



FISHERIES RESEARCH SERVICES



Oestrogenic endocrine disruption of wild Atlantic salmon (*Salmo salar*) parr in Scotland

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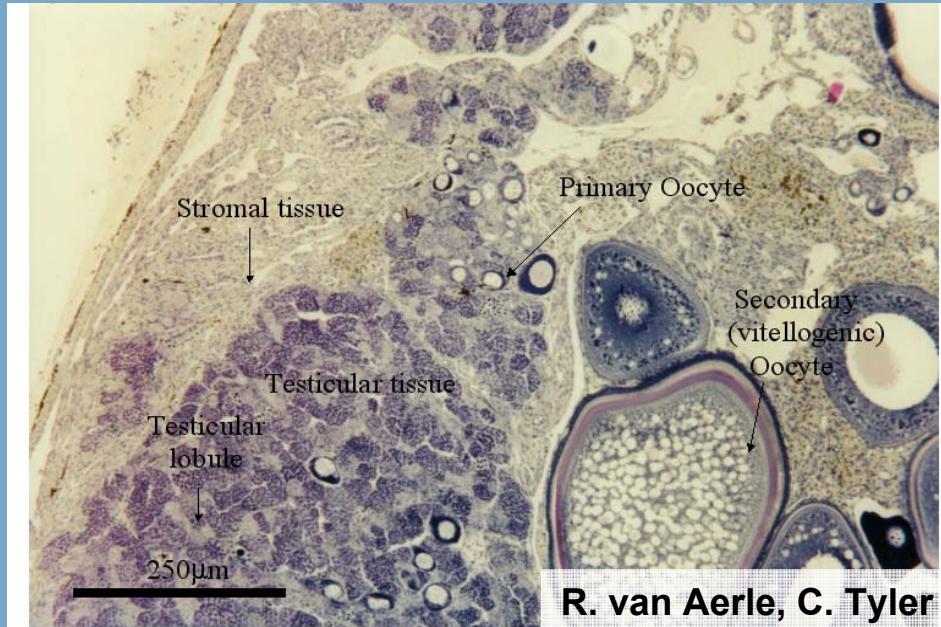
Scope of presentation

- **Background**
 - What is endocrine disruption?
 - Whether/why is it a problem?
 - Scottish situation
 - Aims
- **Methods**
 - Risk assessment
 - Fish sampling
 - Analysis
- **Results**
- **Findings in Context**
- **Summary**
- **New for 2008**

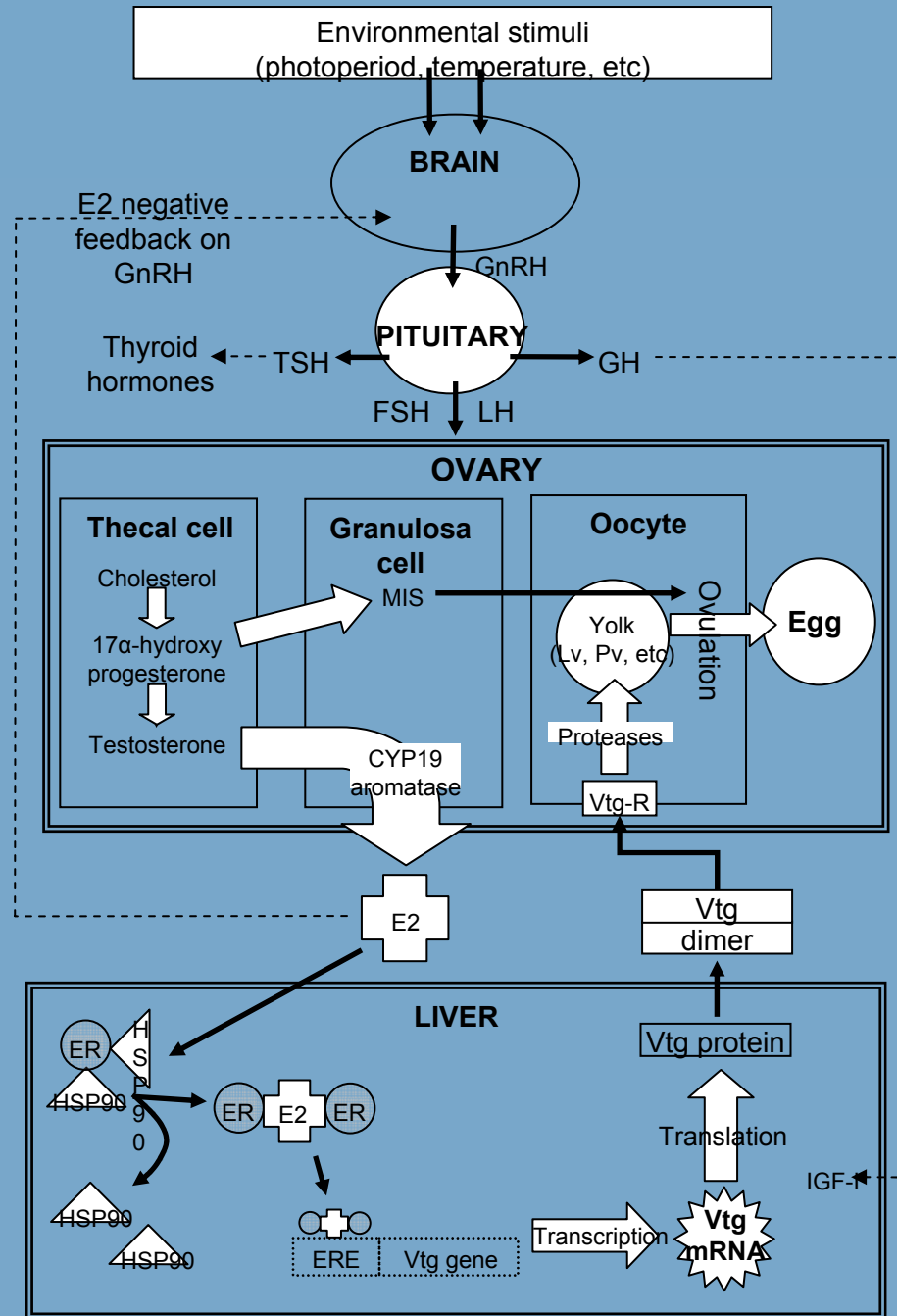


Introduction

- “exogenous substance that causes adverse health effects in an intact organism, or its progeny, consequent to changes in endocrine function” (EC, 1997)



- Widespread feminisation of wild male fish in English rivers (Vtg induction, ovo-testis, reproductive ducts, fertility)





Types of xenoestrogens

- **Steroids**
 - E2
 - E1
 - EE2
 - **(Diethylstilbestrol**
 - **AP/APEs**
 - **Bisphenol A**
 - **Parabens**
 - **Phthalates**
 - **Benzophenones**
 - **Phytosterols**
- **Relative ER α potency**
 - 1
 - 1/10
 - 10
 - 10)
 - 1/1,000 – 1/100,000
 - 1/20,000 ?
 - 1/100,000
 - 1/1,000,000
 - 1/1,000,000

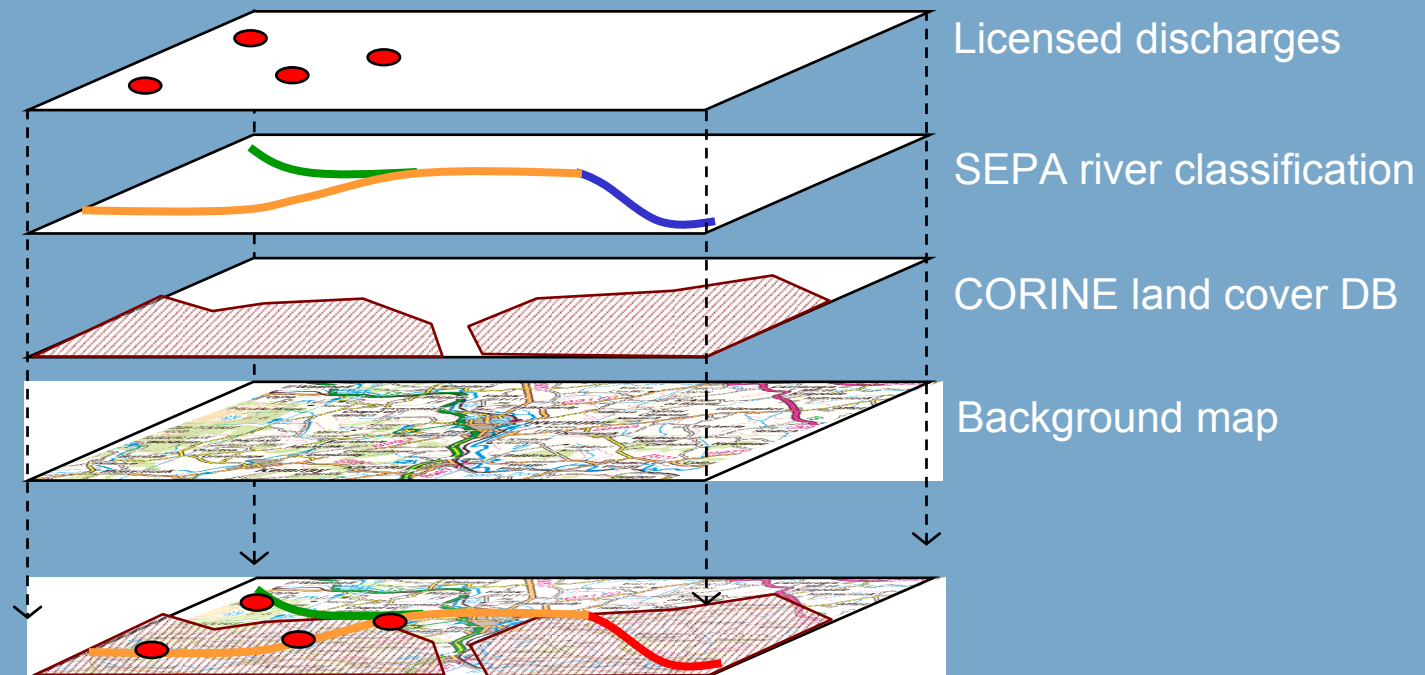


- Link to steroidal hormones in WWTW effluent, level of treatment, residence time, effluent dilution. Brunel, Exeter, CEH, Cefas; EA, Defra
- Netherlands, France, Germany, Scandinavia, Japan, USA, Canada, Australia... **Scotland?**
- **Scotland ≠ England**



Methodology – Risk Assessment

- **Site selection:**
 - low treatment level
 - low dilution (river Q95 / DWF discharge)
 - no other inputs





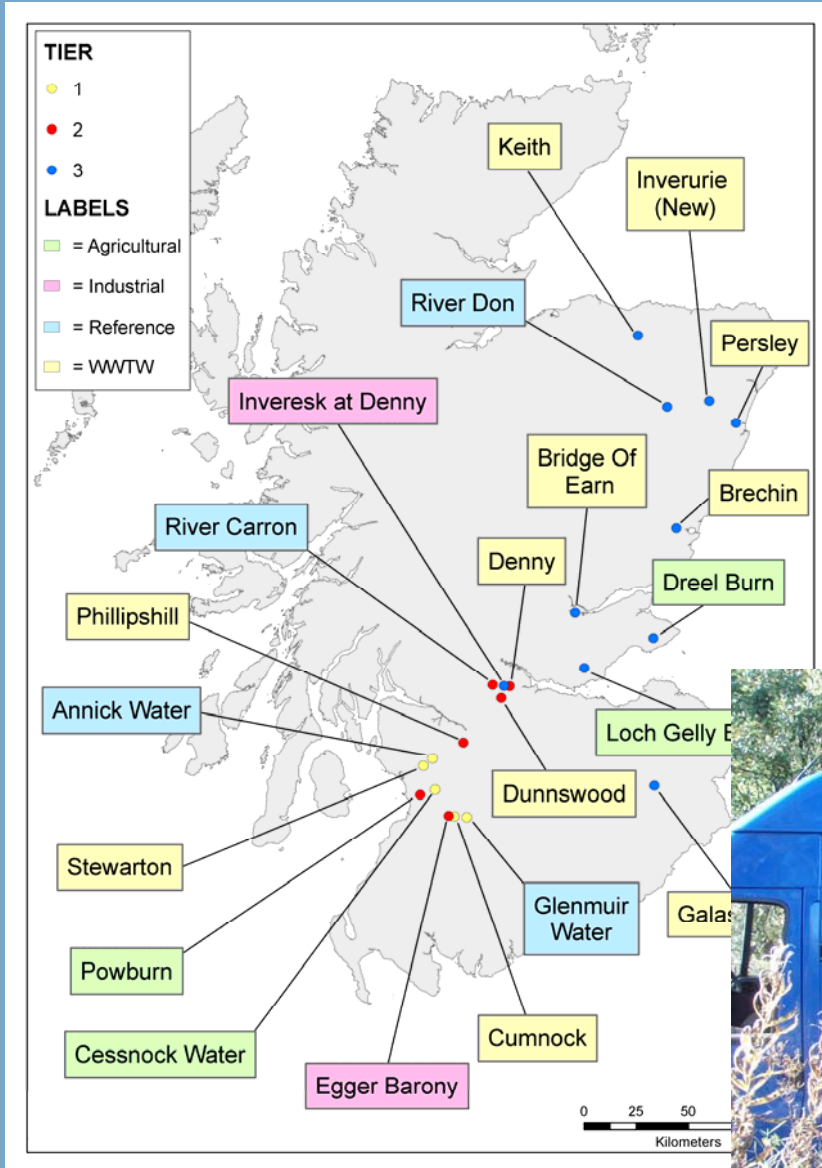
Results – Site Selection



WWTW	Locality	Size (PE)	Treatment	Receiving water	DWF dilution
Stewarton	East Ayrshire	7,000	Secondary Biol. Filt ⁿ .	Annick Water	3.6
Cumnock	East Ayrshire	16,000	Secondary A. Sludge	Lugar Water	5.9
<i>Philipshill</i>	<i>South Lanarkshire</i>	<i>51,960</i>	<i>Secondary A. Sludge</i>	<i>Kittoch Water → White Cart Water</i>	<i>1.1</i>
<i>Dunswood</i>	<i>North Lanarkshire</i>	<i>25,000</i>	<i>Secondary A. Sludge</i>	<i>Red Burn → Bonny Water → R. Carron</i>	<i>3.3</i>
<i>Denny</i>	<i>Falkirk</i>	<i>13,000</i>	<i>Primary</i>	<i>R. Carron, nr estuary</i>	<i>7.3</i>



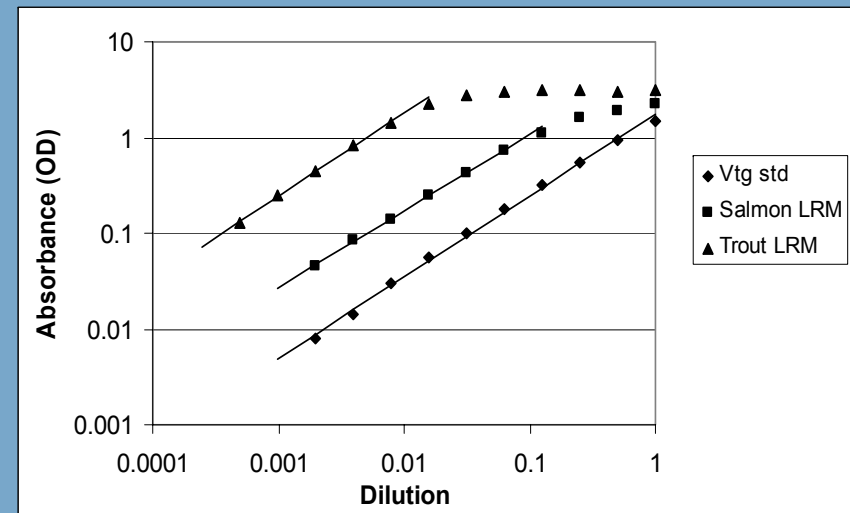
Methodology – Field Survey





Methodology - ELISA

- Blood sampled / centrifuged on site, plasma snap frozen and maintained in liquid N₂
- Homologous antigen-capture sandwich ELISA kit to quantify Vtg in triplicate plasma samples (Biosense Laboratories, Bergen)
- Showed parallel dilution curves of salmon Vtg standard, salmon plasma Vtg and brown trout plasma Vtg
- Samples applied to ELISA plates randomly
- QA by use of aliquots of a plasma sampled from an E2-injected salmon (inter-assay variability = 9%, $n = 3$)

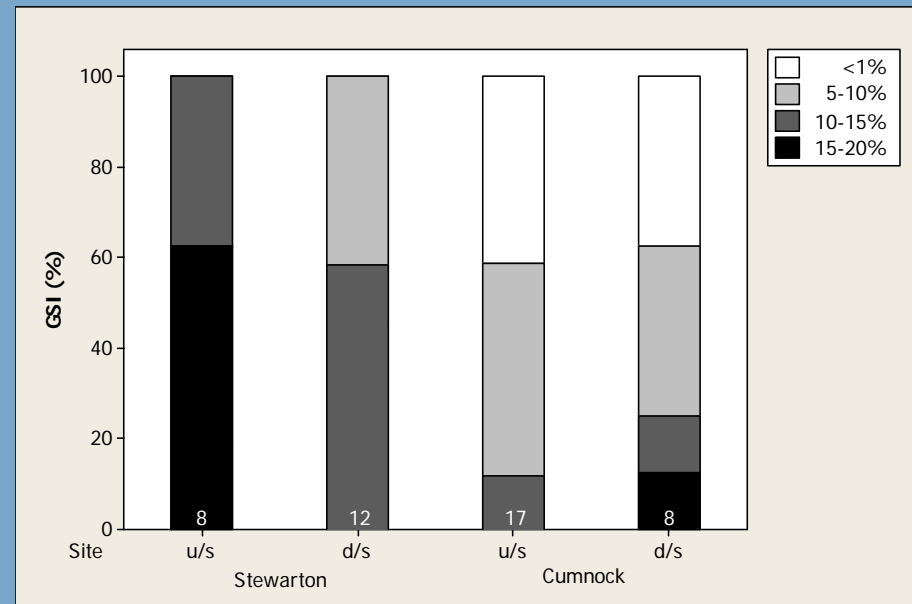
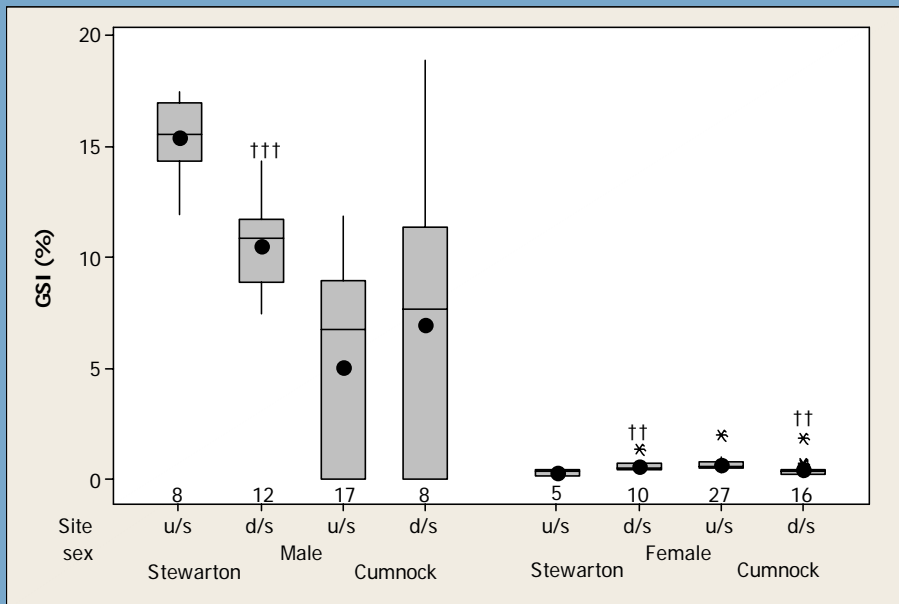




Results - Fish

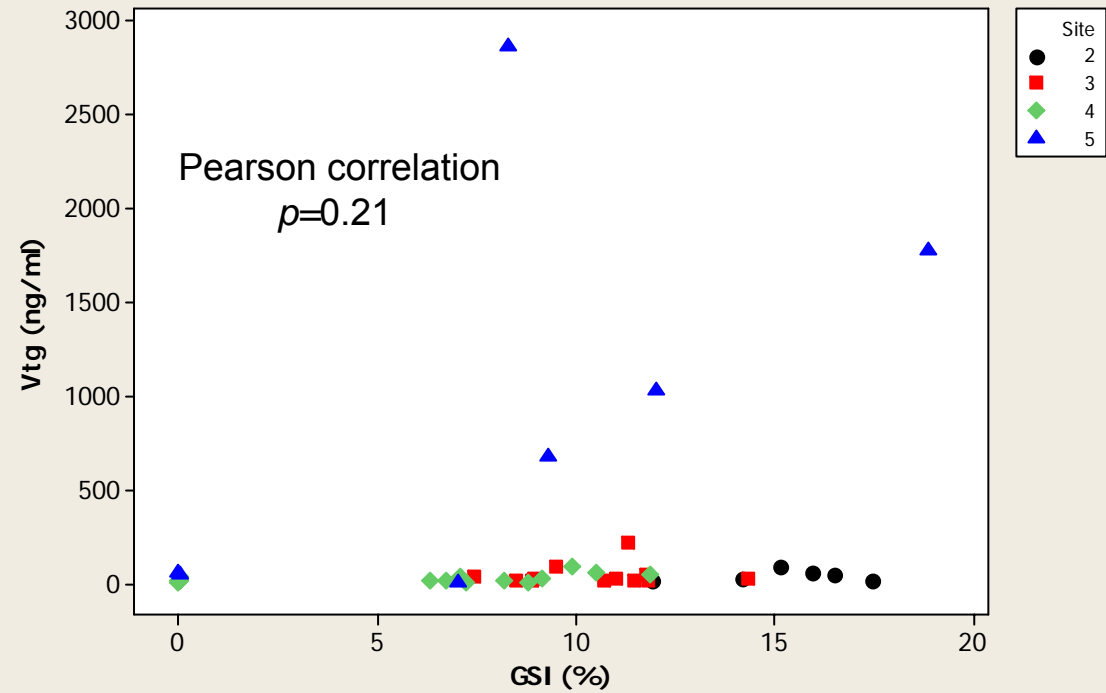
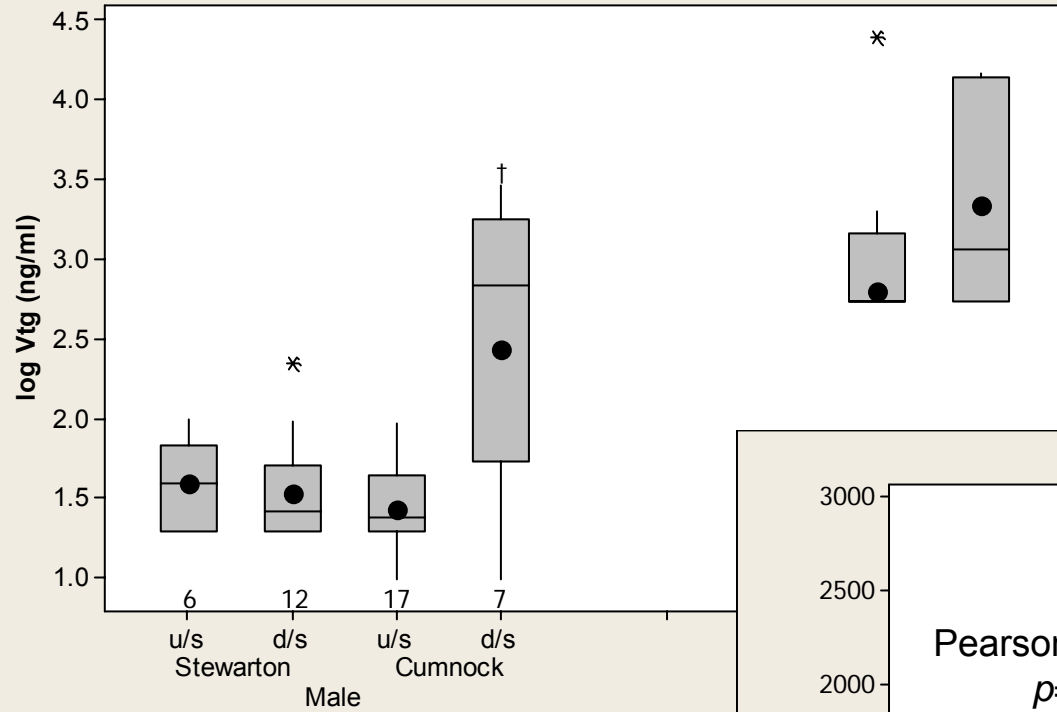
	Stewarton		Cumnock	
	upstream	downstream	upstream	downstream
Length of river fished	~0.8 km	~1 km	~0.3 km	~0.5 km
No. fish caught	13	22	44	24
% male	62	45	32 ^{††}	27 ^{††}
Male length (cm)	13.6±0.9	13.6±1.2	10.7±0.6	12.7±1.6 ^{†††}
Female length (cm)	14.0±1.5	13.8±0.8	10.8±0.7	12.7±1.6 ^{†††}
Male <i>K</i> (l/wt ³ x 100)	1.17±0.04	1.10±0.05 ^{††}	1.02±0.04	1.13±0.05 ^{†††}
Female <i>K</i> (l/wt ³ x 100)	1.16±0.12	1.20±0.05	1.03±0.04	1.19±0.07 ^{†††}

^{††}*p*<0.01
^{†††}*p*<0.001





Results – plasma Vtg





These Findings in Context

- No published papers on plasma Vtg concentrations in wild male salmon
- 30-fold induction of Vtg <0.5 km D/S of Cumnock, no induction 1.8-2.8 km D/S of Stewarton
- In England, rainbow trout caged for 3 weeks had Vtg induction of 40,000 fold immediately D/S of effluent discharges, and 10-100 fold inductions several km D/S (Harries *et al.*, 1996, 1997)
- Experimental exposure of salmonids to oestrogenic EDCs affected smoltification, D/S migration, and saltwater survival (Madsen *et al.*, 2004; McCormick *et al.*, 2005)



Conclusions

- **Wild Atlantic salmon parr were found to be oestrogenically exposed (elevated plasma Vtg) <0.5 km D/S of a Secondary (Activated Sludge) WWTW**
- **Vtg not elevated at 1.8–2.8 km D/S of a Secondary (Biological Filtration) WWTW**
- **The level of Vtg induction observed was relatively low compared to that seen in England**
- **Additional work is required to**
 - further investigate the extent of oestrogenic contamination in these and other Scottish rivers**
 - investigate significance of such exposure on parameters of importance to wild salmonid populations (fertility, smoltification, saltwater survival, natal homing, etc)**



On-going work 2008

- **Kirtle Water – septic tank discharge from Kirkpatrick Fleming, Dumfries & Galloway**
- **Lochar Water – leachate from former Dumfries landfill site?**
- **Electrofished in August 2008**
- **KW: found trout (no salmon) u/s and no fish d/s, until near tidal limit, where were salmon but only 2 trout**
- **LW: only collected salmon**
- **Lower Lochar salmon popn was female biased (22/31), but on u/s site was male biased (12/15; $p < 0.005$)**
- **No diffs in male K, HSI or GSI between u/s and d/s sites**
- **No diffs in female K, HSI or GSI between u/s and d/s sites**
- **Plasma Vtg/hormones? Yet to be analysed**



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 - Shona Kinnear, Lorraine Hawkins, Ian Smith, David Avery, Mike Lees (FRS)
- **References**
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Thank you for listening



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