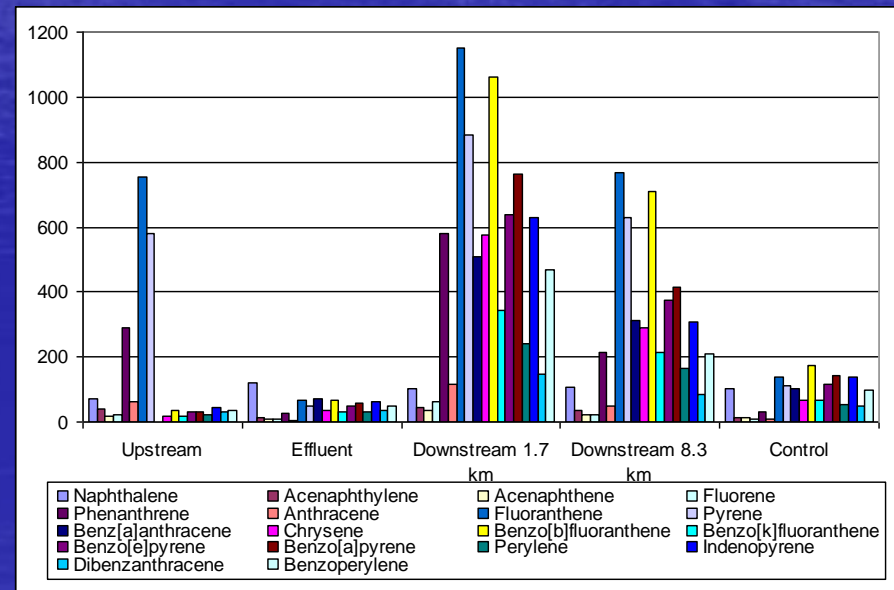
Faecal steroids



Phenols



Synthetic musks

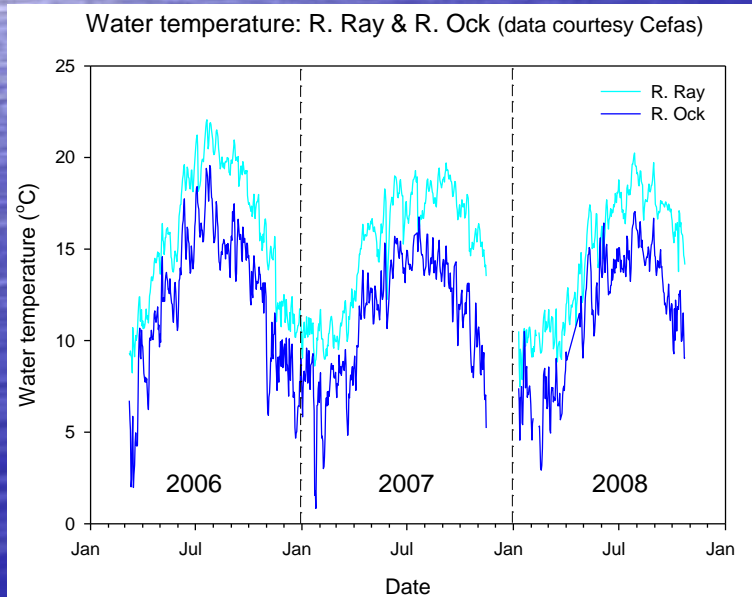
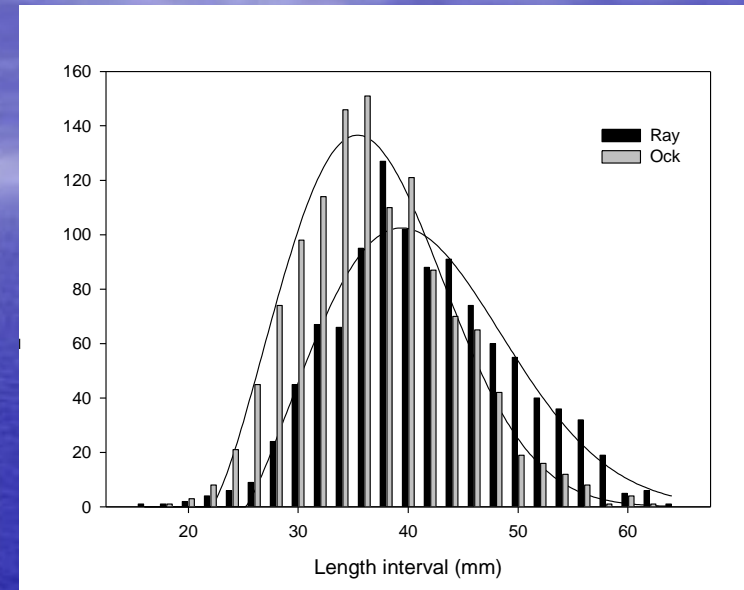


PAH

3-spined stickleback populations

Problem:

- Effluent from Rodbourne STW both warms ($>2^{\circ}\text{C}$) and enriches the Ray. *Fish in the Ray therefore grow more quickly and spawn earlier. The annual cycles are asynchronous so the Ray and Ock cannot be directly compared.*



Solutions:

- Compared matched timepoints (Mar-Nov 07, 08) pre- and post-remediation *within* each river.
- Sought evidence for differences in patterns of change *between* rivers.
- NB: Juvenile fish collected Mar-Nov 08 had hatched and grown on in GAC-treated effluent.

Somatic data

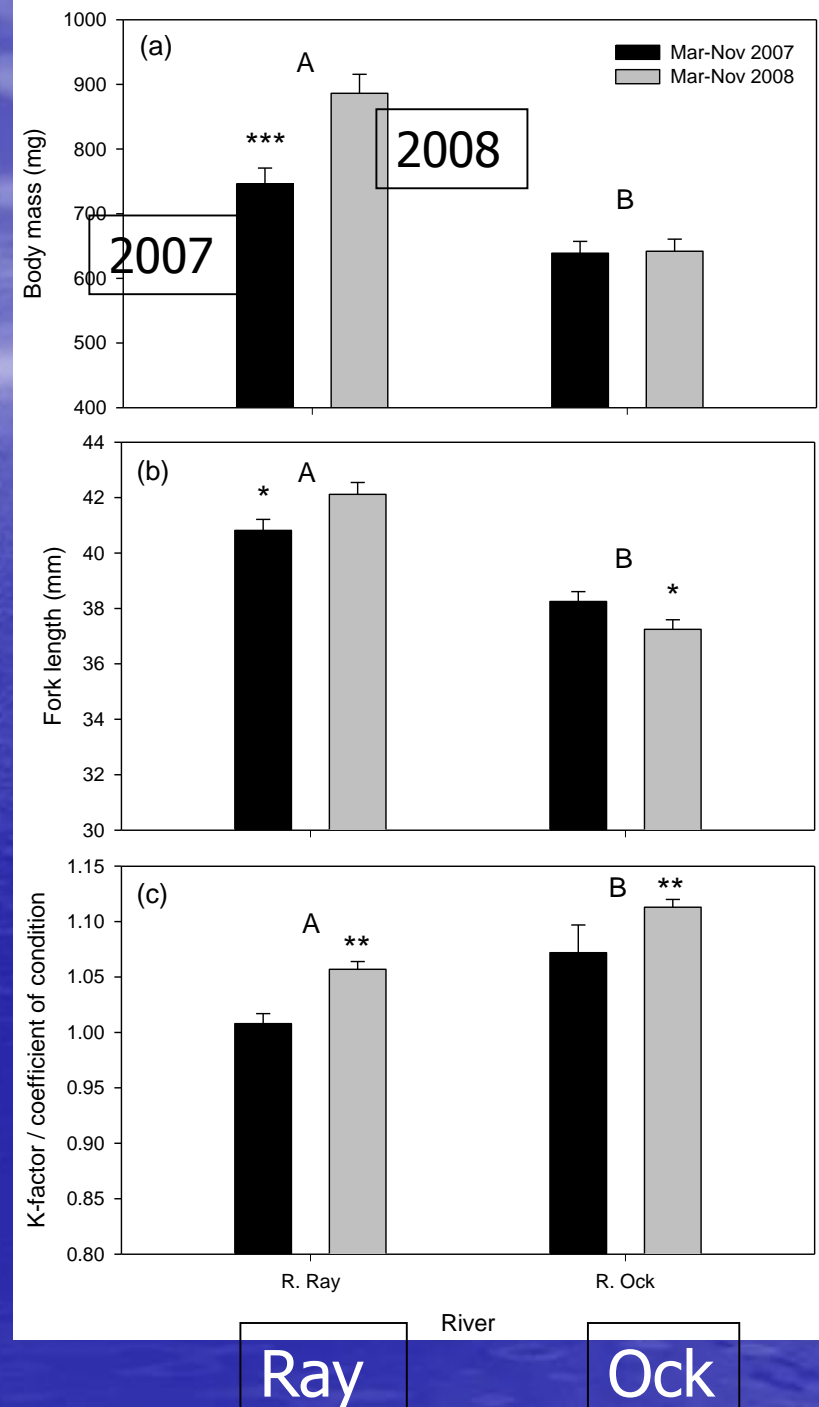
Body mass: Fish are larger post-remediation in the Ray. No change in the Ock.

Fork length: Fish are larger post-remediation in the Ray.

Condition: Improvement in both rivers across 2007/2008 (may be related to adverse weather in 2007)

Sex ratio: M/F = approx 0.7 in both rivers. No intersex found in either river.

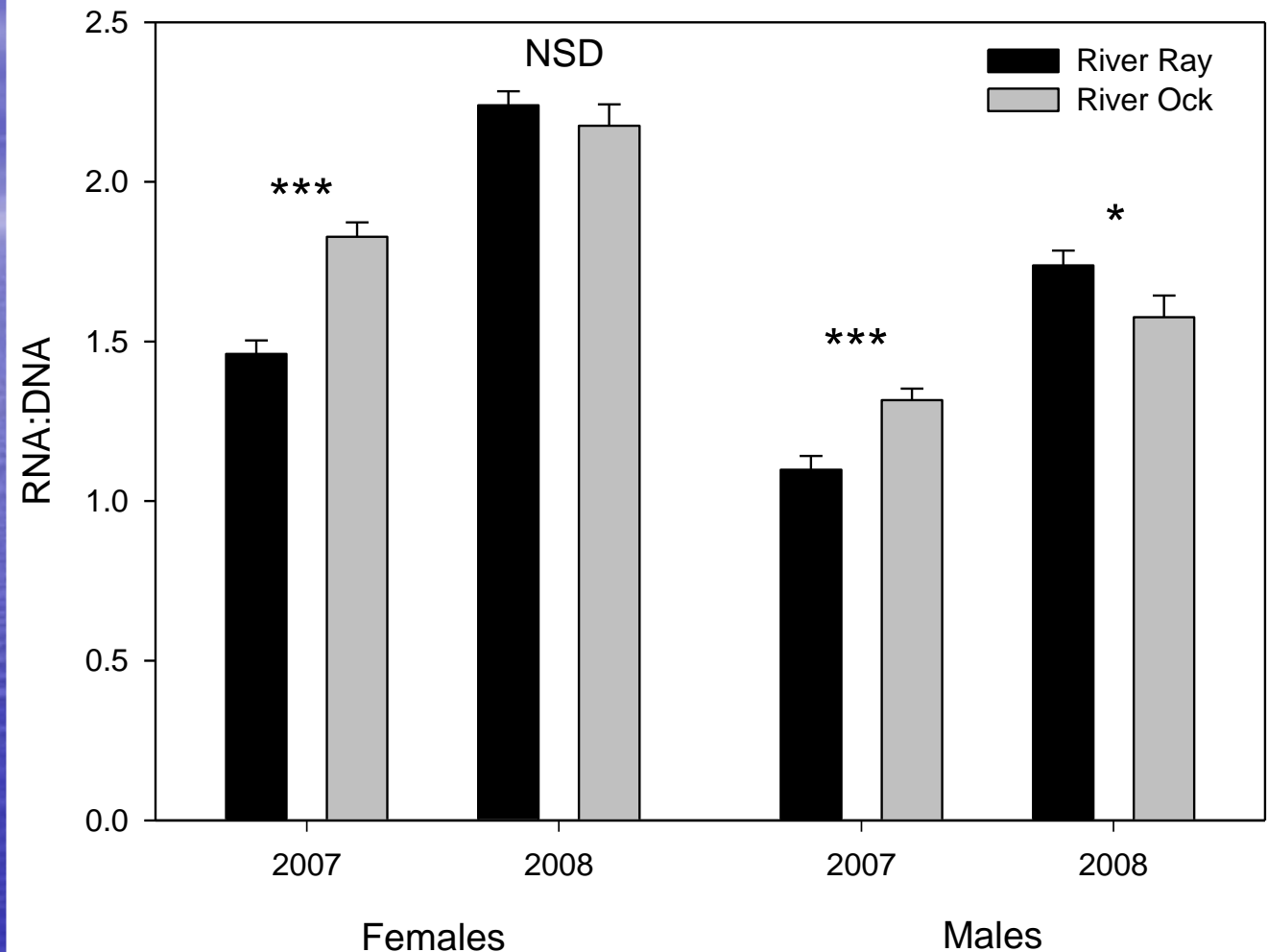
Conclusion: GAC remediation of the Rodbourne STW effluent had a positive effect on growth and condition of sticklebacks in the River Ray



RNA:DNA ratio

RNA:DNA ratio:

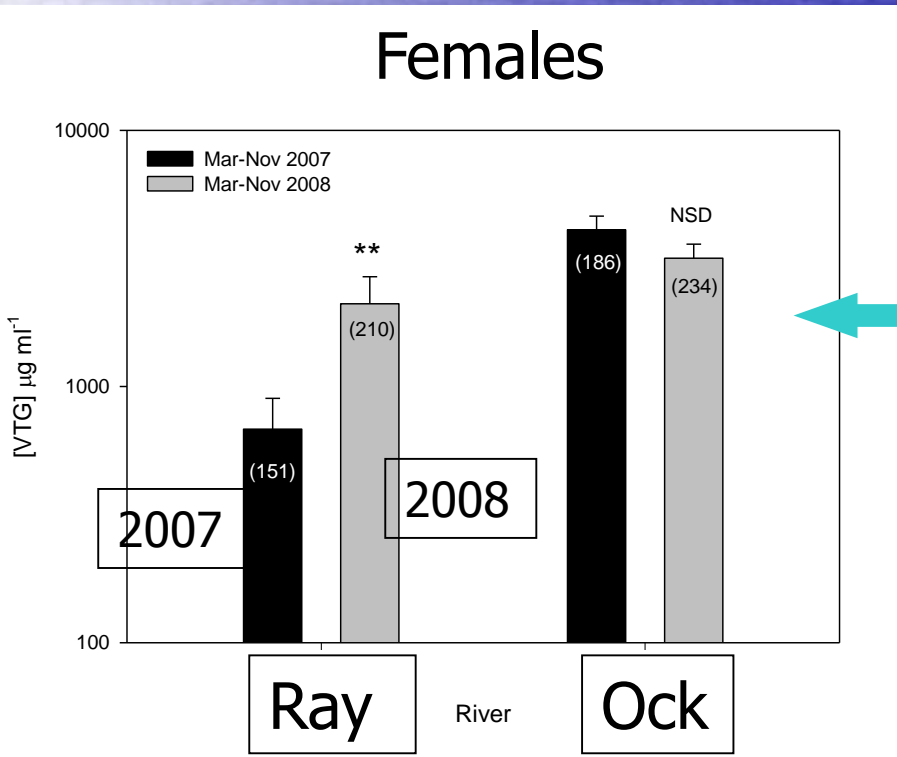
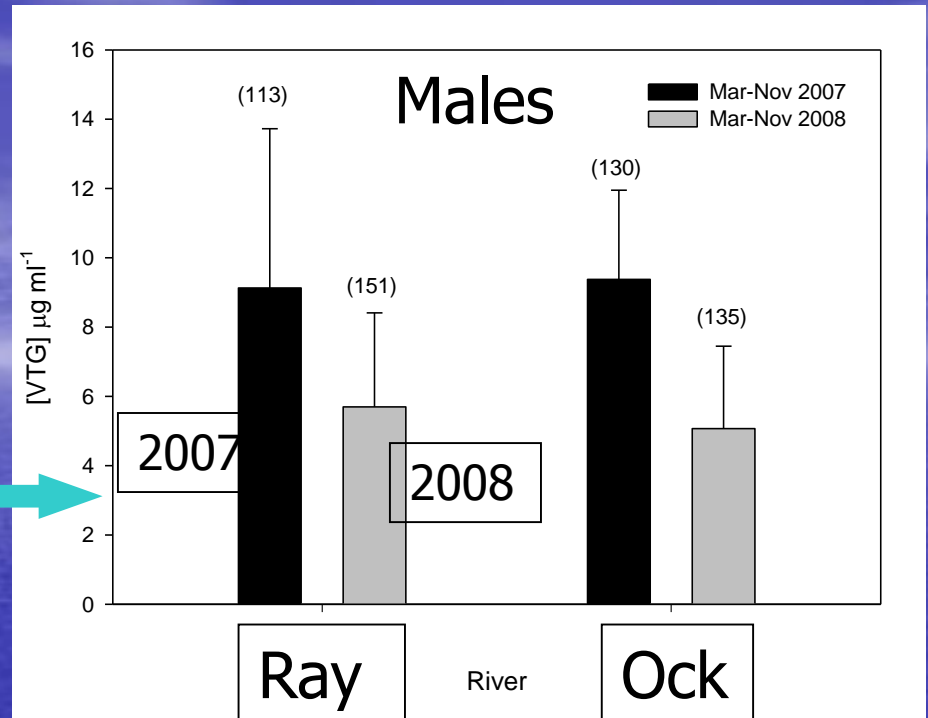
Increase in the Ray post-remediation relative to the Ock



Conclusion: Overall, the somatic data confirm that remediation of the Rodbourne STW effluent with GAC improved the condition of the stickleback population

Plasma Vitellogenin

Males: No significant elevation of VTG
i.e. no evidence of estrogenicity
attributable to effluent prior to
remediation (consistent with
chemistry)

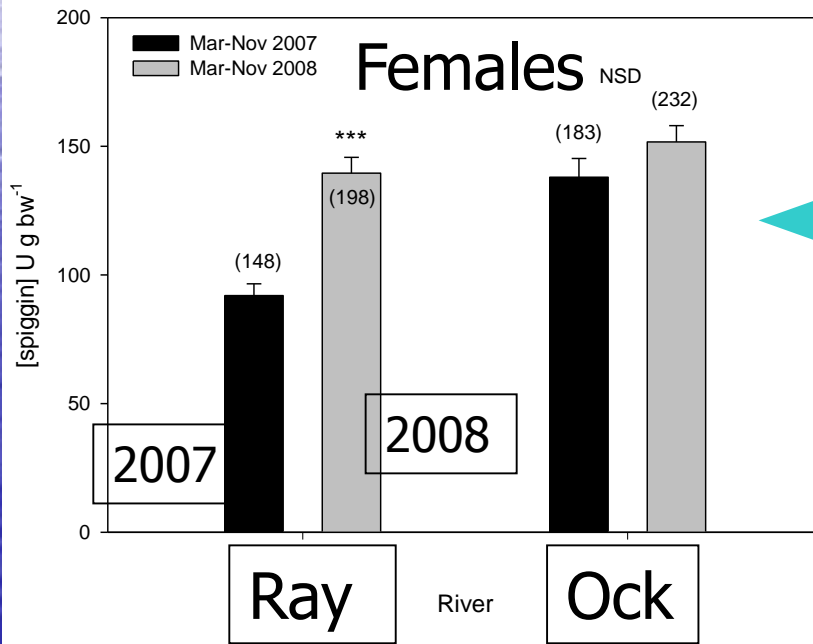
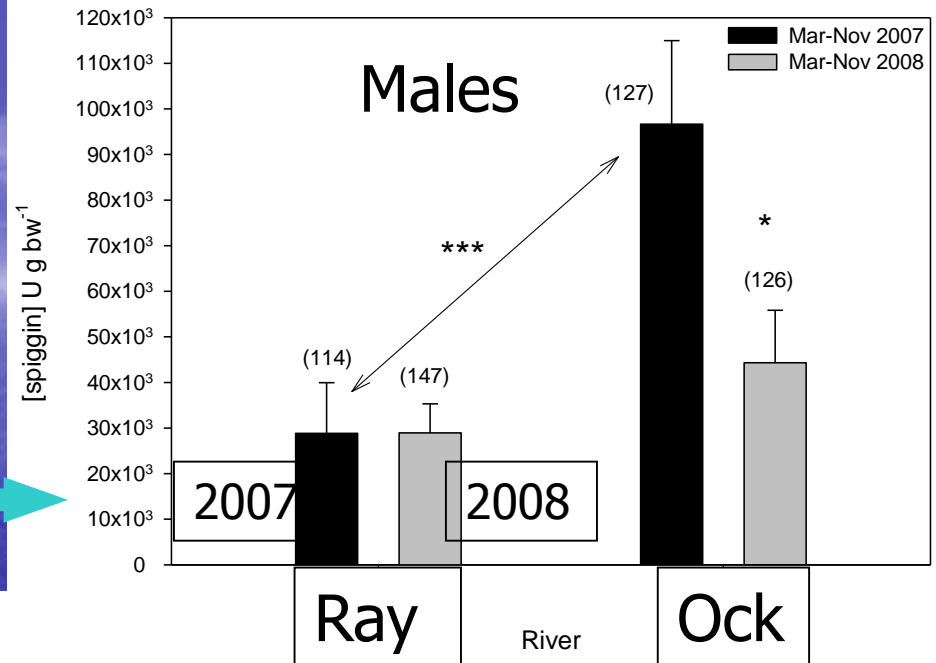


Females: VTG is significantly
lower pre-remediation on Ray.
No significant change on Ock.

Conclusion: Female reproductive
status appears to have been
depressed before the Rodbourne
STW effluent was remediated with
GAC

Plasma Spiggin

Males: No post-remediation depression of SPIG i.e. no evidence of removal of anti-androgenicity by GAC, but SPIG in the Ray is depressed in both years vs. Ock— possible other anti-androgenic sources??



Females: SPIG is significantly lower pre-remediation on Ray. No significant change in the Ock.

Conclusion: Overall, the data support the contention that female reproductive status was improved by GAC remediation. This may be a response to removal of multiple chemicals from the STW effluent.

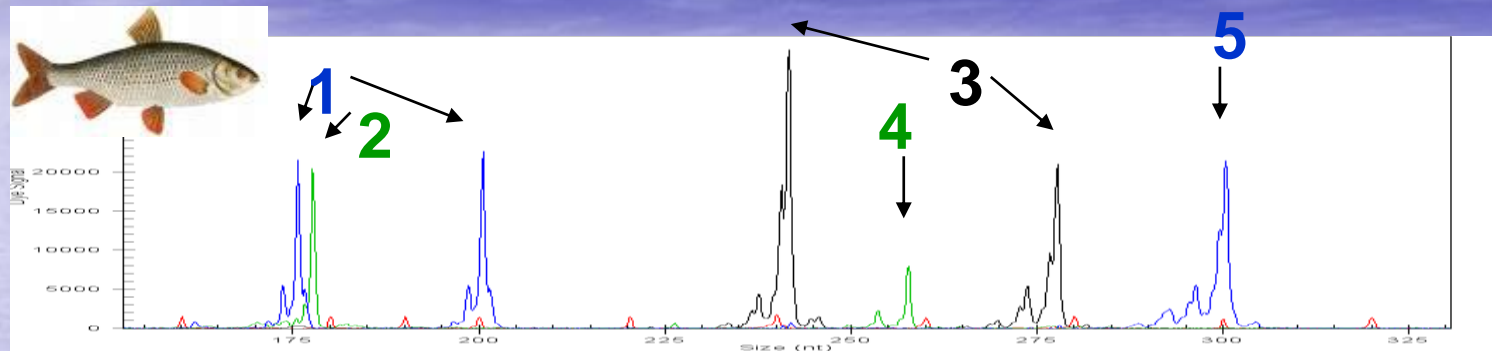
Roach breeding experiments

Aim: To determine if wild intersex roach (*Rutilus rutilus*), when placed within breeding colonies in the laboratory, contribute to subsequent generations, and to what extent

- **Three breeding experiments** undertaken using wild roach *Rutilus rutilus* taken from polluted sites (2006: **River Bourne**; 2007 **Rivers Aire and Nene**; 2008 **River Arun**)
- 3 females and 6-9 male/intersex fish placed in tanks and allowed to breed. Then adults and fry collected from tanks.



Roach parentage analyses



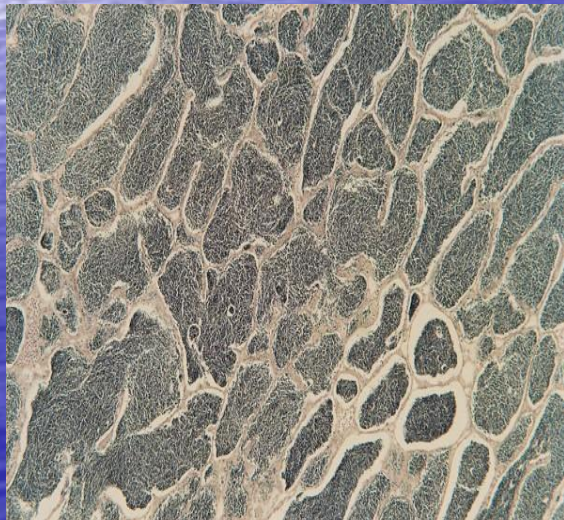
DNA profile of an individual fish

- **DNA profiles** of all parents and ~**50-100 fry** from each tank obtained using **7 variable microsatellite loci**
- Fry assigned to parents using their genotypes - **100% parentage assignment achieved**

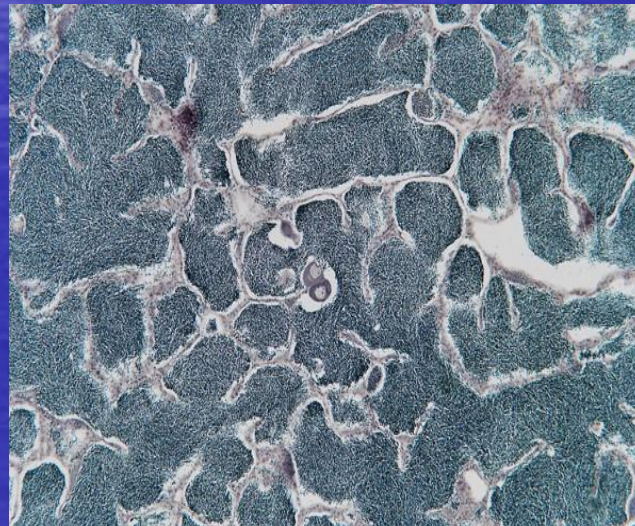
Reference: Hamilton P.B, Tyler C.R. (2008). *Molecular Ecology Resources*. Vol. 8, issue 2, 462-465

Histopathology analysis of parent roach

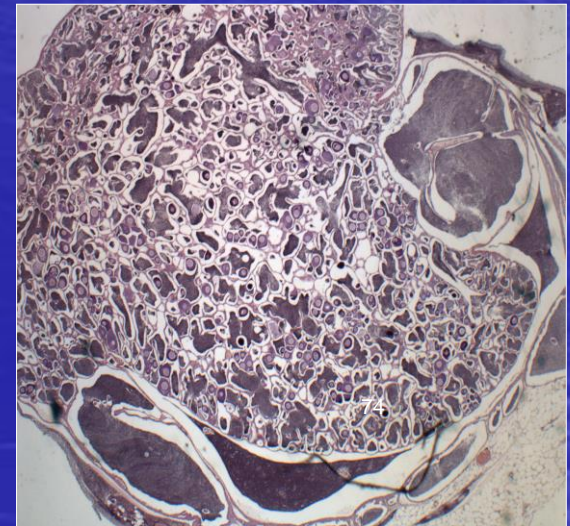
- Gonads of fish were examined to identify intersex status
- Results of histopathology analysis were matched with results from parentage analysis



Normal male fish



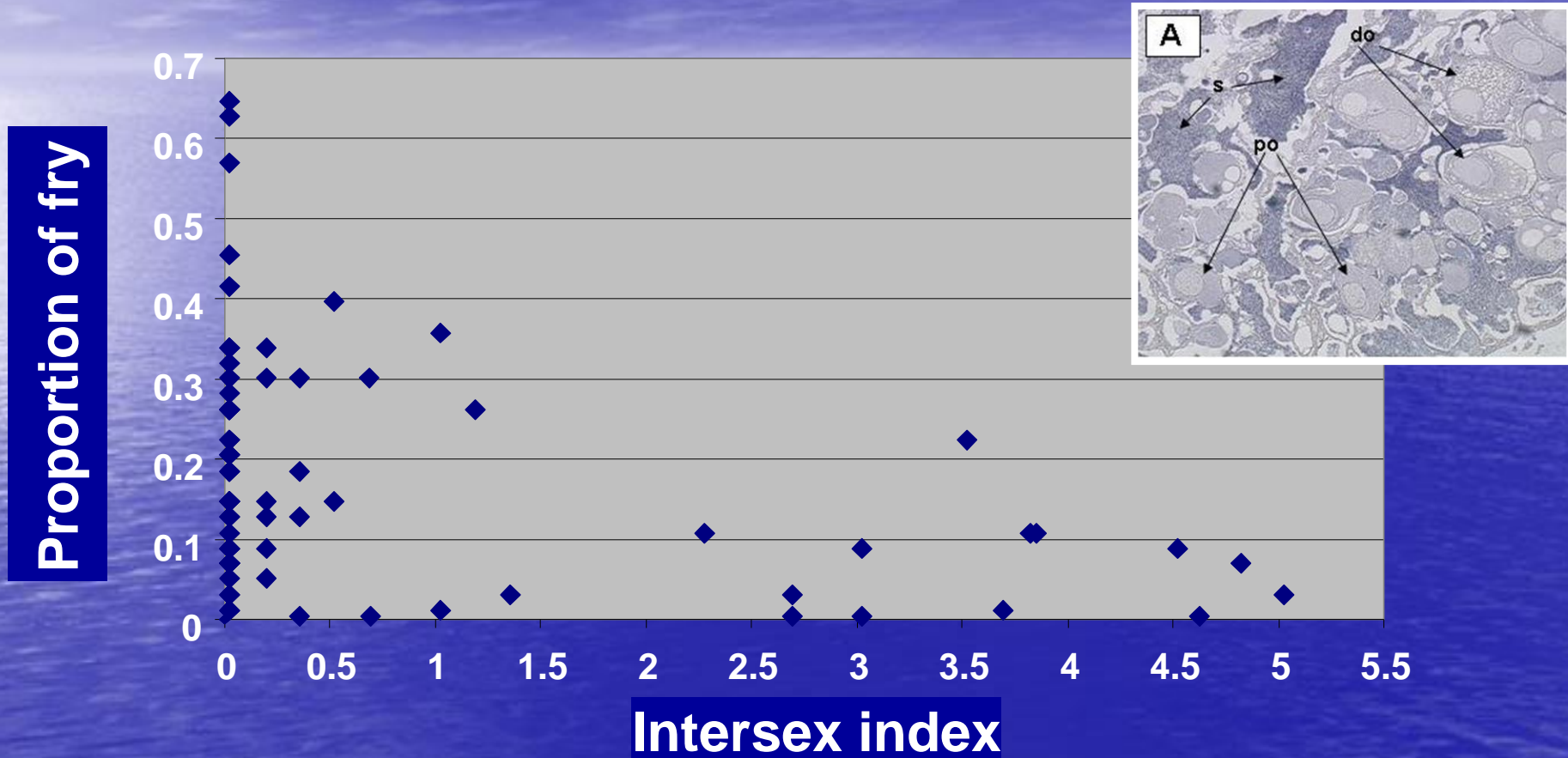
Mildly intersex male fish



Severely intersex fish

Breeding Experiment 3 (2008)

Intersex and reproductive success



Intersex fish are capable of reproducing, but it appears that more severely intersex fish are compromised in their ability to do so.

EDCAT Summary

- Oestrogen levels in rivers are predictable on a seasonal basis
- The River Ray was mildly contaminated with oestrogens and some other pollutants before STW effluent remediation, and GAC treatment led to reduced contamination in water
- GAC treatment was associated with improved stickleback growth, condition, and female reproductive status
- This probably resulted from the removal of a mixture of contaminants, not just endocrine disrupters
- While the reproductive ability of mildly intersex roach does not seem to be compromised, for more severely intersex fish there is a negative relationship between degree of intersex and proportion of fry sired
- This may place roach populations at risk as it is likely to lead to reduced genetic diversity

Read all about it....

<http://www.ceh.ac.uk/edcat>