Interactions Between Inbreeding and Environmental Stressors: Implications for Ecotoxicology

Submitted to the University of Exeter as a thesis for the degree of

Doctor of Philosophy in Biological Sciences

Andrew Ross Brown

May 2013

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.

Signature: A Ross Brown

ABSTRACT

In this thesis the effects of individual and multiple environmental stressors (physical and chemical) are examined in inbred and outbred zebrafish (*Danio rerio*, Hamilton), a model species used in ecotoxicology and environmental risk assessment (ERA). The central question addressed is, are inbred laboratory animals representative and protective of wild populations? That is, are inbred fish equally or more sensitive to chemicals and other stressors compared with more outbred (wild) fish? A combination of tools and approaches incorporating traditional (eco)toxicology and population genetics have been employed, together with more contemporary molecular genetics and population modelling, to compare and contrast a range of responses in inbred and outbred zebrafish exposed to the endocrine disrupting chemical clotrimazole and/or temperature elevation in the laboratory. The choice of test species was based on our broad understanding of its basic biology, extending from the molecular level to the population level, and its wide use as a model organism in (eco)toxicology. Selection of the test chemical clotrimazole and temperature was based on a shared mode of action, aromatase inhibition, and therefore their ability to block oestrogen production, impair reproduction, promote male development and skew population sex ratios in zebrafish.

A cascade of responses were compared in inbred and outbred zebrafish, including changes in the levels of expression of genes for gonadal aromatase and other steroidogenic enzymes, circulating sex steroid hormones, gonadal sex differentiation and development (via gonadal histopathology) and reproductive fitness (female fecundity, paternity and viability of embryos). Amongst the most striking results were directional skews in sex ratio towards males in response to clotrimazole (Chapter 5) and elevated temperature exposure (Chapter 7). Inbred fish were generally more responsive compared to outbreds, which showed evidence of physiological and developmental compensation, resulting in lower male-sex skews and superior fitness in terms of male reproductive success (paternity and viability of embryos). The greater effects observed in inbred fish were attributed to inbreeding×environment interactions and the amplification of inbreeding depression. Although no empirical genetic evidence of this mechanism is presented (loss of heterozygosity at quantitative trait loci and concomitant loss of heterosis and/or the expression of recessive, deleterious alleles in homozygotes), supporting evidence was provided by increased phenotypic variance in some apical endpoints in inbred fish, including specific growth rate and fecundity. This increased variance also has the potential to counteract the higher levels of response observed in inbreds, because the power to detect statistically significant changes in responses is reduced. This trade-off was demonstrated for specific growth rate.

Crucially, significant male-sex ratio skews (>80%) were induced at substantially lower clotrimazole exposure concentrations (1.7 µg l^{-1}) in combination with elevated temperature (33°C), compared with exposure concentrations (43.7 µg l^{-1}) generating similar sex ratio skews at the standard test temperature of 28°C. These temperatures represent current and predicted 2100 (elevated) mean temperatures in the zebrafish's native India and Bangladesh. Although the lowest observed effect concentration was an order of magnitude above the predicted environmental concentration for clotrimazole, it is conceivable that combined environmental exposures to similarly acting chemicals (e.g. other azole compounds used in crop protection, veterinary and human medicine) could produce similar effects to those we observed. The consequent effects of sex ratios skews and reduced fitness (fecundity and embryo viability) on per capita population growth rate (r) and extinction probability were predicted in inbred versus outbred zebrafish populations using stochastic population viability analysis. The results showed that the observed male-skews >80% threaten small zebrafish populations with fewer than 100 breeding adults (<20 adult females). However, small reductions of 2-3% in embryo-juvenile (age 0+) survivorship (including simulated inbreeding depression) were more influential on r and extinction probability than large sex ratio skews and/or reduced female fecundity.

The results presented in this thesis support the contention that chemical effects may be exacerbated by other environmental stressors, but also illustrate the importance of considering biological (genetic), as well as physical and chemical interactions in cumulative ERA. Greater sensitivity of inbred versus outbred organisms to the effects of environmental stressors on sexual differentiation and reproductive fitness offers a margin of safety to ERA and the protection of wildlife populations (excluding those that are severely inbred and critically endangered). This is because, as originally stated, laboratory organisms used in ERA are generally more inbred than their wild counterparts. Nevertheless, more attention should be paid to the origin, breeding history and genetics of laboratory strains. This will help to ensure consistency between studies and testing laboratories and provide more confidence in extrapolating the results to wild populations.

CONTENTS

Page