Assessing population impacts of toxicant-induced disruption of breeding behaviours using an individual-based model for the three-spined stickleback.

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Abstract

The effects of toxicant exposure on individuals captured in standard environmental risk assessments (ERA) do not necessarily translate proportionally into effects at the population-level. Population models can incorporate population resilience, physiological susceptibility, and likelihood of exposure, and can therefore be employed to extrapolate from individual- to population-level effects in ERA. Here, we present the development of an individual-based model (IBM) for the three-spined stickleback (*Gasterosteus aculeatus*) and its application in assessing population-level effects of disrupted male breeding behaviour after exposure to the anti-androgenic pesticide, fenitrothion. The stickleback is abundant in marine, brackish, and freshwater systems throughout Europe and their complex breeding strategy makes wild populations potentially vulnerable to the effects of endocrine disrupting chemicals (EDCs). Modelled population dynamics matched those of a UK field population and the IBM is therefore considered to be representative of a natural population. Literature derived dose-response relationships of fenitrothion-induced disruption of male breeding behaviours were applied in the IBM to assess population-level impacts. The modelled population was exposed to fenitrothion under both continuous (worst-case) and intermittent (realistic) exposure patterns and population recovery was assessed. The results suggest that disruption of male breeding behaviours at the individual-level cause impacts on population abundance under both fenitrothion exposure regimes; however, density-dependent processes can compensate for some of these effects, particularly for an intermittent exposure scenario. Our findings further demonstrate the importance of understanding life-history traits, including reproductive strategies and behaviours, and their density-dependence, when assessing the potential population-level risks of EDCs.

Keywords: *endocrine disrupting chemicals, population resilience, density-dependence, exposure regime, fenitrothion, reproductive strategies*

1. Introduction

Many of the ecological factors which affect the susceptibility of wildlife populations to chemicals are considered in current environmental risk assessment (ERA) schemes via the application of arbitrary (and often conservative) assessment factors. Population resilience, which determines whether effects on individuals translate into effects on the population, is generally not considered, despite substantial evidence for density-dependent regulation of population abundance in a range of wildlife species, including fish (Brook and Bradshaw, 2006; Forbes, 2001; Rose et al., 2001). Population models have the potential to help bridge the gap between individual-level endpoints, obtained from traditional regulatory testing, and population effects (Forbes et al., 2009; Hommen et al., 2010; Thorbek et al., 2010) and to support more realistic ERAs. Matrix models are currently the most common method for analysing the effects of toxicant exposure on fish populations (e.g. Brown et al., 2014; Ibrahim et al., 2014; Miller and Ankley, 2004) due to their minimal data requirements, but they have limited ability to incorporate complex behaviours and density-dependent regulation (Caswell, 2001). Individual-based models (IBMs), on the other hand, enable key life-history traits, behaviours and inter-individual-environment interactions, including density-dependent processes, to be modelled explicitly (Grimm and Railsback, 2005). Understanding the mechanisms of density-dependence allows a more in-depth exploration of the limits to population resilience.

Endocrine disrupting chemicals (EDCs) pose a particular challenge in ERA because their effects in fish are often complex and can include subtle behavioural and/or transgenerational effects that have potential for impacting populations (WHO, 2013). The reproductive effects of EDCs on fish are widely reported and they include intersex (the co-occurrence of male and female gonads) (Harris et al., 2011; Jobling et al., 2002; Tetreault et al., 2011) and reduced fecundity (Ankley et al., 2003; Nash et al., 2004; Paulos et al., 2010) and there is evidence that these effects may disrupt whole populations (Jobling et al., 2002; Jobling et al., 1998; Kidd et al., 2007; Schwindt and Winkelman, 2016; Schwindt et al., 2014). More recently, the potential impacts of EDCs on fish behaviours has received increased attention, with reported effects including significant changes to behaviours such as schooling (Ward et al., 2006; Xia et al., 2010), impairment of predation and predator avoidance behaviours (Weis et al., 2001), and alteration of reproductive behaviours (Brian et al., 2006; Dzieweczynski, 2011; Sebire et al., 2008; Sebire et al., 2011). Although these EDC-induced behavioural impairments are likely to have impacts at the population-level, behavioural effects are not currently considered specifically within regulatory standard risk assessment frameworks. Population models provide a tool to potentially capture these effects (Mintram et al., 2017).

Here, we developed an IBM for the resident freshwater form of the three-spined stickleback (*Gasterosteous aculeatus*) and illustrated its application in the assessment of EDC effects on stickleback populations. The three-spined stickleback was chosen as a model species because of its widespread abundance in water bodies across semi-natural and modified agricultural landscapes and it is widely adopted as an experimental model in ecotoxicology and regulation (Katsiadaki et al, 2007). Sticklebacks have a complex breeding strategy that includes courtship, nest-building behaviours and parental care that are controlled by sex hormones and are thus potentially vulnerable to disruption through exposure to EDCs in the natural environment (Sebire et al., 2008; Sebire et al., 2009; Aoki et al, 2011). The model incorporates density-dependent growth, mortality, reproduction and reproductive behaviours (territoriality, courtship and nest guarding) and was parameterised using data available from the literature. As a case study, we simulated the population-level effects of impaired breeding behaviour resulting from exposure to the organophosphate pesticide, fenitrothion. Fenitrothion primarily inhibits acetylcholinesterase and is thus a potent neurotoxin; however also has anti-androgenic effects (European Commission, 2000), including in sticklebacks (Sebire et al., 2009). We simulated fenitrothion exposure under both a ‘worst-case’ chronic (continuous) and a more environmentally relevant pulsed exposure pattern using literature data derived from laboratory tests. We used the stickleback IBM to determine the extent by which individual-level behavioural effects translate into effects at the population-level.

2. Methods

2.1. Models species

The three-spined stickleback (*Gasterosteus aculeatus*) is widespread throughout Europe and other temperate regions across North America, Canada and Asia (Froese and Pauly, 2016; Ostlund-Nilsson et al., 2006; Wootton, 1984). It is one of the most well-studied fish species in ecology and evolution, and is used regularly as a model species in ecotoxicological studies (Katsiadaki et al., 2007). Sticklebacks are generalist feeders (Sánchez-Gonzáles et al., 2001) and display a polygamous mating system, characterised by nest building and guarding by territorial males (Froese and Pauly, 2016; Wootton, 1984). Their lifespan in the wild is usually one year, with the majority of individuals dying after completion of their first breeding season (Allen and Wootton, 1982*b*; Giles, 1987; Wootton et al., 2005). The wealth of ecological and ecotoxicological data sources describing the detailed natural life-history of the stickleback and chemical effects, including on breeding behaviour, make it an ideal species to model for this case study.

2.2. Model description

The model description follows the ODD (Overview, Design Concepts, Details) protocol (Grimm et al., 2006; Grimm et al., 2010). The model was implemented in Netlogo 6.0.1 (Wilensky, 1999) and is available in the Supplementary Information (SI) under the General Public Licence vs 2. The main paper includes the Overview; the Design Concepts and Details sections are presented in the SI.

2.2.1. Purpose

The model was developed to simulate realistic population dynamics of the three-spined stickleback and to provide assessments on the population-level effects of toxicant exposure. Specifically here, the model has been used to explore the compensatory role of density dependence in the resilience of populations under various regimes of exposure to a toxicant that disrupts breeding behaviours via an anti-androgenic mechanism.

2.2.2. Chosen toxicant

Fenitrothion was chosen as the case study toxicant. It is classed as a 'red list' contaminant and discharges are currently controlled by multiple international directives (Connor et al., 2017). Fenitrothion is now prohibited in the EU (EC No 1107/2009) and its use is restricted in Canada (Directorate, 1995); however, it is still used routinely in the USA, Australia and Africa (Paranjape et al., 2014). In this study, fenitrothion was used as the model EDC because it has been shown to disrupt reproductive behaviours in the stickleback (Sebire et al., 2009).

2.2.3. Entities, state variables and scales

The entities in the model are the spatial units (comprising the landscape) and individual fish. The overall environment is additionally characterised by the breeding season (May to July; Wootton et al., 1978).

Spatial units are characterised by the state variables habitat type: open water non-breeding ground, open water breeding ground, vegetated breeding ground; and male ownership: territories (0.063 – 0.54 m^2) acquired by males in the breeding season are exclusive to one male and cannot overlap. The waterbody scales are user-defined, but in the present study the model system represents a pond measuring 20 m^2 (10,000 L) divided into 500 patches, each measuring 20 cm (length) * 20 cm (width) * 50 cm (height). These patch dimensions are representative of the likely short-term territory sizes for non-breeding, resident small fish species. The patches have a fixed location with an explicit set of neighbouring cells. Additional abiotic pond conditions are not modelled explicitly; however, temperature and food availability are implicitly incorporated via seasonal growth (Table. 1, Eq. 4).

Individual fish have four life stages: eggs, larvae, juveniles and adults. All sticklebacks are characterised by the state variables age (days post fertilisation (dpf) for eggs and days post hatch (dph) for the remaining life-stages), body weight (wet weight, g), and position within the pond, and all life-stages excluding eggs are characterised by length (cm, total length from the snout to the tip of the tail).

Juveniles and adults are further characterised by sex (male or female). Adult males possess the state variable breeding status: Boolean; if they establish territories they exhibit nesting behaviour. Additionally, an individual adult male's territory-size (m^2) is determined by total (global) adult male density and the territory-size an individual male holds determines its courtship success probability. Adult females have an inter-spawning interval (days between spawnings; 3 – 9 d), and batch size (eggs per spawning event) which is determined from fish length (cm).

The time step in the model is one day.

2.2.4. *Process overview and scheduling*

Each of the following processes (in bold) will occur over each time step in sequential order. Eggs undertake survival and development; larvae undertake survival, development, and growth; juveniles undertake survival, development, growth, and movement; adult females undertake survival, development, growth, movement, and reproduction; adult males undertake toxicant-effect, survival, development, growth, movement, and reproduction (Fig. 1). Entities are processed in a random sequence and individual fish update their state variables each day.

Update time and landscape: Date, breeding season and habitat patches are updated.

Toxicant-effect: Applying the anti-androgenic toxicant fenitrothion alters the courtship success probability of adult males and the probability that they will build a nest. Toxicant exposure (at the levels simulated here) only affects adult males, due to the specifics of the empirical data used for this test (Sebire et al., 2009). The level of effect depends on the concentration of toxicant (concentration is consistent throughout the pond) and the exposure pattern (see section 2.5).

Survive: An individual's daily mortality rate is determined by four main factors: developmental mortality (eggs only), senescence (adults only), density-dependent cannibalism (eggs only), and a general mortality rate which represents all other sources of mortality (all life-stages excluding eggs)

Age/develop: Fish age and change life stage. Larvae leave the nest when independent feeding begins at 4 dph and are then classified as juveniles. Juveniles develop into adults at the onset of the following annual breeding season.

Grow: Individual growth is dependent upon age, season, and the strength of density-dependent competition from conspecifics. Seasonal growth is an enforced mechanism within the model (Table. 1, Eq. 4) which implicitly incorporates seasonal variations in food and temperature to alter growth rates throughout the year. Female fecundity is directly proportional to body length (Wootton, 1979). Body mass determines survival probability for larvae, juveniles, and adults, and the inter-spawning interval of females. Larger males out-compete smaller males for breeding territories and all males lose body weight when exhibiting nesting behaviour to account for additional metabolic costs.

Move: Individuals move in search of vegetated habitat patches. At the beginning of the breeding season, adult males move to acquire nesting sites and establish territories, whilst adult females move in search of a mate.

Reproduce: Fish reproduce during the breeding season if males establish territories and successfully attract females. Both male courtship behaviour and territory quality are criteria that females use to choose where they deposit their eggs.

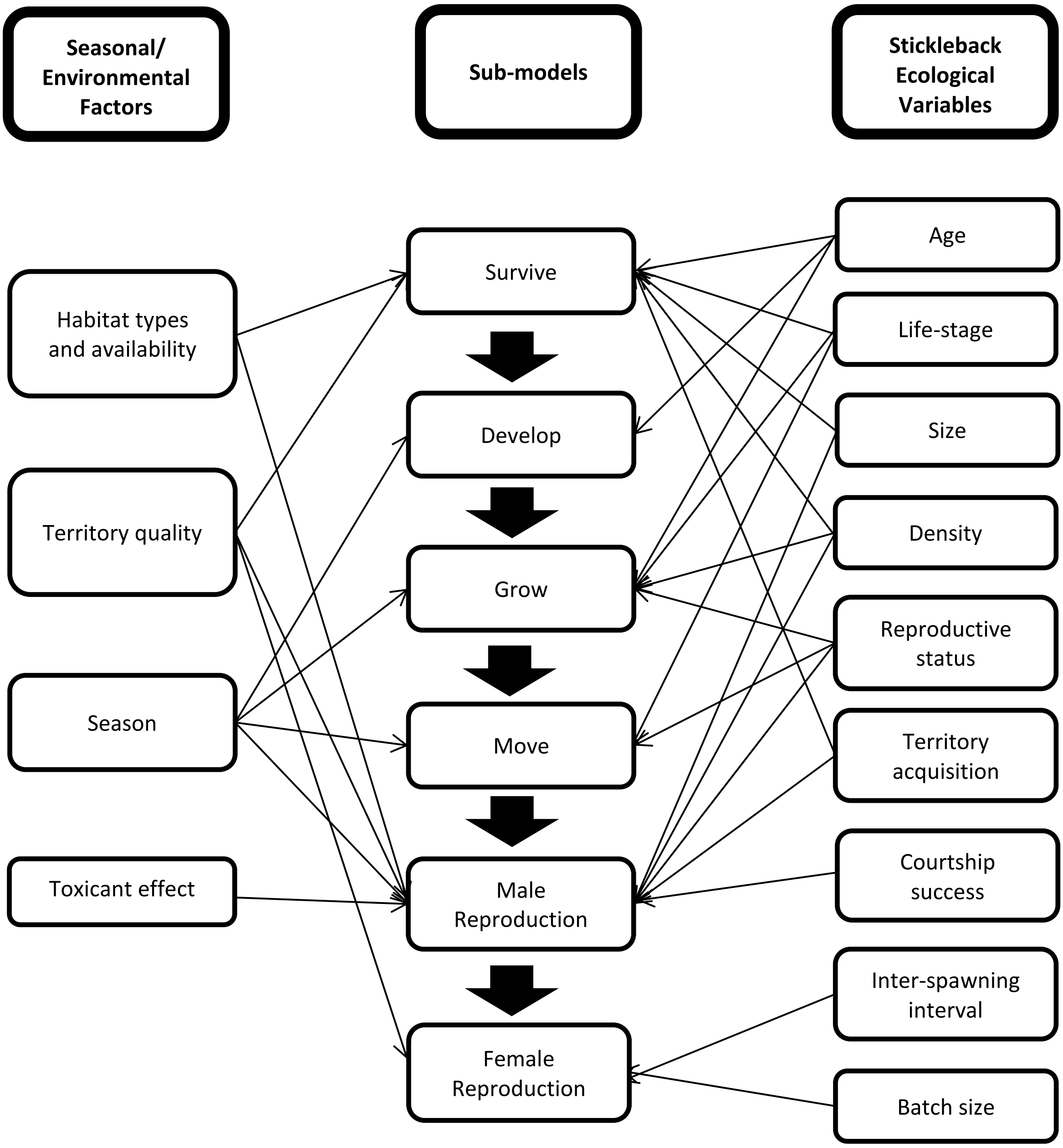

Fig. 1. *Conceptualisation of the key processes (sub-models) undertaken by the stickleback and the ecological and environmental variables which influence them. Small arrows indicate interactions and large arrows indicate the order of processes.*

Table. 1. *Model names, algorithms, parameter values and sources.*

Sub-model	Equation name	Equation	Parameter values	Refs
Toxicant-effect	Eq 1. Fenitrothion dose-response	ND = $\left(\frac{1}{1+e^{a+C\cdot b}}\right)$	ND: Nesting disruption probability C: concentration (µg l^{-1})	Sebire et al. (2009)

	relationship for nest building disruption.		a : ND intercept b : ND gradient a = -0.42 b = 0.40	
	Eq 2. Fenitrothion dose-response relationship for courtship behaviour disruption	CD = $\left(\frac{1}{1+e^{d+C\cdot f}}\right)$	CD: Courtship disruption probability d : CD intercept f : CD gradient d =-1.01 f = 0.36	
Growth	Eq 3. Body length – biomass density relationship	$L_{inf_B} = L_{inf_L} - Gr \cdot B$	L_{inf}_B: Asymptotic length at a given population biomass density (cm) L_{inf_L}: Limiting asymptotic length as biomass density approaches zero (cm) Gr: Strength of density-dependence (cm m^{-2} g^{-1}) B: Population biomass density (g (wet weight) m^{-2}) L_{inf}_L = 5.9 * Gr = 0.1	Lorenzen and Enberg (2002); Cefas Animal Production Unit (APU) data (2013-2015)
	Eq 4. seasonal von Bertallanfy growth function	GR= $L_{inf}\left\{1 - exp^{-\left[\begin{matrix} K(t-t_o)+\left(\frac{C\cdot K}{2\pi}\right)sin2\pi(t-t_s)- \\ \left(\frac{C\cdot K}{2\pi}\right)sin2\pi(t_o-t_s)\end{matrix}\right]}\right\}$	GR : Growth rate (cm day^{-1}) L_{inf}: Asymptotic length (cm) K: Growth constant (cm $year^{-1}$) t: Age (years) t_0: Hypothetical age at which length is equal to zero (years) t_s: Start of the convex segment of a sinusoid oscillation (years) C: Relative amplitude of the seasonal oscillation. K = 1.96 L_{inf} = 6.33 t_0 = -0.02 t_s = -0.042 C =1.30	Somers, (1988); Hoenig and Choudary-Hanumara, (1982); Snyder (1991); Allen and Wootton (1982*b*);Cefas APU data (2013-2015)
	Eq 5. Length:Weight relationship	$W = aL^b$	W: Weight (g) a : Weight constant n: Weight exponent a = 0.0068 b = 3.28	Froese and Pauly (2016)

Reproduction	Eq 6. Territory size (m_2)	If D > 20 fish m^{-2} TS = 0.063 If D < 1.3 fish m^{-2} TS = 0.54 If 20 > adult male density > 1.3 fish m^{-2} $TS = aD^b$	TS : Territory-size (m^2) a : TS constant D: Male density (fish m^{-2}) b : TS exponent a = 0.65 b = -0.80	Van den Assam (1967)
	Eq 7. Courtship success (probability of successfully courting an individual female)	$CS = aLn(TS) + b$	CS : Courtship success probability a : CS constant TS : Territory size (m^2) b : CS intercept a = 0.058 b = 0.90	Van den Assam (1967)
	Eq 8. Reproduction rate (viable eggs $female^{-1}$ day^{-1})	$F = aL^b f$	F : Fecundity (eggs per spawning event) a : Fecundity constant b: Fecundity exponent L : Fish length (cm) f: Fertilisation rate a = 0.82 b = 3.18 f = 0.94	Hagen (1967); Barber and Arnott (2000); Frommen et al. (2008)
	Eq 9. Interspawning interval	If weight >=0.94 ISI = 3 If weight <= 0.49 ISI = 9 if 0.94>weight >0.49 $ISI = aW + b$	ISI : Inter-spawn interval (days) a : ISI constant W : weight (g) b : ISI intercept a = -13.22 b= 15.44	Wootton (1974); Brown-Peterson and Heins, (2009); Wootton et al. (1995)
Survival	Eq 10. Natural mortality	$M_w = M_u W^b$	M_w: Daily natural mortality probability at weight W M_u: Natural mortality probability at unit weight (1 g) W: Weight (g) b: Allometric scaling factor M_u = 0.00781 b = -0.43	Lorenzen (1996) W_u parameter changed from annual, as reported, to daily.
	Eq 11. Egg cannibalism	$EC = aD + b$	EC: Daily egg cannibalism probability a : EC constant D : Global adult and juvenile (fish length >= 1.5 cm) density (fish m^{-2}) b : EC intercept a = 0.0037	Whoriskey and FitzGerald (1985)

			b = -0.0036	

** Adapted to allow for a larger maximum length for German validation data (see section 2.3).*

2.3. Model calibration and validation

The model was calibrated using the growth sub-model. The density-dependent growth algorithm used in the model was taken from Lorenzen and Enberg (2002), and is based on the assumption that as fish density (measured as biomass, g (wet weight) m^{-2}) increases (i.e. approaching carrying capacity), growth rates of juveniles and adults decrease as a result of exploitative competition. There is no wild stickleback population data quantifying density-dependent growth in the wild, therefore the Gr parameter (strength of density-dependence, Table. 1, Eq. 3) was calibrated to provide model outputs of stickleback abundances known to occur in the wild outside of the breeding season (2 – 27 fish m^{-2}, (Krokhin, 1970; Reimchen, 1990; Reimchen, 1994; Wootton and Smith, 2000)) and to produce an adult length of 4.5 cm at the start of the breeding season (Froese and Pauly, 2016, Add-My-Pet, 2014). Calibration was achieved iteratively by visually assessing patterns of population abundances and body lengths.

Model validation was undertaken using stickleback population abundance data from the UK (Wootton, 2007; Wootton et al., 2005) and size distribution data from both the UK (Wootton, 2007) and Germany (Whirzinger et al., 2007). The UK data were derived from wild populations of the resident freshwater form sampled in spring (Feb/March) and autumn (October) from a 200 m^2 river inlet of the River Rheidol (Aberystwyth, UK) between the years of 1972 and 1998. The data from Wirzinger et al. (2007) were size structure data from a stickleback population (unspecified form) in Germany sampled in April and August (2002). Since the field data collected in Germany displayed much larger individuals than the field data collected by Wootton et al. (2007) in the UK, the model could not match the mean fish size for both sets of data. For example, in Germany, the modal fish length was reported to be 4 cm by August compared to the UK population which did not reach 4cm until March. Therefore, for validation against the data collected from Germany, the parameter which determines the absolute maximum length an individual can reach (L_{inf}_L, Table. 1. Eq. 3) was increased in the model to allow fish to grow to a longer length. Validation of the model outputs under default growth settings against the German field data can be found in SI (Fig. A10). The model was allowed to stabilise for 10 years (spin-up) and then data from the subsequent years was used for comparison with the field data. Preliminary analysis had shown that 15 replicate model runs were necessary to get robust means and standard deviation. Replicate number was considered to be robust once the difference in the average and the standard deviation of the population abundance became independent of replicate number (± 5%).

To compare modelled annual population abundances to field data from Wootton et al. (2005) and Wootton (2007) we calculated the total population abundance each year on the 15th of October and 1st March for 21 and 11 years, respectively, to represent the mid values of the field data collection periods. To compare the size distributions, we calculated the proportion of individuals within each size class between 1.8 and 5.8 cm in October and February/March as displayed by (Wootton, 2007), and between 2.5 and 7 cm in April and August as displayed by (Wirzinger et al., 2007) for five years. Modelled size distributions represent the mean frequency of individuals across the whole of each sample period.

2.4. Model sensitivity analysis

A local sensitivity analysis was performed, where parameters from each sub-model formulation were altered by ± 10 %, with the exception of egg and larval development time which was altered by ±1 day. Additionally, the duration of the breeding season in the field is heavily influenced by fluctuations in abiotic conditions (e.g. temperature (Baggerman, 1958; Wootton et al., 1978; Wootton, 1984)) and therefore the sensitivity of the model to breeding season duration was also assessed. A sub-set of parameters (strength of density dependent growth (Gr); percentage of vegetated patches at initialisation; length (cm) of juveniles capable of egg cannibalism), were additionally altered by 25%. The effects of the changed parameters were assessed by comparing the mean population abundance at a single time point over 10 years following a 10 year spin up period.

2.5. Model application: Effects of fenitrothion on stickleback populations

The potential population-level impacts of disrupted male breeding behaviours following exposure to the anti-androgenic pesticide fenitrothion were explored under two exposure scenarios; chronic (continuous exposure for 10 years) and intermittent (a 10 day exposure pulse during the breeding season (10th – 20th June) once a year for 10 years) and included a 10 year recovery period post-exposure. The intermittent exposure scenario is designed to represent a more realistic exposure from agricultural use of fenitrothion (NUFARM, 2013) but is not based on actual empirical or modelled environmental fate data, whilst chronic exposure represents an extreme 'worst-case' scenario. A scenario series with the concentrations used in the empirical laboratory study (0, 1, 50 and 200 µg L^{-1}) (Sebire et al., 2009) was run for both continuous and intermittent exposure to assess the population relevance of the observed individual-level effects.

Effects on individuals were predicted from a concentration-response relationship (Table 1. Eq. 1; Eq. 2) parameterised from published data quantifying disruption to male breeding behaviour (courtship and nest building) after exposure to fenitrothion (Sebire et al., 2009). In the study, the average percentage reduction in nests built by exposed males compared to control males was 25%, 65%, and 85% after exposure to concentrations of 1, 50, and 200 µg L^{-1} fenitrothion, respectively. The average percentage of exposed males which failed to display courtship behaviour compared to control males (specifically leading behaviour; the final stage of the courtship display) after exposure to increasing fenitrothion concentrations (1, 50, and 200 µg L^{-1}) were 60%, 90% and 90%, respectively. In the model, we subtract the level of effect calculated from the concentration-response relationship from the default courtship success probability/nesting probability of each individual male. We assume that if a male does not build a nest or court a female, he will not acquire any eggs.

Population-level effects of fenitrothion were investigated by comparing the mean population abundance of control and exposed populations on January 1st each year, as well as cumulative recruitment to each life stage each year. First, the model was allowed to stabilise for 10 years (spin-up period) followed by an exposure period of 10 years, which again was followed by a recovery period of 10 years, when all input parameters in the model were maintained at their default values. The maximum deviation from the mean control population abundance on January 1st over 200 years following a 10 year spin up period was 15%; thus population level effects were considered relevant if population abundances deviated by > 15% of the mean control value on January 1st. Population abundance was recorded in January because this was the time point where the population was most stable and displayed the least annual variability. The population was considered to have recovered once abundances returned within 15 % of the mean control value. We assumed toxicant effects occurred only during exposure, i.e. once exposure was removed there was immediate organism recovery and no delayed effects. For fenitrothion this is not an entirely unreasonable assumption due to the very low accumulation potential and measured clearance time in fish tissues (Fish bio concentration factor (BCF) = 29 L kg^{-1} ; clearance time (CT_{50}) = 0.19 days) (PPDB 2017).

3. Results

3.1. Validation

3.1.1. Population abundance

The population abundances predicted by the model had a good match to those recorded in the field in both spring and autumn (Fig. 2); thus the average abundances were similar (modelled: 4.0 and 17.2; observed: 4.7 and 13.3 fish m^{-2} in Feb/March and October, respectively). However, the between year variability in the field data was higher than in the model outputs. The mechanism behind this discrepancy was likely due, in part, to the fact that in the field environmental conditions, such as temperature and food availability, varied between years, whereas the model was run with same conditions each year.

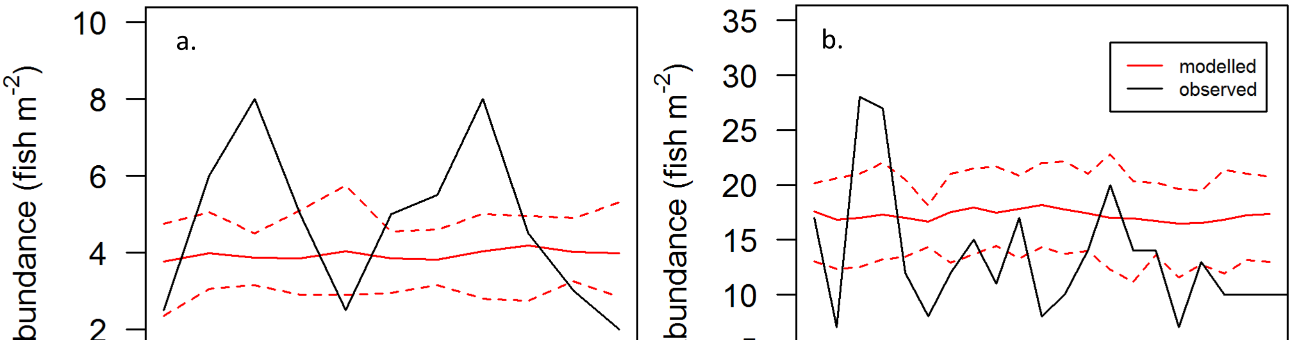

Fig. 2. *Modelled and observed population abundance in Feb/March (a) and October (b) for 11 and 21 years, respectively. Modelled outputs were recorded on 1st March and 15th October and are displayed as the mean abundance of 15 simulations. Dashed lines represent min and max values. Field data was obtained from a demographic study of a UK stickleback population after Wootton et al. (2005) and Wootton (2007). Differences in the number of years sampled between seasons reflect the available data.*

3.1.2. Population size distribution

The size distributions of the modelled simulations generally matched the UK (Wootton, 2007) and the German (Wirzinger et al., 2007) population data well for both seasons, where the parameter which determines maximum length (L_{inf}_L) was increased for the latter (see section 2.3). The model captured the average body lengths along with some of the variation seen in the field populations (Fig. 3). Following the same trends as the field data, modelled growth accelerated in the summer and almost ceased in the autumn and winter as a result of the enforced seasonal growth equation (Table. 1, Eq. 4). Juveniles had their most rapid period of growth in their first 3 – 5 months of life between the breeding season and autumn. In the UK population, individuals grew in body length from 0.45 cm (length at hatch) to 3.6 cm, and grew only an average of 0.4 cm between October and March and this is reflected in the model (Fig 3a, b). In October, the modelled size distribution is more skewed towards smaller individuals, with the modal fish body length representing fish spawned in May (Fig 3b). Further model analysis revealed that this was a result of lower rates of egg cannibalism at the beginning of the breeding season and longer periods of higher growth rates throughout the summer. This size skew was probably more evident in the model outputs than in the field data because annual changes in environmental conditions (e.g. temperature, food availability), which increase variability were not included in the model scenarios. Additionally, the modelled size distribution in April (Fig. 3c) displayed the least variation of all modelled size distributions because individuals were approaching their maximum body length, so the range of body lengths was narrowed at this time point.

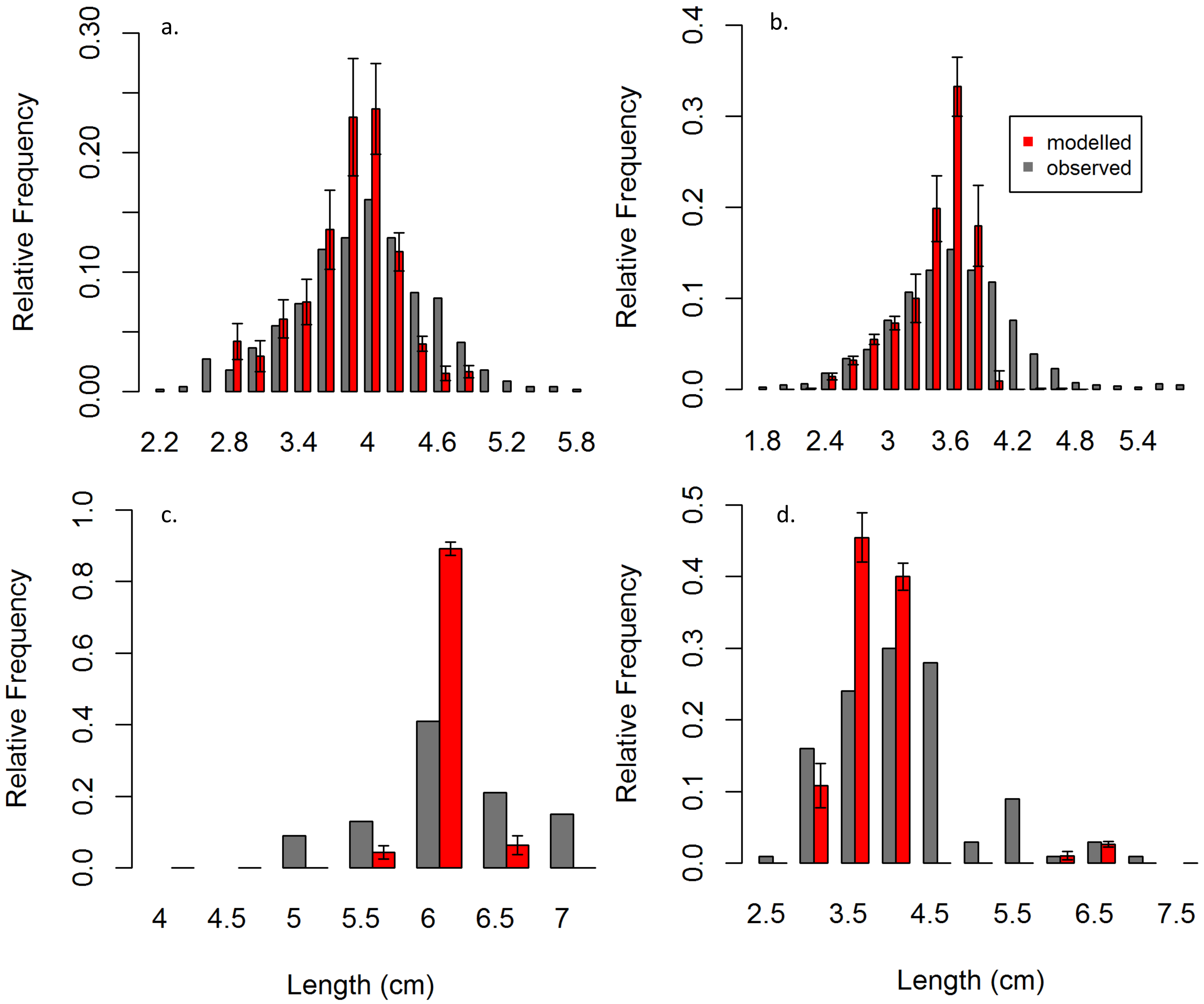

Fig.3. *Modelled and observed size distribution data for stickleback in the UK in March (a) and October (b), and in Germany, with an increased maximum length, in April (c) and August (d). Modelled data represents the mean value of 15 simulations (± s.d).*

3.2. Sensitivity analysis

The model was generally robust to changes (± 10 %) in the majority of input parameter values, and no parameter alterations resulted in a change in the population abundance of more than 10% from control simulations based on default parameter values (Fig. 4).

The model was most sensitive to changes in the duration of the reproductive season and changes to sex ratio. Sticklebacks have a relatively low fecundity and are limited to a three month breeding season in the model; consequently the population has a low buffering capacity to changes in key reproductive parameters, particularly those which directly affect the total number of eggs spawned. Therefore, despite the reduced levels of density dependent competition following lower annual egg recruitment, the breeding strategy of the stickleback did not allow the population to recover fully from a reduction in the duration of the breeding season or a skewed sex ratio. The model was less sensitive to changes in life-stage development time, growth, or other reproduction parameters indicating effective regulation of population numbers via density-dependent growth, competition for mates and nest sites, and survival.

Results of the full local sensitivity analysis can be found in SI (Table A3).

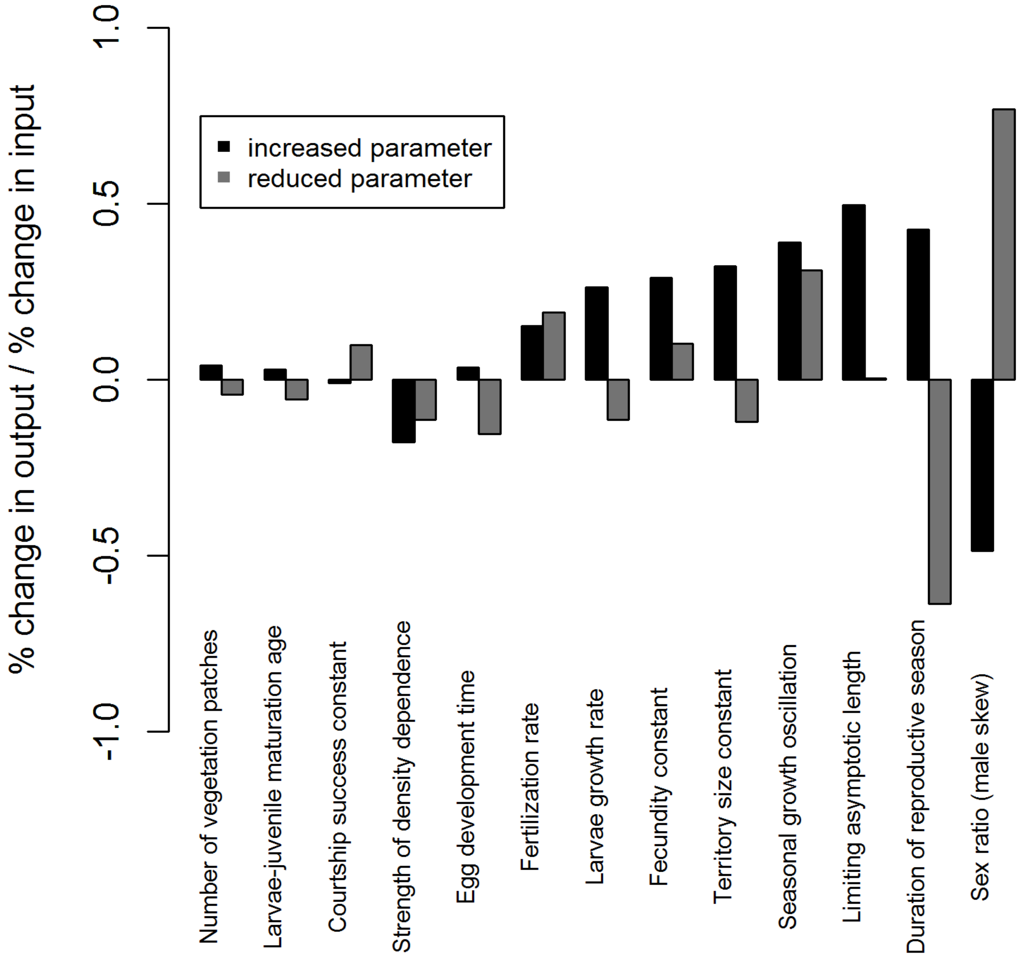

Fig 4. *Local sensitivity analysis of key parameters within the model displayed as the ratio of the percentage change in population abundance and the percentage by which the parameter was increased/decreased (mean value of 15 simulations).*

3.3. Population-level effects of fenitrothion-induced disruption of breeding behaviour

In the model, continuous exposure to fenitrothion affected population abundance at all the simulated concentrations spanning 1 to 200 µg L^{-1}. A concentration of 1 µg L^{-1} fenitrothion caused a maximum reduction from the mean control population abundance of 43% during the 10 year exposure period. However, the population made a full recovery 4 years after exposure ceased. Exposure to 50 µg L^{-1} and 200 µg L^{-1} fenitrothion caused population extinction after 8 and 3 years, respectively (Fig. 5a).

As expected, the effects of intermittent exposure to fenitrothion were much less detrimental to population abundance than the continuous exposure. Thus, populations subject to intermittent exposure showed a maximum reduction from the mean control population abundance of 9%, 16%, and 41% after exposure to concentrations of 1, 50, and 200 µg L^{-1}, respectively. The reduction in population abundance displayed at 1 µg L^{-1} fenitrothion was not considered significant as a reduction of 9% falls within the range of population fluctuations displayed under default settings (±15%). Relevant reductions in population abundance after exposure to 50 µg L^{-1} fenitrothion were transient occurring at years 8 (exposure period) and 11 (recovery period), but population deviations were only 1% outside of the range of control fluctuations. Although the reduction in population abundance occurred during the recovery period; the number of juveniles present in January will still be a reflection of the reduced recruitment caused by fenitrothion exposure in the previous breeding season. After exposure to 200 µg L^{-1} fenitrothion, the populations recovered in the 4th year of the recovery phase (Fig. 5b).

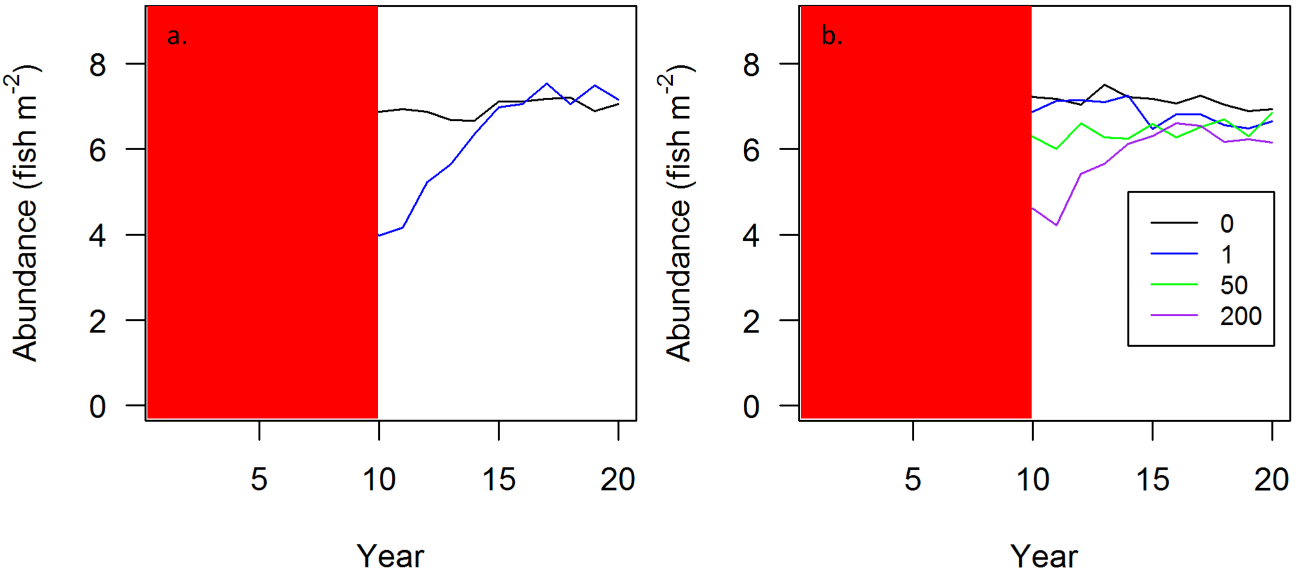

Fig.5. *Mean modelled annual total population abundance (mean value of 15 simulations) on the 1st January each year for (a) continuous and (b) intermittent exposures to fenitrothion. Legend refers to*

fenitrothion concentration (μg L^{-1}). Red and white sections divide exposure and recovery time period: 10 year exposure; 10 year recovery.

The results of the analysis on recruitment to different life stages (data not shown) showed that fenitrothion exposure impacted most on egg numbers and least on adult numbers; for example, intermittent exposure to 1, 50, and 200 μg L^{-1}, respectively caused a maximum annual reduction in total egg abundance of 17, 27, and 69% compared to adults, for which the maximum reduction was 12, 16, and 36%, relative to control abundances. Further analysis of the model revealed that this pattern emerged as a result of density-dependent compensation when population abundances are low, thus, lower densities of larvae resulted in reduced competition for food, faster juvenile growth rates and a consequent reduction in size-dependent mortality, as well as reduced competition for good quality (sheltered) habitat patches where mortality rates were lower and conditions more optimal for nesting males in the breeding season.

In order to assess the role of density-dependent processes at each life stage in the model in more detail, we assessed how the asymptotic length parameter, which determines maximum body length, oscillated with annual changes in population abundances. Asymptotic length is a good measure of the strength of density-dependence because in the model, growth is affected by competition and this is implemented by increasing the asymptotic length as the population biomass of fish decreases (see SI, Details section). Thus, the less biomass in the system, the larger the individuals can grow. Larger individuals are less susceptible to size-dependent mortality (Lorenzen, 1996) and larger females produce more eggs; this mechanism can, to some degree, therefore compensate for low population abundance. Monitoring this parameter also gives an indication of the effects of population abundance on density-dependent life history processes, including growth, mortality (egg cannibalism) and reproduction (competition for territories).

Throughout the exposures, the asymptotic length of the control population remained stable (5.55 – 5.59 cm) but increased with increasing concentration of fenitrothion (Fig. 6). Intermittent exposure to 1, 50 and 200 µg L^{-1} resulted in a maximum mean asymptotic length of 5.59, 5.62, and 5.78 cm, respectively, during the 10 year exposure period. This density-dependent compensation allowed for some population recovery in between annual exposures and resulted in relatively stable populations throughout the exposure period, following an initial decline in abundance, as observed in the case of the two highest exposure concentrations. Comparatively, chronic exposure to 1, 50 and 200 µg L^{-1} caused a maximum asymptotic length of 5.74, 5.89 and 5.89 cm but the extent of the density-dependent compensation in growth was insufficient to prevent populations going extinct.

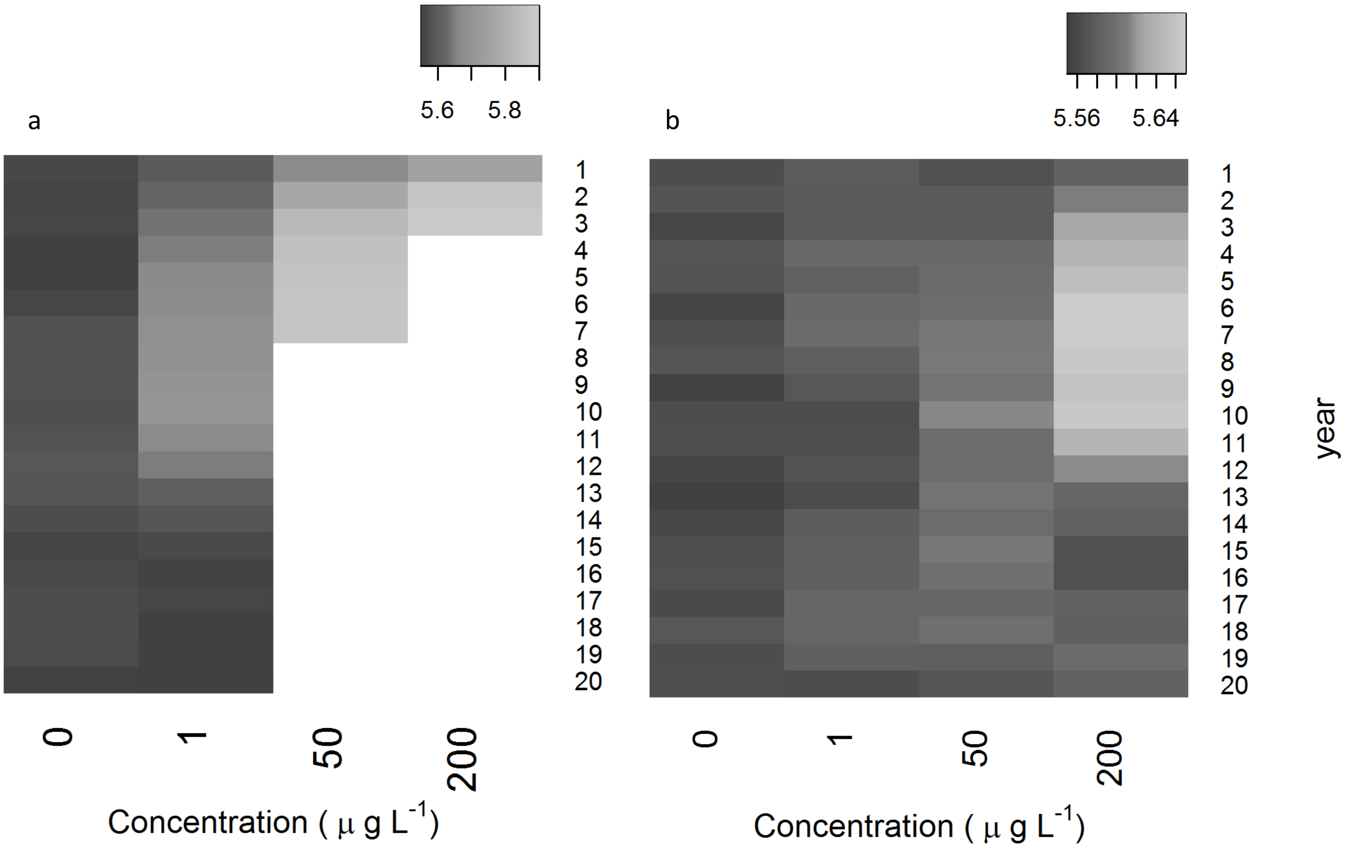

Fig. 6. *Annual fluctuations in asymptotic length (cm) after exposure to 1, 50, and 200 µg* L^{-1} *fenitrothion under an intermittent (a) and a chronic (b) exposure regime (10 year exposure; 10 year recovery). Light grey represents high asymptotic length, dark grey low asymptotic length and white*

space marks population extinction. The colour scale is adapted for each graph and is expressed in the colour keys.

4. Discussion

We developed a stickleback IBM and applied it to a case study to assess the relevance of individual-level chemical endocrine disruption effects on populations. Specifically, we looked at the potential population effects of disrupted breeding behaviour (male nest building and courtship) for intermittent and constant continuous exposures to the anti-androgenic pesticide fenitrothion.

Overall, the stickleback IBM provided a good fit to the available UK stickleback population data, indicating that the model provides a good representation of an extensively monitored natural system. However, the body size discrepancies between wild sticklebacks in the UK and in Germany meant that the model, with current parameterisation, cannot simultaneously provide a good fit for both populations. We chose to use the UK population data as the main body of validation as the data is more extensive than the data generated from the study sites in Germany. The UK data represents up to 21 years of sampling and records both population abundance and size class distributions for resident freshwater stickleback. In contrast, the data from Germany only recorded size class data in a single year and the fish were not accurately aged, meaning that the size distributions could represent a mixture of 0+ and older cohorts. Additionally, the model is representative of the low-plated resident freshwater form of stickleback and it is possible that the data from the German sites, located close to the sea, includes the genetically different anadromous sticklebacks which grow faster and to a larger maximum size (Wootton, 1984; Schluter, 1995).The larger body lengths displayed in the German population may also be a result of an earlier breeding season and/or differences in abiotic parameters such as temperature (Allen and Wootton 1982*b*), photoperiod (Guderley et al., 2001), or food availability (Allen and Wootton 1982*b*). Importantly, however, the model does reflect seasonal differences in growth observed from both sets of population data. The stickleback is a temperate fish species and seasonal fluctuations in temperature and food availability affect growth rates in the wild, resulting in high growth rates in the summer and low growth in the winter (Allen and Wootton 1982*a*, Allen and Wootton 1982*b*). In the model, seasonal growth is one of the key mechanisms driving population dynamics (SI Ap. 12 for more details). We incorporated seasonal growth using an adapted version of the von Bertalanffy equation (where parameters *ts* and *c* enforce seasonal oscillations (Table. 1, Eq. 4)) which predicts temperature-dependent growth accurately according to the UK validation results. A more mechanistic approach to incorporating seasonal growth, such as adding an energy-based element (Martin et al., 2012, Sibly et al., 2013), could better extend the model's application to different latitudes and regions.

The sensitivity analysis revealed that the model was most sensitive to alterations in the duration of the breeding season and the operational sex ratio. Stickleback invest a high proportion of energy into nest guarding and egg brooding by males, and females display relatively low fecundity compared to other fish species whose reproduction requires less investment post spawning (Bone and Moore, 2008). The model's sensitivity to parameters which directly affect egg recruitment is therefore an emergent property of the stickleback's life history strategy. As a comparison, the zebrafish has a high fecundity and in most latitudes may be able to spawn all year round (Spence et al., 2007). A sensitivity analysis of a zebrafish IBM (Hazlerigg et al., 2014) demonstrated that this model species was more resilient to changes in reproductive parameters, such as a reduction in the duration of the breeding season, than the stickleback IBM. . The comparison between these models demonstrates how life history strategy can pre-determine the capacity for population resilience and therefore the choice of focal species for risk assessment needs to be carefully considered, if these factors are to be taken into account.

It is well documented that different EDCs can induce different types of physiological effects on individual fish (e.g. masculinisation and reduced fecundity from androgens (Morthorst et al., 2010); feminisation and reduced fecundity from anti-androgens (Jensen et al., 2004) and oestrogens (Nash et al., 2004); impaired growth rates from thyroid disruptors (Liu et al., 2008)) and behavioural effects have also been widely reported (Dzieweczynski, 2011; Dzieweczynski et al., 2014; Ward et al., 2006; Weis et al., 2001; Xia et al., 2010). There are, however, very few examples of studies on population level-effects of EDCs in fish. A notable example is in the work by Kidd et al. (2007), where a whole Canadian lake (Lake 260) was treated with ethinylestradiol (EE_2) at concentrations between 5-6 ng L^{-1} for a period of 3 years which resulted in the feminization of male fathead minnows and the collapse of the fathead minnow population (Kidd et al., 2007). Breeding behaviours in the fathead minnow have been shown to be disrupted after laboratory exposures to the same concentrations (Majewski et al., 2002), and this may have contributed to the subsequent population crash. The breeding strategy of the fathead minnow is similar to that of the stickleback (e.g. nest guarding by males), and the population-level impacts of disruption to the same behaviours would therefore be expected to be similar for both species. In addition to the physiological and behavioural effects of EDCs, indirect effects may occur via the disruption of food web interactions. These interactions are particularly relevant for pesticide risk assessment since these chemicals target invertebrates and are therefore likely to have adverse effects on the prey of fish species. Specifically, fenitrothion is highly toxic to aquatic invertebrates (PPDB, 2017) and effects have been reported on species which contribute to the diet of the stickleback (Fairchild and Eidt, 1993; Choi et al., 2002). Since the current study aims to predict the effects of a single behavioural endpoint on population abundance, food-web interactions are not considered here. However, disruption of invertebrate communities would likely compromise some of the density dependent compensation observed in the model following fenitrothion exposure.

Using the stickleback IBM, we showed that exposure duration, as well as exposure concentration, affected population responses and effect levels were markedly greater in populations subjected to a continuous chronic exposure regime compared to a more realistic intermittent regime. For example, concentrations that only caused negligible effects under pulsed exposure scenarios caused marked decreases or even extinction in continuous exposure scenarios. This is consistent with an empirical study, whereby compensatory responses allowed a fathead minnow population to recover following pulsed exposure to toxicants (Ali et al., 2017). Further analysis of the model (e.g. tracking changes to density dependent parameters) revealed that the compensatory capacity for the investigated effects was driven by density-dependent competition for resources leading to increased growth and survival in early life stages and increased availability of spawning territories for adults. In particular we assessed the extent to which growth (asymptotic length - L_{inf}) was affected by density. In the continuous exposure scenario, the capacity for L_{inf} to increase and compensate population biomass was exhausted at 50μ L^{-1} fenitrothion, whereas for the pulsed exposure that did not occur even at a fenitrothion concentration of 200μg L^{-1}. The compensatory effects of density-dependence also vary between different life-stages. For example, effects were consistently greater for eggs and larvae than for juveniles and adults, and this was particularly evident at the highest exposure concentration. Studies on invertebrates have demonstrated that exposing resource limited populations to toxicants can reduce intra-specific competition and therefore lessen the negative effects of the toxicant (Liess, 2002; Moe et al., 2002). However, empirical studies which validate the interaction between resource competition and chemical effects in fish are limited and it is therefore, as yet, difficult to confirm the realism of these modelled results. In addition, since the current model assumes constant environmental conditions (e.g. food availability and temperature), the exposure scenarios simulated here do not consider potential interactive effects that may be associated with extremes in environmental conditions and this should be taken into consideration when interpreting these results. The capacity for compensation in natural systems generally is greatest when a population is close to carrying capacity and populations can recover faster in systems with rich resources (Beverton and Holt, 1957). As a consequence the effect of chemical exposure should be seen in the context of resource availability and considered together with other stressors. Moreover, both stressors and resources fluctuate seasonally, so it is important to understand the environmental context and life history strategies of focal species when extrapolating in risk assessment. With the current level of detail, the stickleback IBM has proved to be useful in risk assessments for assessing the population-level consequences of individual-level endpoints relating to behaviour, growth, survival, and reproduction.

Conclusions

Ultimately, for EDC induced behavioural effects to have a population level impact, they will need to impair growth, reproduction, dispersal and/or survival. The extent to which such effects translate into population level effects depends on exposure concentration, duration and timing of the toxic effects as well as on life-history strategies contributing to the resilience of the population.

Using the stickleback IBM, we showed that under a semi-realistic exposure regime (pulsed exposure) the individual-level behavioural effects of fenitrothion exposure are greater than effects on whole populations, because of the buffering capacity of ecological processes, such as density dependence. Mechanistic effect models, like the IBM used here, can incorporate more relevant endpoints based on the life-history strategy of the species, population-level interactions, and the likely exposure regime of the chemical. Therefore such models can be applied to help inform our understanding of what level of EDC or other chemical effects on individuals are likely to be ecologically relevant at the population level.

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