# INTERACTIVE EFFECTS OF INBREEDING AND EXPOSURE TO AN ENDOCRINE DISRUPTING CHEMICAL ON LIFE HISTORY TRAITS IN FISH

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## Introduction:

The protection of wildlife populations is an implicit goal in environmental risk assessment. It relies on the meaningful extrapolation of data from ecotoxicological studies based on model test organisms to predict population level effects. Such model organisms are often obtained from selectively bred laboratory strains with reduced genetic variation [1]. Whilst this helps to ensure responses are consistent between individuals it may not be reflective of the potential responses of wildlife populations. It has been argued that the use of outbred strains may be more appropriate for ecotoxicology, since they may better represent wild populations [2].

Inbreeding can have negative fitness consequences for natural populations because it causes inbreeding depression, and in some cases this reduced fitness has been shown to be exacerbated under chemical stress [3,4]. Indeed, the few reports available (all of which are on invertebrates) have shown that inbreeding increases the impacts of chemical exposure in laboratory maintained animals.

A significant environmental pressure on wildlife populations is exposure to chemicals discharged as a consequence of anthropogenic activities, and one group of chemicals causing widespread concern are endocrine disrupting chemicals (EDCs). Exposure to EDCs is associated with deleterious impacts on reproduction and other aspects of health in a wide range of wildlife species [5], and may also negatively impact on their population dynamics [6,7,8]. Furthermore, EDCs have the potential to lead to compounding (interactive) effects on inbred populations, since reproductive fitness traits often show most inbreeding depression.

The aim of this research project was to test the hypothesis that inbred zebrafish (*Danio rerio*) differ in their susceptibility to the effects of chemical exposure compared with outbred zebrafish (representing wild populations). This was done via exposing hybrid WIK laboratory strain/Wild Bangladesh zebrafish to clotrimazole, a known EDC and priority hazardous substance [9] and assessing effects on various life history traits, including reproductive competitiveness.

## Methods:

Controlled zebrafish matings were conducted to generate inbred (theoretical inbreeding co-efficient F=0.25) and outbred (F=0) family lines. Zebrafish were exposed over a 96 day period (between 37 and 133 days post hatch) to nominal clotrimazole concentrations of 0, 5 and 50 $\mu gL^{-1}$. Various end points were recorded to determine effects on somatic growth, sexual differentiation and gonadal development. These included: *in vivo* measurements to determine specific growth rates; sexual differentiation and germ cell development assessed via histology; plasma concentrations of 11-ketotestosterone (11-kt); and the expression of a number of key target genes assessed via quantitative real-time PCR.

Following exposure to 5 $\mu gL^{-1}$ clotrimazole, competitive breeding trials were conducted and measures of reproductive output (number of eggs spawned and embryo viability) and competitive fertilization success were determined, the latter via paternity assessments of the offspring using microsatellite genotyping.

## Results:

Exposure of inbred and outbred zebrafish to 5 $\mu gL^{-1}$ clotrimazole had no effect on survival, growth or gonadal development. However, exposure of both lines to 50 $\mu gL^{-1}$ clotrimazole caused a male-biased sex ratio compared with controls (87 % vs. 55 % and 92 % vs. 64 %, for inbred and outbreds, males vs. females, respectively), advanced germ cell development, and reduced plasma 11-kt concentrations. We also found outbred males (but not inbred) exposed to the high level of clotrimazole developed testis that were more than twice the weight of control males. This corresponded with an increase in the relative proportion of Leydig cells, as well as maintenance of the expression of gonadal aromatase (*cyp19a1a*) and insulin-like growth factor (*igf1*), both genes of which were significantly down regulated in inbred males.

In competitive breeding trials, exposure of zebrafish (inbred and outbred) to 5 $\mu gL^{-1}$ clotrimazole had no effect on fecundity, but significantly reduced embryo viability in inbred (but not outbred) fish. However, this reduction in viability was not observed when inbred and outbred males were directly competing for fertilisation

success within the same tank. Paternity analysis revealed a trend towards a reduction in the proportion of embryos sired by inbred exposed males compared to outbred exposed males. Furthermore, irrespective of clotrimazole exposure, plasma 11-kt concentrations were reduced in inbred compared to outbred males.

Inbreeding depression coefficients indicate the additional stress caused by exposure to clotrimazole amplified the effects of inbreeding on embryo viability ($\sigma$ = -1.4 and 24.0, for control and exposed fish respectively) and a key male fitness component, siring success ($\sigma$ = -12.8 and 21.3).

**Conclusion:**

This is one of the first studies reporting that effects of exposure to an EDC (clotrimazole) on some traits (including sexual development and reproductive success) can differ between inbred and outbred animal strains. We show that inbreeding may be an important consideration in ecotoxicology and that EDCs may potentially affect inbred wild populations differently to outbred wild populations. Our data indicate the importance of better understanding interactions between inbreeding and chemical exposure for environmental risk assessment and protection of wildlife populations.

**References:**

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