# The population-level impacts of endocrine disrupting chemicals in fish

*Submitted by* **Tobias Sayer Coe** *to the University of Exeter as a thesis for the degree of Doctor of Philosophy in Biological Sciences, April 2010*



I certify that all material in this thesis which is not my own work has been identified and that no material has previously been submitted and approved for the award of a degree by this or any other University.

……………………………………

# Abstract

Endocrine disrupting chemicals (EDCs) are one of the many anthropogenic pollutants released into the environment. There is a substantial body of literature that has shown a detrimental impact on wildlife, particularly aquatic species, following exposure to these pollutants. In particular, exposure to EDCs can cause disruptions in sexual development and reproductive function. The majority of the effects observed are alterations in the physiology and behaviour of individuals and a key research question that has yet to be fully addressed is whether the effects seen in individuals exposed to EDCs manifest in measurable changes in population parameters.

Whilst some work has been conducted previously investigating the population-level impacts of EDC exposure, this work has generally used mathematical models to examine whether measured short-term changes in individual fecundity and survival may lead to alterations in population viability and growth in the long-term. Such an approach ignores the potential impact of EDCs on more subtle factors (for example alterations in behaviour and genetics) that can potentially affect fish population dynamics.

The work presented in this thesis used two model species, the zebrafish, *Danio rerio* and stickleback, *Gasterosteus aculeatus*, to examine whether exposure to EDCs had an effect on previously ignored factors that determine group dynamics, including behaviour, parentage and reproductive success. In groups of zebrafish (two males and two females) exposure to the model oestrogenic compound ethinylestradiol ($EE_2$) at 10 ng/L caused a reduction in the paternity success of the most successful male, reducing the skew in paternity, relative to controls. This disruption in the reproductive

hierarchy was associated with a suppression of 11-ketotestosterone (11-KT) concentrations in exposed males. Such alterations in reproductive hierarchies have implications for population genetic diversity as well as usual patterns of sexual behaviour and selection.

In a second study, males were exposed to $EE_2$ (females were not exposed) and then placed into colonies of varying size with either 1, 2 or 4 males competing to breed with one female. The reproductive success of the most reproductively successful male in colonies containing two males and one female was unaffected by previous exposure to $EE_2$, relative to controls, but was significantly affected for the most reproductively successful male in tanks containing four males competing to breed with one female. This finding suggests that the impact of EDC exposure on reproductive hierarchies and success is dependent on the group structure and is not a straightforward monotonic effect.

It is well known that there exist so-called 'windows of sensitivity' for the effects of EDCs on sexual development in fish. Exposure to EDCs during this period can cause dramatic alterations in development, including complete sex reversal if the magnitude and/or duration of exposure are sufficient. In the third study in this thesis, zebrafish were exposed during the key window of sexual development, from 20-60 days post fertilisation (dpf). The reproductive success of both mature males and females was then examined in competitive breeding scenarios. Whilst there were no obvious effects of the early life $EE_2$ exposures on the gonadal phenotypes in either males or females at maturity, the reproductive success in males exposed to 2.76ng $EE_2$/L was increased. In contrast, exposure of females to 9.86 ng $EE_2$ /L during early life reduced their subsequent reproductive success. Given the importance of female reproductive capability in population demographics and dynamics, the effect of exposure to $EE_2$ on female reproductive success may therefore have significant implications for exposed fish populations.

Whilst conducting the $EE_2$ exposure experiments, it became clear that the genetic diversity in the laboratory strains of zebrafish was much lower than that previously published for wild zebrafish. A study was therefore conducted in which several laboratory strains of zebrafish and a population of zebrafish obtained directly from the wild in Bangladesh were genotyped in order to elucidate the genetic diversity in the different strains. The results showed that the genetic diversity in commonly used laboratory strains (even those described as outbred or 'wild type') was significantly lower than that of wild zebrafish. Given the impact of reductions in genetic diversity and variation on fitness at both the individual and population level, this has implications for studies that extrapolate results from laboratory studies to wild populations, as exposure to EDCs may have a different impact on laboratory strains than their more genetically diverse wild counterparts.

The final study conducted examined populations of wild sticklebacks (*Gasterosteus aculeatus*) from either clean sites or sites with a history of exposure to anthropogenic pollutants, particularly EDCs from sewage effluent. All the populations examined from sites with a history of pollutant exposure showed evidence of population bottlenecks, whereas populations from clean sites did not. Fish from the different populations were then placed into competitive breeding mesocosms. Each mesocosm contained an equal number of males from a clean, control reference site and from one of the polluted sites. The same number of females from a clean, control reference site was also added to the mesocosm. Males from all polluted sites were able to compete and breed successfully when placed in these competitive breeding scenarios and there was no evidence that they had reduced reproductive success. The implication of this finding is that even if exposure to anthropogenic EDCs has impacted on the genetics of a wild population, the reproductive potential of individuals may not necessarily be altered.

# Acknowledgements

The work for this thesis was carried out at, and funded by the University of Exeter with some work at the Centre for Environment, Fisheries and Aquaculture Science (Cefas) laboratory at Weymouth. Travel grants were obtained from the University of Exeter.

A huge number of people have contributed, either directly or indirectly, to this thesis. First and foremost, I would like to thank my supervisors Prof. Charles Tyler and Dr. Dave Hodgson for their help, support and encouragement throughout the past four years. Both have shown immense patience when dealing with what must have been at times a churlish and impatient student! It feels overly clichéd to wax lyrical about 'dwarfs standing on the shoulders of giants' and also not strictly true. The reality was more one of being held aloft.

Several other people were instrumental to the successful completion of this thesis. I could not have completed the work that I have were it not for the constant support and advice of Dr. Patrick Hamilton. It has been immensely appreciated. Dr. Anke Lange, Dr. Andrew Griffiths, Dr. Gregory Paull and Dr. Amy Filby also came to my aid at moments when I was lost, or simply baffled by the work at hand. I am equally indebted to Jan Shears for her unstinting (if occasionally brusque!) advice and help. All work needs a levelling presence and Jan, you were most definitely it throughout my PhD! Marta Soffker was a patient lab-partner and co-author for a significant proportion of my PhD and none of the behavioural work conducted would have been possible without her help. Thanks are also due to Jessica Miller from the University of

Harvard for providing samples of different strains of zebrafish and Dr. Abdul Wahab from Bangladesh Agricultural University for the provision of wild zebrafish samples.

A great number of people were there for me, both at work and play, over the past four years. I will not list them here. You know who you are but perhaps not how much I have valued your constant support and encouragement, whether it was serious discussions about work or a session of spasmodic dancing and rum and cokes at 2am in the Angel. I would, however, like to individually thank Emma for her support when I needed it the most.

To Kate I owe a special thank you. It must be inordinately frustrating to have to put up with someone with such ‘tunnel vision’ and an oft practiced gift of stubbornness. Your patience, kindness and generosity of spirit know no bounds.

Finally I would like to thank my family. Mum and Dad – you are always there for me and your unwavering support is a constant within my life. I would like to dedicate this thesis to you.

# Table of Contents

**Gregory C. Paull, Amy L. Filby, Hannah G. Giddins, Tobias S. Coe, Patrick B. Hamilton and Charles R. Tyler** (2010) Dominance hierarchies in zebrafish (*Danio rerio*) and their relationship with reproductive success. *Zebrafish* (in press)

**Lange, A.**, **Paull, G.C., Coe, T.S., Katsu. Y., Urushitani, H. Iguchi, T. and Tyler, C.R.** (2009) Sexual reprogramming and estrogenic sensitization in wild fish exposed to ethinylestradiol. *Environmental Science and Technology*, 43: 1219-1255

**Lange, A.**, **Katsu, Y., Ichikawa, R., Paull, G.C., Chidgey, L.L., Coe, T.S., Iguchi, T. and Tyler, C.R**. (2008). Altered sexual development in roach (*Rutilus rutilus*) exposed to environmental concentrations of the pharmaceutical 17alpha-ethinylestradiol and

associated expression dynamics of aromatases and estrogen receptors. *Toxicological Sciences*, 106: 113-23

# List of (non research paper) Figures and Tables

# Research papers and author's declaration

**Research Paper I: Tobias S. Coe**, Patrick B. Hamilton, David Hodgson, Gregory C. Paull, Jamie R. Stevens, Katie Sumner and Charles R. Tyler (**2008**) An environmental estrogen alters reproductive hierarchies, disrupting sexual selection in group-spawning fish. *Environmental Science and Technology*, 42 (13): 5020-5025

**Research Paper II: Tobias S. Coe**, Patrick B. Hamilton, David Hodgson, Gregory C. Paull and Charles R. Tyler (**2009**) Parentage outcomes in response to estrogen exposure are modified by social grouping in zebrafish. *Environmental Science and Technology,* 43: 8400-8405

**Research Paper III: Tobias S. Coe**, Marta K. Söffker, Amy L. Filby, David Hodgson and Charles R. Tyler (**2010**) Impacts of early life exposure to estrogen on subsequent breeding behavior and reproductive success in zebrafish. *Submitted to Environmental Science and Technology.*

**Research Paper IV: T. S. Coe**, P. B. Hamilton, A. M. Griffiths, D. J. Hodgson, M. A. Wahab and C. R. Tyler (**2009**) Genetic variation in strains of zebrafish (*Danio rerio*) and the implications for ecotoxicology studies. *Ecotoxicology*, 18: 144-150

**Research Paper V**: Patrick B. Hamilton, **Tobias S. Coe**, Eduarda M. Santos, Jonathan S. Ball, Alastair Cook, Ioanna Katsiadaki and Charles R. Tyler (**2010**) Impacts of pollution on the population genetics and breeding resilience of the three-spined stickleback. *Manuscript in preparation.*

**Other papers – included in the Appendix**

Gregory C. Paull, Amy L. Filby, Hannah G. Giddins, **Tobias S. Coe**, Patrick B. Hamilton and Charles R. Tyler (**2010**) Dominance hierarchies in zebrafish (*Danio rerio*) and their relationship with reproductive success. *Zebrafish* (in press).

Lange, A., Katsu, Y., Ichikawa, R., Paull, G.C., Chidgey, L.L., **Coe, T.S**., Iguchi, T. and Tyler, C.R. (**2008**). Altered sexual development in roach (*Rutilus rutilus*) exposed to environmental concentrations of the pharmaceutical 17alpha-ethinylestradiol and associated expression dynamics of aromatases and estrogen receptors. *Toxicological Sciences*, 106: 113-23.

Lange, A.**,** Paull, G.C., **Coe, T.S.**, Katsu. Y., Urushitani, H. Iguchi, T. and Tyler, C.R. (**2009**) Sexual reprogramming and estrogenic sensitization in wild fish exposed to ethinylestradiol. *Environmental Science and Technology*, 43: 1219-1255

Harris, C.A., Hamilton, P.B., Runnalls, T.J., Vinciotti, V., Henshaw, A., Hodgson, D., **Coe, T.S.**, Jobling, S., Tyler, C.R. and Sumpter, J.P. (2010) The consequences of feminisation in breeding groups of wild fish. *Environmental Health Perspectives*, *in press*

**Statement:** I, Tobias Sayer Coe, was involved in the following manner in the papers presented in this thesis: I planned and wrote the General Introduction. I co-planned and carried out the experiments for **papers I** and conducted all genetic analyses. I planed and carried out the experiments for papers **II and IV**. I co-planned and carried out the experiments for **paper III**. Amy Filby assisted with the running of the exposure for **paper III**. I performed all the genetic and parentage work for **papers I-V**. I performed all the statistical analyses for **papers I, II, IV and V**, all statistical analyses apart from those on behaviour in **paper III**, with guidance from David Hodgson at times and the genetic analyses on **paper IV**. Analyses of behaviour (including statistical analyses) for **paper III** were performed by Marta Soffker. The Phylogenetic analyses for **paper IV** were lead by Patrick Hamilton and Andrew Griffiths. The population genetic analyses in **paper V** were conducted by Patrick Hamilton. RIAs conducted for **papers I-III** were performed with assistance from Alex Scott. I was responsible for writing, and co-ordinating the manuscripts for **papers I, II and IV** with valuable input from co-authors. I co-wrote **papers III and V** with Marta Soffker and Patrick Hamilton respectively.

For the papers included in the appendix I performed all the genetic and parentage analyses for the paper “Dominance hierarchies in zebrafish (*Danio rerio*) and their relationship with reproductive success”. I performed the statistical analyses for the papers “Altered sexual development in roach (*Rutilus rutilus*) exposed to environmental concentrations of the pharmaceutical 17alpha-ethinylestradiol and associated expression dynamics of aromatases and estrogen receptors” and “Sexual reprogramming and estrogenic sensitization in wild fish exposed to ethinylestradiol”.

For all chapters and papers my primary supervisor Prof. Charles Tyler played an advisory and editorial role, advising on the planning, design and implementation of the experiments conducted and editing manuscripts and thesis chapters where necessary. My second supervisor, Dr. David Hodgson, also played a similar role and in addition provided detailed guidance and help on statistical analyses.

# List of Abbreviations

| | |
|---|---|
| **11-KT** | 11-Ketotestosterone |
| **A** | Number of Alleles |
| **$A_R$** | Allelic Richness |
| **AR** | Androgen Receptor |
| **BCF** | Bioconcentration Factor |
| **BPA** | Bisphenol-A |
| **DDE** | Dichlorodiphenyltrichloroethylene |
| **DDT** | Dichlorodiphenyltrichloroethane |
| **dph** | Days post hatch |
| **DNA** | Deoxyribonucleic Acid |
| **$E_1$** | Oestrone |
| **$E_2$** | 17β-oestradiol |
| **$E_3$** | Estriol |
| **EDC** | Endocrine Disrupting Chemical |
| **$EE_2$** | 17α-ethinyloestradiol |
| **ELISA** | Enzyme-linked immunosorbent assays |
| **ER** | Oestrogen receptor |
| **FSH** | Follicle-stimulating hormone |
| **GLM** | General(ised) Linear Model |
| **GLMM** | Generalised Linear Mixed Model |
| **GnRH** | Gonadotropin Releasing Hormone |
| **GSI** | Gonadosomatic Index |
| **GTH** | Gonadotropin |
| **$H_E$** | Expected heterozygosity |
| **$H_O$** | Observed heterozygosity |
| **HPG** | Hypothalamic-Pituitary-Gonadal |
| **IBM** | Individual Based Model |
| **LH** | Luteinising Hormone |
| **mRNA** | Messenger RNA |
| **Methyltestosterone** | MT |
| **MHC** | Major Histocompatibility Complex |
| **MRS** | Most Reproductively Successful |
| **Nonylphenol** | NP |
| **Octylphenol** | OP |
| **PCR** | Polymerase Chain Reaction |
| **PIC** | Polymorphic Identification Content |
| **QSAR** | Quantitative Structure-Activity Relationship |
| **RAPD** | Random Amplified Polymorphic DNA |
| **RFLP** | Restriction Fragment Length Polymorphism |
| **RNA** | Ribonucleic Acid |
| **RO** | Reverse Osmosis |
| **SNPs** | Single Nucleotide Polymorphisms |
| **VTG** | Vitellogenin |
| **WwTW** | Waterwater Treatment Works |
| **UK** | United Kingdom |
| **US EPA** | United States Environmental Protection Agency |

# List of Species' Names

| | |
|---|---|
| African clawed frog | *Xenpus laevis* |
| Atlantic croaker | *Micropogonias undulatus* |
| Atlantic salmon | *Salmo salar* |
| Brown trout | *Salmo trutta* |
| Bull trout | *Salvelinus confluentus* |
| Carp | *Cyprinus carpio* |
| Chinese rare minnow | *Gobiocypris rarus* |
| Copepod | *Nitocra psammophila* |
| Copper redhorse | *Moxostoma hubbsi* |
| Fathead minnow | *Pimephales promelas* |
| Flounder | *Platichthys flesus* |
| Goldfish | *Carassius auratus* |
| Gudgeon | *Gobio gobio* |
| Japanese medaka | *Oryzias latipes* |
| Killifish | *Fundulus heteroclitus* |
| Marine Cunner | *Tautogolabrus adspersus* |
| Mosquitofish | *Gambusia holbrooki* |
| Perch | *Perca fluviatilis* |
| Rainbow trout | *Oncorhynchus mykiss* |
| Roach | *Rutilus rutilus* |
| Sacramento sucker | *Catostomus occidentalis* |
| Sea bream | *Sparus aurata* |
| Spotted halibut | *Varaspar variegates* |
| Three-spined stickleback | *Gasterosteus aculeatus* |
| Turbot | *Psetta maxima* |
| White sucker | *Catostomus commersoni* |
| Zebrafish | *Danio rerio* |