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Population relevance of toxicant mediated changes in sex ratio in fish: An assessment using an individual-based zebrafish (*Danio rerio*) model

Charles R.E. Hazlerigg[a,b,c], Charles R. Tyler[b], Kai Lorenzen[a,c], James R. Wheeler[d], Pernille Thorbek[d,*]

[a] Division of Biology, Imperial College London, Silwood Park, Ascot, Berkshire SL5 7PY, UK
[b] Ecotoxicology and Aquatic Biology Research Group, Hatherly Laboratories, University of Exeter, Prince of Wales Road, Exeter EX4 4PS, UK
[c] Fisheries and Aquatic Sciences, School of Forest Resources and Conservation, University of Florida, 7922 NW 71st Street, Gainesville, FL 32653, USA
[d] Environmental Safety, Syngenta Ltd., Jealott's Hill International Research Centre, Bracknell, Berkshire RG42 6EY, UK

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ABSTRACT

Ecological risk assessments (ERAs) of toxicants are predominantly based on data from laboratory tests on individuals. However, the protection goal is generally at the population level. Ecological modelling has the potential to link individual-level effects to population-level outcomes. Here we developed an individual-based zebrafish population model to study the possible population-level relevance of toxicant-mediated changes in sex ratio. The model was structured with sub-models based on empirical data (e.g. growth, reproduction, mortality) derived from a combination of our own laboratory and field experiments, the literature and theoretical concepts. The outputs of the default model were validated against size distributions for wild populations of zebrafish sampled in Bangladesh. Sensitivity analysis showed that population abundance was most sensitive to changes in density-dependent survival and the availability of refugia for juveniles.

The model was then used to determine the population-level relevance of changes in sex ratio caused by an androgenic (dihydrotestosterone) and oestrogenic (4-tert-octylphenol) substance. Both were investigated under acute (10 day) and chronic (1 year) exposure regimes. Acute exposures to the test chemicals had little effect on population-level endpoints at any of the concentrations tested. Chronic exposures decreased population abundance at higher concentrations for both chemicals and most strongly with DHT. However, these concentrations were far in excess of environmentally realistic levels. Our study demonstrated that ecological models can be applied to link laboratory derived ecotoxicity data at the individual level to impacts at the population level and in our study we found different modes of action and potencies caused different levels of population perturbation. Ecological models can therefore help in assessing the ecological relevance of different organism-level effects of toxicants aiding future environmental protection strategies.

1. Introduction

To perform effective ecological risk assessments (ERAs) clear protection goals are necessary and the European Food Safety Authority (EFSA) has developed protection goals based on the ecosystem services concept (EFSA, 2010). Delivery of ecosystem services typically depends upon functioning populations and communities rather than on individuals (e.g. EFSA, 2010; Luck et al., 2003) and ERA regulations implicitly state that their desired protection goal is at the population level (e.g. EU Regulations 528/2012, 1107/2009). Whilst some higher tier tests focus on population or community-levels (e.g. aquatic mesocosms) these are expensive, time consuming, can provide limited understanding to real-world scenarios, are not suitable for all species and larger spatial scales and it is unclear to what extent the results can be extrapolated outside the specific conditions of the study (Forbes et al., 2010; Hommen et al., 2010; Schindler, 1998). Consequently, most ecotoxicity testing focuses on measuring effects on individuals (e.g. fecundity, growth and more recently in endocrine-specific test methods sexual differentiation). Therefore, there is currently a shortfall between what is measured in ecotoxicity tests and the protection goal of ERAs, which is handled by applying conservative assessment factors. The link between individual-level effects and population-level effects is complicated by factors such as

* Corresponding author. Tel.: +44 01344416558.
E-mail address: pernille.thorbek@syngenta.com (P. Thorbek).

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life-history, landscape structure and density dependence (Ferson et al., 1996; Forbes and Calow, 2002; Grant, 1998). Modelling has been suggested as a tool to link individual-level ecotoxicity data to significance at the population level for ERAs (Barnthouse et al., 2007; Forbes et al., 2008; Galic et al., 2010; Pastorok et al., 2002; Thorbek et al., 2010) and European regulatory authorities, industry and academia anticipate that ecological models will be integrated into future ERAs of plant protection products (Hunka et al., 2012).

Existing ecological models for ERAs of fish are primarily demographic matrix models, whereby an individual's contribution to the population is measured through recruitment and survivorship with all individuals in a size class identical (e.g. Meng et al., 2006; Miller and Ankley, 2004; Schäfers and Nagel, 1993). Incorporating population-level processes such as density dependence and temporal variation into these models is important, but difficult to implement (Caswell, 2001). Density dependence is fundamental for population regulation (Rose et al., 2001) and is a driving force in population recovery after a reduction in abundance following disturbances such as chemical exposure or harvesting. An et al. (2009) omitted density dependence from their model investigating the effects of intersex (i.e. altered gonadal development) on roach populations, whilst Lin and Meng (2009) proposed a matrix model for medaka to compare acute and chronic toxicity without including density dependence, migration, predation or competition, limiting the applicability of their model to laboratory conditions. However, density-dependent processes are essential to understand the effects at the population-level (e.g. Barnthouse et al., 2007; Ferson et al., 1996; Pastorok et al., 2002). ERAs are increasingly concerned with the effects of endocrine disrupting chemicals (EDCs, e.g. Regulatory Working Groups EDTA, EDSTAC), whose effects are often sub-lethal, may affect a multitude of life-history traits and can occur at very low concentrations (e.g. Goodhead and Tyler, 2009; Gross et al., 2003; Purdom et al., 1994). Consequently, density dependence and behavioural interactions in population models to allow for potential compensatory processes are essential when assessing potential impacts on wild populations. We suggest individual-based models (IBMs) could be a useful tool for application in understanding the effects of EDCs (e.g. Baldwin et al., 2009; Madenjian et al., 2011).

Here, we developed an IBM for zebrafish (*Danio rerio*) aimed at linking individual-level endpoints observed in laboratory tests to responses at the population level. We included density-dependent effects on growth and survival in the model, with relationships parameterised with data from the literature and our own previous experiments (Hazlerigg, 2012; Hazlerigg et al., 2012). To demonstrate how this model may improve ERAs, we assessed the ecological relevance, i.e. population-level impact, of changes to sex ratio resulting from exposure to two compounds, dihydrotestosterone (DHT) and 4-tert-octylphenol (4-OP) using data from laboratory tests (OECD, 2011a,b,c). Changes in sex ratio has received increased attention (e.g. Hutchinson et al., 2000), and is the primary endpoint for the newly validated OECD Fish Sexual Development Test (OECD, 2011d).

2. Methods

2.1. Model species

We chose the zebrafish as a model species because it is frequently used in ecotoxicological studies (Hill et al., 2005; Segner, 2008; OECD Test Guidelines), with a well described biology to parameterise an IBM. The zebrafish is a small cyprinid fish (maximum length 5 cm), native to South East Asia (Engeszer et al., 2007; Spence et al., 2007a), inhabiting slow moving water bodies and the edges of rice fields (McClure et al., 2006; Spence et al., 2007*a*). Its lifespan is 1–2 years (Spence et al., 2007*a*) and it is a generalist feeder (McClure et al., 2006; Spence et al., 2007b).

2.2. Model description

The model description follows the ODD protocol (Overview, Design Concepts, Details): a standard format for describing IBMs (Grimm et al., 2006, 2010). Only the overview and design concepts sections of this protocol are included here; the details section is in the Supplementary Material. The model was implemented in the free software platform NetLogo (Wilensky, 1999) version 4.1.1 and is available on request. The sub-models are based on empirical data, (e.g. growth, reproduction, mortality), either derived from our own data and observations in laboratory and field experiments (Hazlerigg, 2012; Hazlerigg et al., 2012), from the literature, and/or based on accepted theoretical concepts.

2.2.1. Purpose

This model was designed to explore the population-level impact of toxicant induced changes to sex ratio. Toxicants often lead to sub-lethal effects before organism damage affects survival and our focus is on those cases. Specifically, we explored how density dependence can potentially compensate for the negative effects of toxicants. The model was designed to use standard ecotoxicological endpoints as inputs.

2.2.2. Entities, state variables and scales

Zebrafish are divided into four life-stages: eggs, larvae, juveniles and adults. Eggs hatch to larvae that change into juveniles at the onset of exogenous feeding and become adults at sexual maturity. All zebrafish are characterised by the state variable age (days post fertilisation (dpf)). All life-stages, except eggs, are also characterised by the state variables sex (undifferentiated, male or female), total length (mm, distance from the most forward anterior point to the tip of the tail, from here-on length) and wet body weight (mg). Further, adult females have an inter-spawn interval determining the time interval between reproductive events. Fish size (length) is the main variable determining fecundity (after Eaton and Farley, 1974) and survival rates (in fish generally see Lorenzen, 1996; Peterson and Wroblewski, 1984), as well as dominance hierarchies and territoriality in zebrafish (after Paull et al., 2008; Pyron, 2003; Spence and Smith, 2005).

The model environment mimics a 36 m^2 pond (18 000 L), divided into 900 patches each 20 cm × 20 cm × 50 cm, reflecting the likely size of zebrafish territories (Spence and Smith, 2005). Patches are characterised by the state variables habitat-type, water volume and number of fish in each life-stage in the patch. There are three habitat types: water (open water), breeding-ground (areas of gravel substrate where reproduction occurs) and vegetation (nursery areas for juveniles). Abiotic pond conditions, including temperature, pH, dissolved oxygen, ammonia and photoperiod, as well as some biotic conditions, including food availability are only modelled implicitly in the sub-models for growth and survival as they are parameterised from data collected over a 6-month period in semi-wild ponds in Bangladesh (Hazlerigg, 2012). The ranges of these variables are as follows, temperature 10–30 °C, pH 7.4–9.9, dissolved oxygen 1.4–10.4 mg l^{-1}, ammonia 0.047–0.67 mg l^{-1} and photoperiod 10 h 40 min to 13 h and 35 min light daily; extrapolation to conditions outside these limits should only be done with great care. Simulations are usually run for 3 or 6 years with 1-day time-steps.

2.2.3. Process overview and scheduling

Six processes occur each time-step in the following order: toxicant-effect, survival, development, movement, growth and reproduction. Toxicant-effect (sex differentiation) only affects juveniles (sex determination in zebrafish is between 20 and 60 dpf,