EFFECTS OF ETHYNYLOESTRADIOL

ON TURBOT (*SCOPHTHALMUS MAXIMUS*) LARVAE

M.J.C. Jordan
Institute of Marine Sciences
School of Biological Sciences, University of Portsmouth
Ferry Road, Eastney, PORTSMOUTH
Hampshire, P04 9LY United Kingdom
Tel: (44) (023) 92845802 Fax: (44) (023) 92845800
Email: medina.jordan@port.ac.uk

C.P.Waring
Institute of Marine Sciences
University of Portsmouth
Tel: (44) (023) 92845806 Fax: (44) (023) 92845800
Email: colin.waring@port.ac.uk

EXTENDED ABSTRACT ONLY - DO NOT CITE

Introduction

Endocrine disruption in fish has been a popular focal point in recent years. Research has (so far) focussed mainly on identifying what substances contaminating aquatic environments have endocrine disrupting qualities. Furthermore, such research has been conducted primarily on adult freshwater fish species. Very little is known about how these substances affect marine fish, in particular their early life stages.

17α-ethynylestradiol (EE_2) is a synthetic oestrogen, a common component of the contraceptive pill. It is normally excreted, in harmless conjugated form, into many waterways. However, research has shown that EE_2 conjugates found in sewage effluent can be deconjugated or rapidly degraded, thus restoring the potential of EE_2 to disrupt fish endocrine systems (Larsson *et al.*, 1999). EE_2 has been found to be a potent xeno-oestrogen in many fish species, mimicking the effects of natural oestrogens.

Purdom *et al*. (1994) tested the effects of EE_2 on immature rainbow trout (*Onchorynchus mykiss*) after finding hermaphroditic fish in the lagoons of some sewage treatment works. They found that exposure to EE_2 could induce vitellogenesis, a process normally restricted to adult female fish at times of sexual maturity. EE_2 exposure was also found to induce vitellogenin production in channel catfish (*Ictalurus punctatus*) (Nimrod & Benson, 1996). Injections of EE_2 was found to cause phenotypic sex reversal of genetic males to females in medaka (*Oryzias latipes*) (Papoulias *et al*., 2000). Nash *et al*. (1997) and Kime & Nash (1999) found that parental exposure of zebra danios (*Danio rerio*) to 5ng/L EE_2 prior to spawning caused cytostatic disruption of embryonic development of eggs during the early gastrula period.

The aim of this investigation was to assess the effects of different concentrations of EE_2 on turbot (*Scophthalmus maximus*) larvae (a marine species) after being exposed to the synthetic oestrogen over a 72-hour period from 2 days post-hatch. Mortality rate, yolk sac volume, larval length and larval morphology were analysed.

Materials and Methods

50 1-day-old turbot larvae (fertilised embryos purchased from the Isle of Man, UK) were placed in 12 moulded glass tanks, each containing 350 ml seawater with antibiotics. The tanks were kept at a constant temperature (15°C) and light intensity.

The larvae were allowed to acclimatise to the tanks for 24 hours before the tanks were inoculated with one of 4 different concentrations of EE_2: 1000 μgl^{-1}, 500 μgl^{-1}, 250 μgl^{-1} and 100 μgl^{-1}. Two control tanks were also set up: an ethanol (solvent) control tank (1000 μgl^{-1} ethanol) and a seawater-only control tank. Two replicate tanks were assigned to each treatment.

Approximately 270 ml of seawater from each tank was replaced each day with fresh, aerated, filtered seawater, and the test tanks were re-inoculated with the nominal concentrations of EE_2 (the ethanol (solvent) control tanks were also re-inoculated daily). Any dead larvae in the tanks were counted and removed with a pipette.

The larvae were exposed to EE_2 for a 72-hour period. At the 24, 48 and 72-hour intervals, dead larvae were counted and removed and live larvae were sampled (for future analysis).

Mortality rate, larval length, yolk sac volume and larval morphology were analysed.

Larval length and yolk sac volume differences between treatments were compared using the Kruskal-Wallis test.

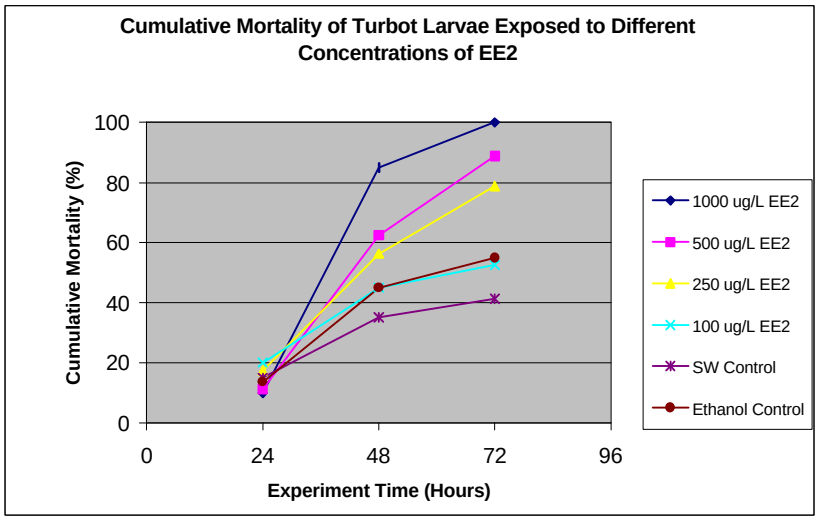

Figure 1. Cumulative Mortality

Results

Results show a marked increase in larval mortality at EE_2 concentration above 250 μgl^{-1} (Figure 1).

Statistical analysis showed no significant difference in total larvae length (from tip of mouth to tip of tail fin) of larvae from the 6 different treatments over the 24-, 48- and 72-hour periods (Figure 2).

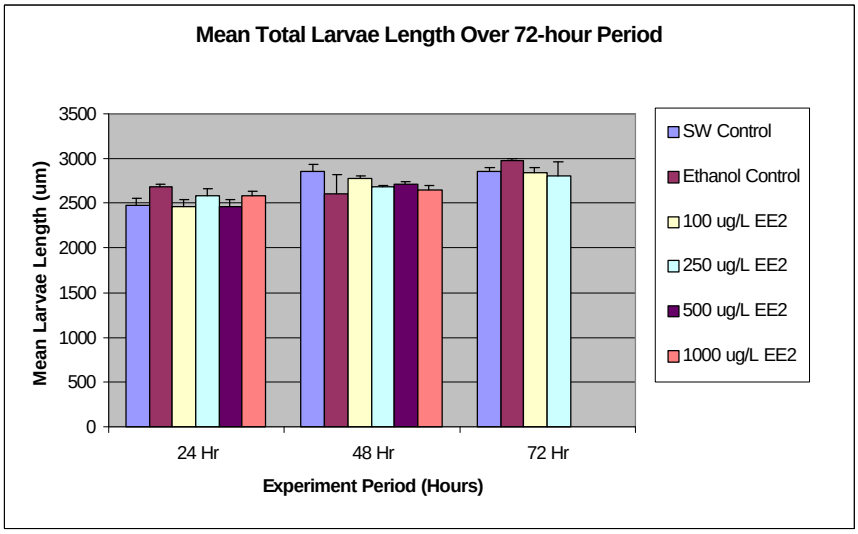

Figure 2. Total Larvae Length Over The Experiment Period.

Differences in larval yolk sac volumes between treatments were also found to be statistically non-significant.

Conclusions

Although EE_2 concentrations above 250 μgl^{-1} were found to be lethal to larvae, the synthetic oestrogen (at any of the four concentrations) was found not to have an effect on larval morphology. This indicates that at certain concentrations, EE_2 appears to induce a physiological response rather than a morphological response. Future experiments will therefore, concentrate on the mode of toxicity of EE_2 to the early life stages of marine fish.

Acknowledgements

I would like to thank Maureen Sims, David Maund and everyone at the Institute of Marine Sciences for their assistance with many aspects of the laboratory work and sample analysis.

References

Kime, D.E., & Nash, J. P. (1999). Gamete viability as an indicator of reproductive endocrine disruption in fish. *Science of the Total Environment,* 233(1-3), 123-129.

Larsson, D.G.J., Adolfson-Erici, M., Parkkonen, J., Pettersson, M., Berg, A.H., Olsson, P.E., Förlin, L. (1999). Ethynyloestradiol - an undesired fish contraceptive? *Aquatic Toxicology,* 45(2-3), 91-97.

Nash, J. P., Winn-Brown, P., & Kime, D. E. (1997). Embryonic mortality caused by parental exposure to low levels of ethynylestradiol in the zebra danio (*Danio rerio*). *Abstr. SETAC-Europe 7th Annual Meeting.* (p.256).

Nimrod, A.C., & Benson, W. H. (1996). Estrogenic responses to xenobiotics in Channel Catfish (*Ictalurus punctatus*). *Marine Environmental Research,* 42(1-4), 155-160.

Papoulias, D. M., Noltie, D. B., & Tillitt, D.E. (2000). An *in vivo* model fish system to test chemical effects on sexual differentiation and development: exposure to ethynylestadiol. *Aquatic Toxicology,* 48(1), 37-50.

Purdom, C.E., Hardiman, P.A., Bye, V.J., Eno, N.C., Tyler, C.R., & Sumpter, J.P. (1994). Estrogenic effects of effluents from sewage treatment works. *Chemistry & Ecology,* 8, 275-285.