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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

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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

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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., 2007a). Its lifespan is 1–2 years (Spence et al., 2007a) 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,

Andersen et al., 2003). Adults engage in all other processes, juveniles in all others except reproduction, larvae in all others except movement and reproduction and eggs in all others except movement, reproduction and growth (Fig. 1 and Table 1). Applying toxicant-effect alters the proportion of individuals that develop into males or females. The level of effect depends on the concentration of the toxicant applied (logit function) and the direction of the effect depends on the toxicant type (androgenic, male skew, DHT; oestrogenic, female skew, 4-OP). The survival sub-model includes density-dependent background mortality rates, predation rates, senescent mortality (adults only) and developmental mortality (eggs only). Abundance counts for use in calculation of density-dependent effects on survival are performed at the start of the survival sub-model. Development determines the progression of individuals through the life-cycle, updating age and life-stage; egg to larvae and larvae to juvenile is age-dependent, whilst juvenile to adult is size-dependent. Toxicant-induced effects on sex are at larva to juvenile transition. Movement determines an individual's location during a time-step and includes territoriality in adults (dependent on fish length, larger individuals dominate spawning patches) and habitat preference in juveniles. Daily growth rate is determined from the fish's current length and density effects (through competition) from other individuals. Density is measured as biomass, summed at the start of the growth sub-model. Reproduction involves sexually mature males and females, with female spawning dependent upon the presence of a mate and breeding territory. Female size and age determines fecundity. Smaller, younger females produce few eggs daily; larger, older females produce many eggs every three days. Reproduction occurs year-round in the model, with no seasonality. Within each process, eggs are updated first followed by larvae, juveniles and finally adults, with individuals progressing to a new life stage adopting the movement, growth and reproduction of its new life-stage that time-step. Individual patches or individuals within each zebrafish life-stage are processed in a randomised sequence and state variables are updated immediately.

2.2.4. Design concepts

2.2.4.1. Basic principles. The concept of density dependence is fundamental in population ecology (Beverton and Holt, 1957; Nicholson, 1933; Ricker, 1975) and for fish in particular (e.g. Barnthouse et al., 1988; Elliott, 1994; Lorenzen and Enberg, 2002; Myers and Cadigan, 1993). Thus, understanding density dependence is key to predicting a population's compensatory reserve and resilience to stressors. We included density dependence at the sub-model level for zebrafish growth and survival, parameterised from empirical data (Hazlerigg, 2012; Hazlerigg et al., 2012), with density dependence at the local scale (one patch) for larvae and juveniles and at the global scale (whole pond) for adults, reflecting mobility and range differences between these life-stages seen in freshwater fish (e.g. Einum and Nislow, 2005). Consequently, reduced population density will result in increased growth and survival rates of remaining individuals. Furthermore, reproductive behaviours and territoriality are also density-dependent, mediated through the availability of, and competition for, breeding grounds. This reflects competition for optimal breeding areas, common in freshwater fish (e.g. Fleming and Gross, 1994; Whoriskey and FitzGerald, 1994).

Basic principles of allometric and geometric scaling are used to model length weight relationships, predation and fecundity (after Lorenzen, 1996; Wootton, 2002).

2.2.4.2. Emergence. Population dynamics, age and size structure emerge from the behaviour and interaction of the model's entities and in response to toxicant exposure (DHT and 4-OP). Density dependence, in part, emerges from model processes such as competition for habitat, and is affected by rules and parameters that make survival and growth dependent on density. The resultant implications of these processes on length-dependent movement, persistence, development and reproduction of individuals are key drivers of emerging population dynamics and responses to stressors.

2.2.4.3. Adaptation. Several fish behaviours in the model, especially movement related, are implicitly adaptive. This includes adult reproductive behaviour, where individuals search for alternative breeding grounds if the current one is occupied by a dominant of the same sex. Dominant individuals prioritise mating with dominant individuals of the opposite sex implicitly accounting for efforts of parents to provide the best genetic base for their young. Juveniles prioritise movement to vegetated areas over open water, as they reduce predation risk.

2.2.4.4. Sensing. Individuals sense the number and size of conspecifics (locally over one patch for larvae and juveniles and globally over the whole pond for adults) and adjust their behaviour and relevant processes accordingly, e.g. territoriality and density effects on growth and survival. Individuals also detect the environmental conditions of their patch (e.g. habitat type, toxicant concentration) which drives specific behaviours and actions (e.g. spawning) depending on their biotic (e.g. presence of mates) and abiotic (e.g. at a breeding ground) environment.

2.2.4.5. Interaction. Individuals interact indirectly through a range of processes, e.g. territoriality and density-dependent growth and survival. Pairwise mating occurs, with one male mating with one female each time-step if located on a breeding ground (no long-term pairing occurs). Therefore, competition to occupy these breeding ground patches in the pond occurs, with successful occupation governed by the lengths of competing individuals. Growth and survival interactions are indirect, via density-dependent effects, where increasing population abundance and biomass result in decreasing growth and survival and vice versa.

2.2.4.6. Stochasticity. All forms of mortality were modelled as stochastic processes. Habitat types were distributed randomly in the pond.

2.2.4.7. Observation. Counts of individuals of each life-stage, population abundance and biomass are taken each time-step and recorded graphically. The size (length) structure of the population is recorded in a histogram daily. Changes in population abundance and size distribution were used to measure toxicant effects.

2.3. Model calibration and validation

The model was calibrated using the predation sub-model and data of wild zebrafish population densities. The sub-model used for predation was based on a mortality model for freshwater fish taken from Lorenzen (1996). Importantly, calibration of the model was possible as this previous model included all mortality modalities, not just predation. This model was adapted for the zebrafish (Table 1), using the same b exponent from this previous study for fish in wild ponds. The Mu parameter was calibrated to provide model outputs of zebrafish pond densities known to occur in the wild. This calibration used a range of parameter values to provide a population density in the model that was within the range of adult population densities found in the wild (range 0.001–0.03 fish l^{-1}).

Knowledge of wild zebrafish population dynamics is currently lacking. However, population size structure can be used for model validation (e.g. Preuss et al., 2009; Schiegg et al., 2005) as it provides a 'snapshot' of the status of a population as well as the history of the population (e.g. periods of high reproduction or mortality

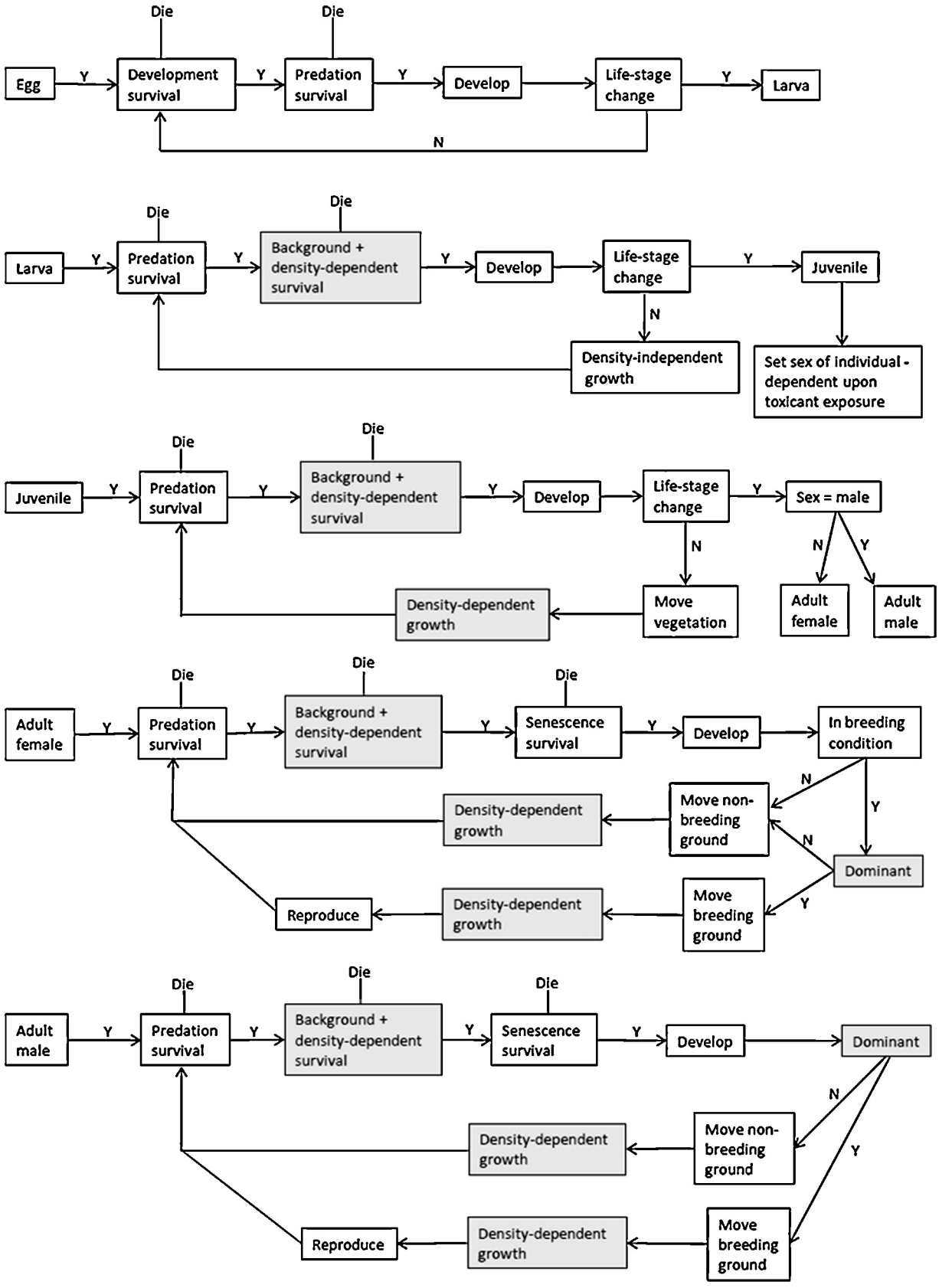

Fig. 1. Full life-history of the zebrafish and the processes that result in state transitions. N denotes 'no', Die denotes death and Y denotes 'yes'. Grey Shaded boxes show the processes affected by population (local or global) density.

Table 1
Algorithms, default parameter values and sources for zebrafish sub-models used in the model. All source data and fitted sub-models can be found in Supplementary Material.

Sub-model	Toxicant dose–response curve	Developmental mortality probability	Predation mortality probability	Density-dependent background mortality probability
Equation	DHT : $PM = \left(\frac{1}{1+e^{-(a+Cb)}}\right)$ 4-OP : $PF = \left(\frac{1}{1+e^{-(f+Ci)}}\right)$	$M = 1 - R$	If adults: $M = M_u W^b$ If juveniles: $M = (M_u W^b)/2$ If eggs: $M = P$	$R = \frac{e^{cLd}}{1+((aL+b)N}$
Parameter description	PM: Proportion of the population males C: Concentration (ng l^{-1} for DHT, µg l^{-1} 4-OP) a: DHT intercept b: DHT gradient PF: Proportion of the population female f: 4-OP intercept i: 4-OP gradient	M: Daily mortality probability R: Daily survival probability	M: Natural mortality probability at weight W M_u: Natural mortality probability at unit weight W: Weight (mg) b: Allometric scaling factor P: Daily egg predation probability	R: Daily survival probability c: Density-independent mortality constant d: Density-independent mortality exponent a: Density-dependent mortality constant b: Strength of density-dependent mortality at length 0 N: Abundance L: Length (mm)
Parameter values	$a = 0.00000185$ $b = 0.004392$ $f = 0.000000896$ $i = 0.011565$	$H = 0.949$	$M_u = 0.040$ $b = -0.382$ $P = 0.025$	$c = 0.884448688$ $d = -2.70443978$ $a = -0.000133$ $b = 0.0041722$
Sources	OECD (2011a,b,c)	Brion et al. (2004); Hill and Janz (2003); Lin and Janz (2006); Paull et al. (2008)	After Lorenzen (1996)	DI parameters c and d from laboratory experiment (Hazlerigg et al., 2012) DD parameters a and b linear fit to Bangladesh data (Hazlerigg, 2012)

Sub-model	Length:weight relationship	Reproduction rate (viable eggs female^{-1} day^{-1})	Growth rate (mm day^{-1})	Inter-spawn interval (days)
Equation	$W = aL^b$	$F = aL^b f$	$\frac{dL}{dt} = k\left(\left(\frac{L_\infty}{1+gB}\right) - L\right)$	If age $\leq$ 215 ISI = 1 If age $\geq$ 548 ISI = 3 If age > 215 and <548 ISI = $aA - b$ (rounded to the nearest integer)
Parameter description	W: Weight (mg) a: Weight constant L: Length (mm) b: Weight exponent	F: Fecundity (eggs day^{-1}) a: Fecundity constant L: Length (mm) b: Fecundity exponent f: Fertilisation rate	K: Growth rate L_∞: Asymptotic Length g: Strength of density dependence B: Biomass (mg) L: Length (mm)	ISI: Inter-spawn interval a: ISI constant b: ISI intercept A: Age (dpf)
Parameter values	$a = 0.004791$ $b = 3.198844$	$a = 0.000002637$ $b = 4.59015$ $f = 0.88$	$K = 0.003197$ $L_\infty = 60.13812$ $g = 0.00075$	$a = 0.006$ $b = 0.29$
Sources	Pers. Data (Supplementary Material)	Balasubramani and Pandian (2008); Brion et al. (2004); Christianson-Heiska et al. (2004); Coe et al. (2008); Eaton and Farley (1974); Ensenbach and Nagel (1997); Fahraeus-Van Ree and Payne (1997); Fenske et al. (2005); Gerlach (2006); Hill and Janz (2003); Lin and Janz (2006); Maack and Segner (2004); M. Soffker (pers. comm. 2010); Nash et al. (2004); Paull et al. (2008); Schäfers et al. (2007); Skinner and Watt (2007); Spence and Smith (2006, 2005); Uusi-Heikkila et al. (2010)	Hazlerigg (2012)	After Eaton and Farley (1974)

would cause changes to population structure that can be seen long after the direct effects). The model size distributions at day 1000 (to stabilise population) from 100 simulations were compared with data from three populations of wild zebrafish that we sampled in Bangladesh where 482 wild zebrafish between 16 mm and 43 mm were collected on May 2009. We calculated the proportion within each size class between 23 mm and 43 mm (lengths chosen as field sampling methods were ineffective in catching smaller individuals) and compared this to proportions calculated from the 100 model simulations (Fig. 2). The three wild populations show variable size

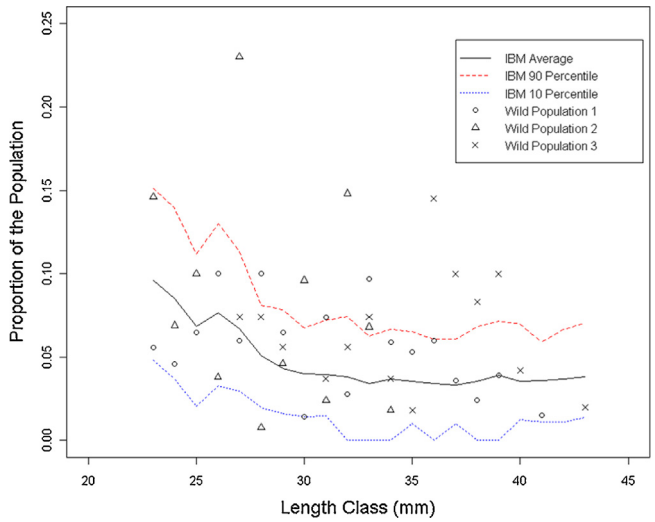

Fig. 2. A comparison of the variability in proportion of the population in each length class from three wild zebrafish populations and from 100 simulations of the zebrafish IBM.

distributions, so our model outputs would not match all equally well. Nonetheless, the match was good for population one, but less so for population three. Overall, our model captured the trends in wild populations with the declining slope of abundance with age consistent between our model and the wild populations and similar variability between our model outputs and the natural populations. The sensitivity of this match was studied further for parameters to which the model is most sensitive (see Section 2.4).

2.4. Model sensitivity analysis

A thorough sensitivity analysis was conducted to understand the robustness of the population outputs to different aspects of the model (Table 2). Firstly, a local sensitivity analysis to investigate effects on population abundance, biomass and adult abundance when outputs from each sub-model formulation was increased and decreased by ten percent (Fig. 3). Secondly, the size distribution produced from a 10% increase in the three parameters with highest sensitivity for population abundance (i.e. number of vegetation and breeding ground patches, strength of density dependence on survival and juvenile adult maturation length) were compared to the default model to check that the general functioning and interactions within the model are not drastically altered. Furthermore, changes in the duration of the reproductive season (2, 5, 8 and 12 month reproductive seasons) on population abundance, biomass and adult abundance were studied.

The sensitivity of the density-dependent components of the model was assessed in further detail, due to their potential importance for population regulation and its response to stressors, investigating the effects at the population level of 25% and 50% increases and decreases in the strength of density dependence in the model. Finally, we tested the sensitivity of the spatial scale of density dependence by altering larval and juvenile density dependence from a local (single patch) to a global (whole pond) level.

2.5. Simulation experiments

After 2 years to stabilise, exposure to chemicals resulting in male bias (DHT) and female bias (4-OP) were simulated to investigate the population-level relevance of these changes in sex ratio (Table 3). All chemical concentrations used in the empirical studies were simulated in the model (DHT – 0, 100, 320 and 1000 ng l^{-1}; 4-OP – 0, 10, 32, 100 and 200 $\mu g\, l^{-1}$). These are nominal (those theoretically delivered), rather than actual (analytically confirmed) concentrations. Using these treatment concentrations and the relevant concentration–response relationship (found in the Supplementary Material), resulted in model populations with 50–98% males (DHT) and 50–90% females (4-OP). Two exposure patterns were simulated; acute exposure lasting 10 days and chronic exposure lasting one year. In both, individuals between 2 and 60 dpf were affected, according to the concentration–response relationship, during the exposure, with the sex ratio of individuals spawned after the exposure period returned to 50:50. Following exposure, population recovery was assessed over 3 years (defined as the time from

Table 2
Sensitivity analyses conducted on the zebrafish IBM.

Analysis	Parameters tested	Amount of parameter change (%)	Endpoints measured	Replicates (*N*)	Duration (Number of time-steps)
Local sensitivity analysis	Number of vegetation/breeding grounds; Strength of density-dependent growth; Strength of density-dependent survival; Adult predation; Juvenile predation; Larvae predation; Egg predation; Larvae juvenile maturation age; Juvenile adult maturation length; Sex ratio (male, and female bias); Fertilisation rate; Fecundity; Inter-spawn interval	±10	Population abundance Population biomass Adult abundance	70	1000
Population size distribution	Number of vegetation/breeding grounds; Strength of density-dependent survival; Juvenile adult maturation length	±10	Population size distribution	70	1000
Reproduction season	Duration of reproductive season	−33 −66 −84	Population abundance Population biomass Adult abundance	70	1000
Density dependence	Strength of density-dependent growth; Strength of density-dependent survival	±25 ±50	Population abundance	70	1000

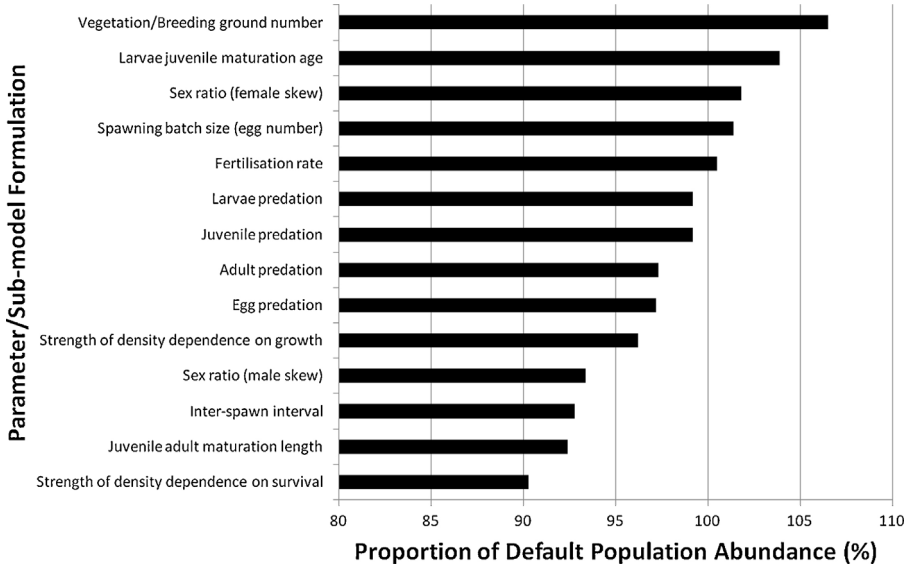

Fig. 3. The mean population abundance as a proportion of the default simulations resulting from a 10% increase in each parameter/sub-model value independently implemented in the model (from 70 replicates). The model is most sensitive to the parameters furthest from the line for 100% of the default population abundance (top and bottom of the graph). See Supplementary Material for data on 10% decrease in each parameter/sub-model value.

Table 3
Nominal (planned) and actual (measured) chemical concentrations and associated experimental and modelled sex ratio for DHT and 4-OP exposure.

Nominal DHT concentration ($ng\,l^{-1}$)	Actual DHT concentration range ($ng\,l^{-1}$)	Experimental sex ratio[a]	Modelled sex ratio[b]	Nominal 4-OP concentration ($\mu g\,l^{-1}$)	Actual 4-OP concentration range ($\mu g\,l^{-1}$)	Experimental sex ratio[a]	Modelled sex ratio[b]
0	0.4–11.5	43♀:57♂	50♀:50♂	0	0	48♀:52♂	50♀:50♂
100	3.3–60[c]	32♀:68♂	40♀:60♂	10	9.5	68♀:32♂	52♀:48♂
320	3.3–145.8	19♀:81♂	20♀:80♂	32	5.7–26.0[c]	76♀:24♂	59♀:41♂
1000	8.7–272.6	18♀:82♂	2♀:98♂	100	17.6–91.5	77♀:23♂	76♀:24♂
				200	42.5–73.1	80♀:20♂	90♀:10♂

[a] Average from data collected at three different laboratories.
[b] Sex ratio for each concentration from a fitted logit dose–response function.
[c] LOEC.

exposure end until population abundance returned within 5% of pre-disturbance levels). Each scenario was replicated 70 times.

3. Results

3.1. Sensitivity analysis

Model output sensitivity to changes in parameter values and algorithms was fairly low (Fig. 3, Supplementary Material). When increased by 10%, some parameters decreased population abundance (e.g. inter-spawn interval, adult predation); whilst others increased population abundance (e.g. vegetation/breeding ground number and fertilisation rate). No single parameter or sub-model (for an increase by 10%) resulted in a change in the population abundance of more than 10% from the default simulations (neither increased nor decreased). The parameters to which the model was most sensitive were the strength of density dependence on survival, juvenile adult maturation length and the number of vegetation and breeding grounds. Model sensitivity was reasonably symmetric, thus when parameter values were decreased by 10% the parameters that resulted in the greatest change to population abundance from the default simulations were the same as those when parameters values were increased by 10%. Primary sensitivity analyses showed that the population abundance was robust to changes in model parameters and sub-models. The size distributions were robust to changes in the three parameters to which the population abundance was most sensitive (data not shown).

Increases and decreases in the strength of density effects on survival had obvious effects on abundance. Changes in the strength of density effects on growth (either increased or decreased) did not significantly affect population abundance. Neither changes in breeding strategy to group spawning, nor disruption of the dominance hierarchies based on female lengths, resulted in significant changes in population abundance (data not shown). Globalising larvae and juvenile density dependence reduced the strength of density dependence on growth and survival in these life-stages, resulting in population abundances roughly two and a half times that of populations under local density dependence (global density dependence = $15\,360 \pm 1699$, local density dependence = 6159 ± 809).

Shortening the reproductive season decreased population abundance outside the reproductive season and reduced the average abundance across the year, but average adult abundance across the year increased. The mechanism behind this was that the shorter reproductive seasons lowered reproductive output, reducing population density and releasing growth and survival from density dependence, resulting in higher adult abundances and a population potentially more resistant to external stressors.

3.2. Population-level effects of changes in sex ratio

Acute exposure (10 days) of DHT had no effect at lower concentrations, but at the highest two concentrations ($320\,ng\,l^{-1}$ and $1000\,ng\,l^{-1}$, corresponding to 80% and 98% males) the maximum

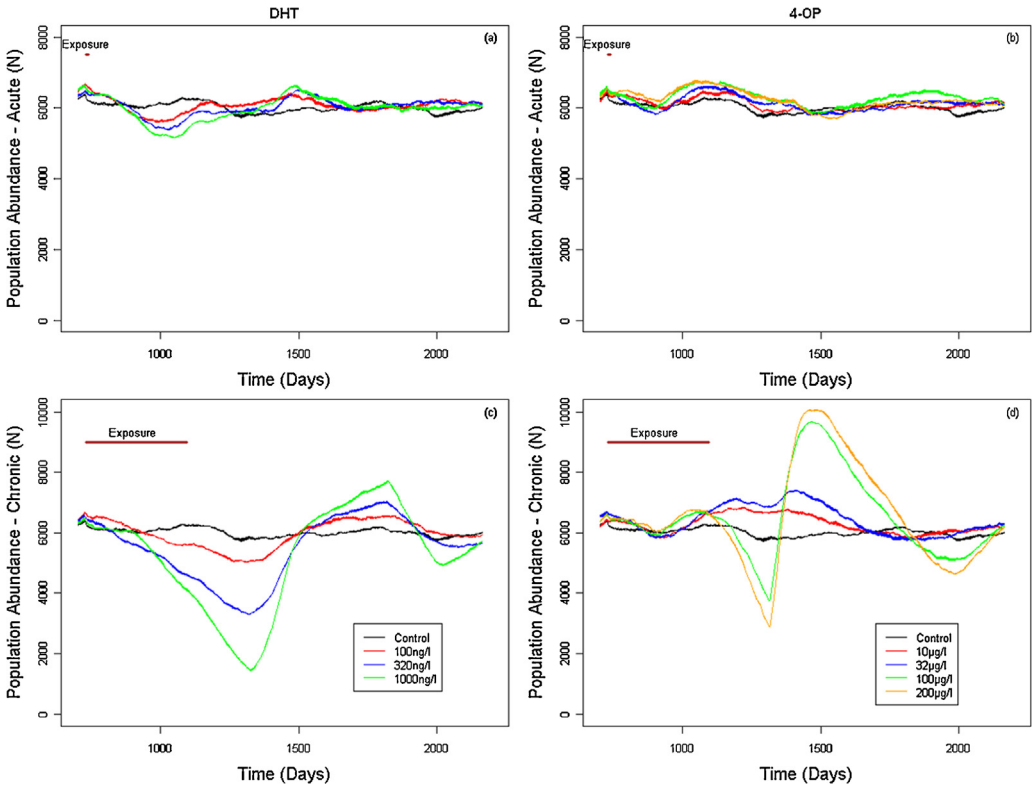

Fig. 4. Mean modelled population abundance and recovery under altered sex ratios due to toxicant exposure (from 70 replicates). DHT and 4-OP exposure, for both acute 10 day (a and b) and chronic 1 year (c and d) exposure profiles.

decrease in population abundance was 12% and 17%, respectively (Fig. 4). The population only recovered after 218 and 310 days for 320 ng l^{-1} and 1000 ng l^{-1}, respectively. The maximum decrease in population abundance occurred around 300 days after DHT application because the reproductive output, and hence abundance, was not affected until the affected individuals were established in the mature adult population and contributing to reproduction. Acute exposure of 4-OP (10 days) had no observed effect on population abundance at any concentration tested (Fig. 4).

Chronic exposure for one year resulted in population abundance changes in both 4-OP and DHT at all concentrations (Fig. 4). Exposure to DHT at 100 ng l^{-1} had limited effect on population abundance, but at 320 ng l^{-1} and 1000 ng l^{-1} it was reduced by 44% and 73%, respectively. At the highest concentration, the maximum loss of population abundance occurs approximately 230 days after the end of the exposure (once the affected individuals reached maturity). Recovery of the population to pre-exposure levels occurred after a further 150 days, though there was a tendency for an over-compensation at concentrations of 320 and 1000 ng l^{-1}, by 15 and 31%, respectively (Fig. 4). Chronic exposure to 4-OP also had significant effects on population abundances. Initially, population abundance increased under all 4-OP exposure concentrations, by 10–23% (Fig. 4). For lower concentrations of 4-OP, this slight increase in abundance remained until reverting back to pre-exposure levels once exposure ceased. However, for the two highest exposure concentrations (100 and 200 µg l^{-1}) the population abundance decreased following this initial rise and reached a maximum reduction of 36% and 51%, respectively. Once exposure ceased, the population over-compensated, resulting in a 64% and 71% higher abundance than control simulations for 100 and 200 µg l^{-1} exposures, respectively, before returning to pre-exposure levels around 800 days after exposure ended (Fig. 4). The highest concentrations of DHT and 4-OP exposure resulted in a secondary, slight reduction in population abundance before levelling out to pre-exposed levels. This probably reflects the dynamic oscillation resulting from the behaviour of density dependence in the system.

The response of individual life-stages under chronic exposure showed that changes in egg and larva abundance reflected those of the overall population for DHT (Fig. 5) and 4-OP (data not shown) exposure. For example, when exposed to 1000 ng l^{-1} of DHT, population abundance decreased by 73% whilst larvae and egg abundances decreased by 86% and 89%, respectively. Then during the over compensation period, population abundance increased by 31% whilst larvae and egg abundances increased by 35% and 34%, respectively. Juveniles showed a population decrease following DHT exposure, however, there was no over compensatory response during the recovery period. Adult female abundance decreased with DHT exposure, whilst adult male abundance increased with DHT exposure (Fig. 5). It should be noted that adult female numbers declined significantly, by 90% from the control simulations following DHT exposure of 1000 ng l^{-1}.The decrease in juveniles, larvae and eggs was not unexpected, as egg production was lower as DHT exposure skewed the sex ratio and led to fewer spawning pairs. Less eggs and larvae resulted in fewer juveniles, which led to some release from density-dependent mortality and consequently more juveniles reached maturity. This result was consistent with our sensitivity analysis which found the model to be most sensitive to the strength of density-dependent mortality.

To investigate the different recovery dynamics between chronic DHT and 4-OP exposure (specifically why the population overshoots the pre-exposure level significantly following exposure to 4-OP but less so with exposure to DHT), changes in size structure (Fig. 6) and daily egg production during recovery were investigated for 1000 ng l^{-1} (DHT) and 200 µg l^{-1} (4-OP). At the beginning of recovery (time = 1315) individuals in reproductive condition were similar under both exposure scenarios

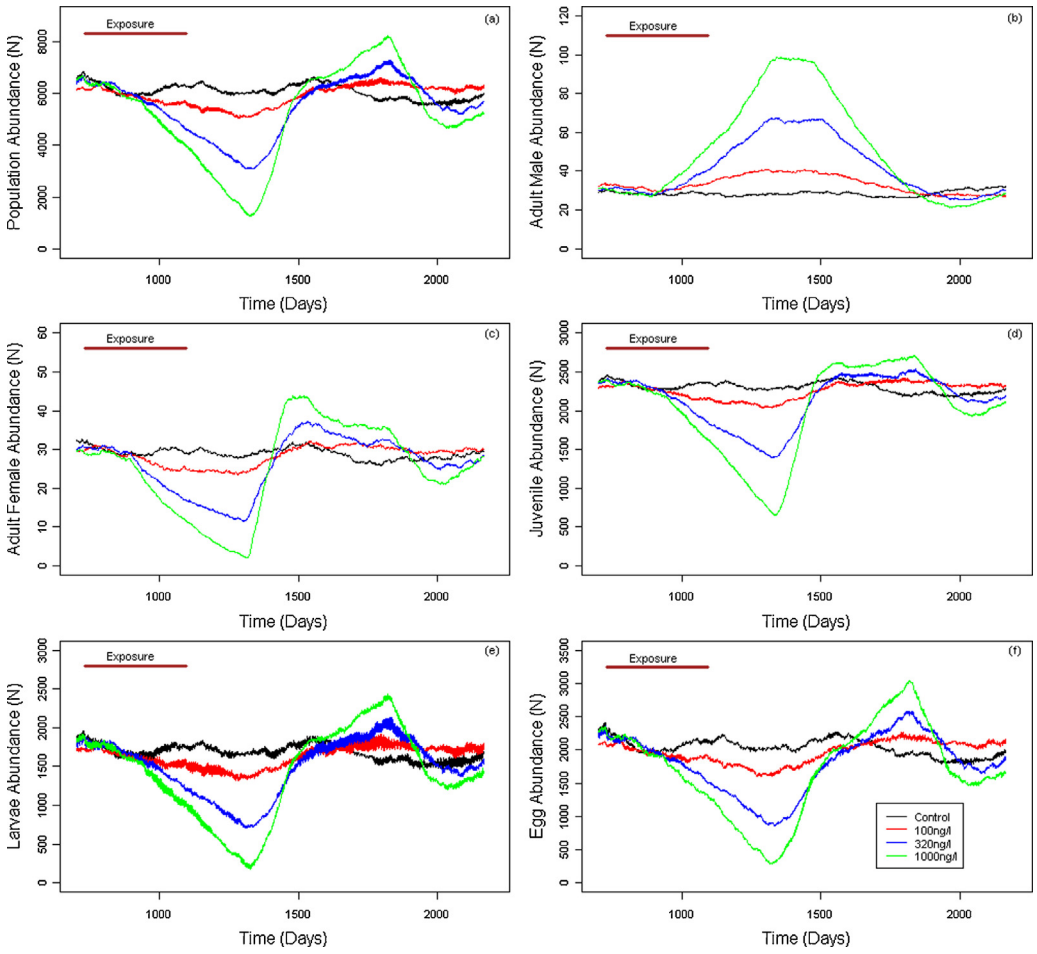

Fig. 5. Mean abundance and recovery of each life-stage in the model when the population was exposed to a range of concentrations of DHT (from 70 replicates).

(average female length in DHT = 46.3 ± 1.93, in 4-OP = 47.8 ± 1.37; number of pairs mating in DHT = 4, in 4-OP = 4). However, when new individuals mature, increasing the number of potential mating pairs, the females involved in reproduction differ, as females in the DHT exposure are newly matured small individuals mating with the older, longer males, whilst females in the 4-OP exposure are older, longer individuals mating with the newly matured small males (after time = 1345 average reproductive female length in DHT = 33.1 ± 1.91, in 4-OP = 43.3 ± 1.02). As egg production increases exponentially with fish length, the larger females reproducing after 4-OP exposure produce considerably more eggs per day than those following DHT exposure (after time = 1345 daily egg production in DHT = 153 ± 180, in 4-OP = 796 ± 230). Density-dependent mortality cannot initially compensate for this large increase in egg production in the 4-OP scenarios. This combination of large egg numbers and delayed density-dependent mortality allows the rapid increase in abundance resulting in the population overshooting significantly the pre-exposure levels. Meanwhile, the lower rate of egg production in the DHT scenarios where the mature females are smaller allows density-dependent mortality to compensate for the increasing population numbers, reducing the over-compensation at the population level.

4. Discussion

4.1. Validation

The size distribution and variability of modelled zebrafish populations resembled those collected from wild zebrafish in Bangladesh. As the size distribution of a population integrates a multitude of different life-history traits this correlation between our model and wild populations suggests that we captured the essence of the natural system in our model.

4.2. Sensitivity analysis

Population abundance was robust against changes in model parameters, indicating good structural integrity of the model. The model was most sensitive to parameters related to juvenile survival (the strength of density dependence on survival and the number of vegetation patches) as well as recruitment to the spawning stock (length at maturity). These parameters often regulate wild fish populations, as density-dependent mortality of juveniles regulates abundance (e.g. Elliott, 1994; Myers and Cadigan, 1993; Rose et al., 2001), whilst length at maturity affects recruitment (Olsen et al., 2004). This consistency between our model and this literature, matched with our use of data from empirical studies to parameterise these aspects of the model suggests that our model reliably captures fundamental aspects of fish population dynamics.

Shortened reproductive seasons resulted in more variable population abundance, higher adult abundances and resulted in annual cohorts similar to those observed in wild zebrafish populations (Spence et al., 2007a). However, zebrafish in reproductive condition have been found throughout the year in Bangladesh (Spence et al., 2007a; pers. obs.), suggesting that reproduction may depend on food rather than time of year. Future research into the role of food availability and energy demands of reproduction may determine the mechanism behind the apparent reproductive season in wild

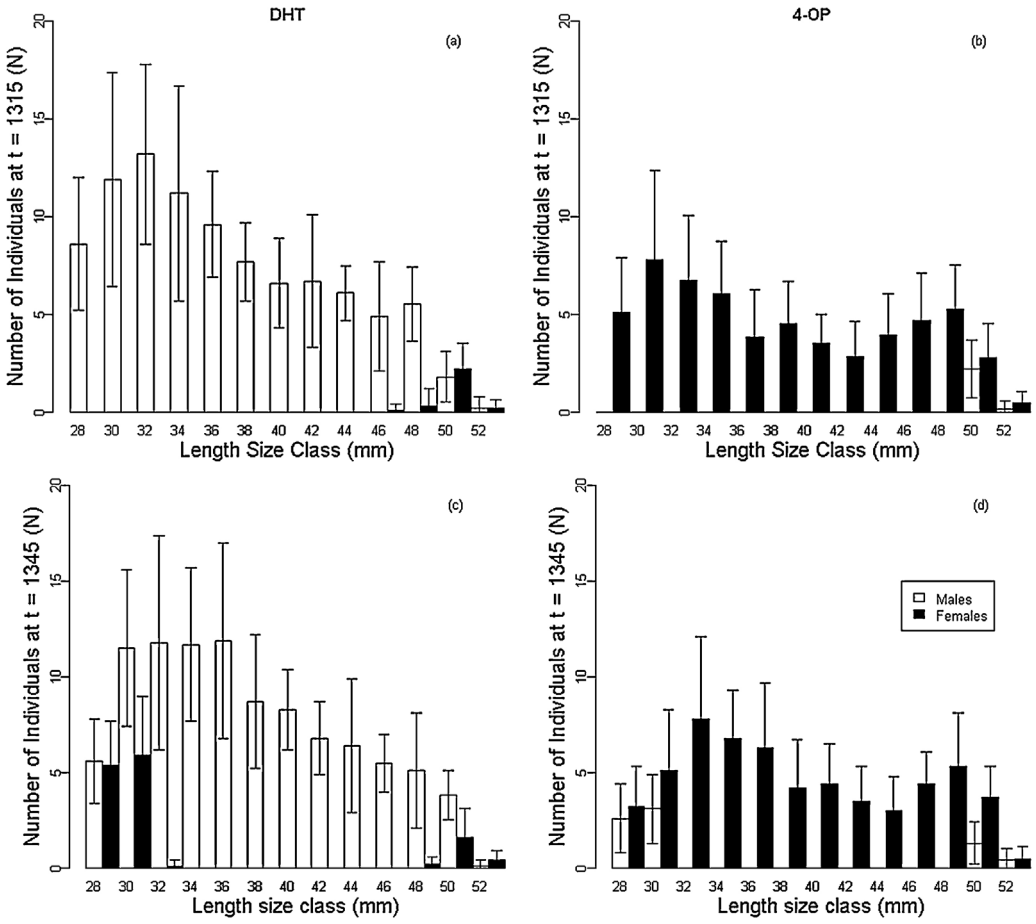

Fig. 6. Mean size and sex distribution of modelled adult zebrafish populations (±s.d.) following toxicant exposure (from 70 replicates). At the beginning of population recovery (time = 1315, a and b) all reproducing females under DHT and 4-OP exposure are more than 46 mm in length (although there are a range of female sizes in the 4-OP exposed population (between 28 mm and 52 mm) only the larger females are reproducing due to the pairwise mating system used in the model, the short inter-spawn intervals for each individual female (maximum 3 days), the fact that larger females outcompete smaller females for breeding grounds and a lack of male mates—see Section 3.2 of the main text for a full discussion on this). After 30 days recovery (time = 1345, c and d), reproducing females following 4-OP exposure are all more than 40 mm in length (once again outcompeting their shorter rivals for limited numbers of male mates) whilst those following DHT exposure are predominantly less than 32 mm in length.

populations. Furthermore, investigating the variable levels of feeding success and energy use between individuals could explain the presence of fecund females outside the breeding season. The higher adult abundances resulting from shortened reproductive seasons could have implications for ERAs as the adult life-stage is often the least sensitive to chemical exposure, especially with endocrine disrupting modes of action (Maack and Segner, 2004), potentially making the population more resistant to chemical exposure. Thus, results from the current model with continuous reproduction and lower average adult abundances may be conservative for certain chemicals.

No population-level effects occurred when spatial aspects of the model (such as fish movement and dominance hierarchies) were switched off, suggesting that the model does not need to be spatially explicit. However, it should be noted, as the distances over which the fish sense and select habitat patches is high compared with individual patch size and the habitat setup is randomised, spatial effects are likely to be small. A more structured spatial setup (e.g. vegetation/breeding grounds at the pond edges and open water in the middle), with larger water bodies and/or a reduced range of sensing may produce stronger spatial effects. Detailed movement and behaviour functions were initially included in the model as previous studies have shown that they can have important influences on population regulation (Railsback and Harvey, 2011; Turchin, 1998). Furthermore, there is growing concern over the effects of EDCs on dominance and mating behaviours as demonstrated in the zebrafish (Coe et al., 2008) and other species (Brian et al., 2006; Zala and Penn, 2004). The process of inclusion and then removal was previously used in the modelling cycle (see Grimm and Railsback, 2005) when investigating the importance of density dependence on fecundity (where the literature showed higher zebrafish stocking densities resulted in lower egg production due to behavioural interference during mating attempts (Spence and Smith, 2005)), which is why it does not feature in the present model. Should the model be adapted in the future to investigate the importance of the flow of genetic material or to investigate how different exposure profiles may result in different population-level responses then these movement behaviours and the spatial setup of the pond may be important and should be maintained.

4.3. Sex ratio results

Laboratory observed sex ratio changes (for empirical data see Supplementary Material) elicited significant population-level responses under chronic, but not acute, exposure. Higher concentrations markedly decreased population abundance, but no population went extinct under any of these scenarios. Laboratory observed individual-level effects were greater than model observed population-level effects (e.g. with an exposure to 4-OP of 200 ng l^{-1}, laboratory studies observed a sex ratio skew to 90% female, though this only resulted in a population decrease of 51%). Male biased sex ratios resulted in greater decreases in population abundances than

female biased sex ratios. During recovery, exposures resulting in strong male or female bias led to a dynamic oscillation in population abundance before returning to pre-exposure levels. This response was strongest under high concentrations of the oestrogenic toxicant (4-OP).

Our results are consistent with the literature, where observational and theoretical studies have found little or no effect of reduced numbers of males on populations (e.g. Dyson and Hurst, 2004; Milner-Gulland and Lhagvasuren, 1998) and mathematical studies showing female bias promotes population growth (Ovidiu Vlad, 1989). In male biased populations, less sexually mature females produce significantly less eggs and density-independent mortality results in low recruitment to maturity. Meanwhile, female biased populations have less males, but the low energy costs of sperm production and multiple mating by low numbers of males can sustain the population. However, whilst this is true with a small female bias in sex ratio in our model, significant changes still occur at higher female bias in sex ratio. The reproductive strategy used by the zebrafish (short-term pair-wise mating) limits the compensation potential of males for significant decreases in male numbers. Therefore, population effects due to exposure from feminising compounds are heavily dependent on the species' breeding strategy.

No population went extinct within these simulations, even though sex ratio was more than 90% skewed in each direction. However, we investigated the population-level impact of chemical-mediated changes in sex ratio in isolation of other sub-lethal endocrine effects, such as direct effects on reproduction. In a life-cycle test with zebrafish, Segner et al. (2003) reported reduced fertilisation success after 78 days of exposure to 4-OP at 28 $\mu g\,l^{-1}$. Another study, reported a reduction in growth, fecundity and fertility with a NOEC (no observed effect concentration) of 12 $\mu g\,l^{-1}$ (Wenzel et al., 2001). These concentrations are lower than those found to have population-level effects through sex ratio skews in this study, meaning that multiple sub-lethal effects may co-occur. Meanwhile other studies have observed changes in sexual behaviour and reproductive success of medaka with NOECs of 10 $\mu g\,l^{-1}$ (Gray et al., 1999) and inhibited development of sperm ducts in sand gobies at concentrations of 28 $\mu g\,l^{-1}$ after six months (Robinson et al., 2004). A recent meta-analysis of 78 studies of induced environmental sex reversal through exposure to EDCs found exposed fish were subject to other EDC-induced fitness-related effects (e.g. reduced growth) that future theoretical studies into sex reversal should take into account (Senior et al., 2012).

Additionally, genetic diversity of populations may be fundamental to their sustainability, especially in changing environments (Brown et al., 2009). In our model we do not explicitly model genetic sex as this aspect of zebrafish development needs further clarification (Liew et al., 2012). However, masculinisation/feminisation of a population (e.g. due to exposure by EDCs) can result in the eradication of certain sex-chromosomes and potentially cause population extinction once the cause of environmental sex reversal is removed (e.g. Cotton and Wedekind, 2008; Hurley et al., 2004; Stelkens and Wedekind, 2010). The development of future models could incorporate sex-determination in species where it is understood.

4.4. Consequences for ERA

There is general concern about the ecological and population-level relevance of individual endpoints commonly studied in laboratory ecotoxicity tests (Forbes et al., 2010; Hommen et al., 2010). Defining the population relevance of endpoints may become important for the criteria being developed by the European Commission to define 'endocrine disrupting properties' in various legislative contexts (Bars et al., 2011, 2012; Wheeler et al., 2012). The endpoint tested in this analysis, sex ratio, shows high individual-level effects have relatively low population-level impact. This result suggests that ERAs based on laboratory collected ecotoxicity data may not capture the true effect of a chemical on wild populations and specifically, the density dependence mechanisms in our model may be of importance in ERAs generally. However, we concede that this analysis was conducted in isolation and contributory factors may alter this conclusion under different situations (i.e. toxicant impacts on reproduction). The OECD thresholds for sex ratio skew for these chemicals are far lower than the levels that caused significant changes in the population in this study and so for sex ratio skews, in isolation, the ERA would be conservative.

5. Conclusions

We have shown how an ecological model can be used to explore the ecological relevance of organism-level ecotoxicological endpoints and thus improve ERAs. Density-dependent survival is identified as having an important role in population regulation, potentially compensating for the effect of negative stressors such as chemical exposure. The model, by itself, does not answer the question which endpoints should be used or prioritised in the future, but it could be used to initiate a process in which accumulating insights into the relation between individual- and population-level endpoints will lead to better, i.e. ecologically more realistic, ERAs.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ecolmodel.2013.12.016.

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