

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

3 Kate S. Mintram¹, A. Ross Brown¹, Samuel K. Maynard², Chun Liu³, Sarah-Jane Parker⁴, Charles R.
4 Tyler¹, Pernille Thorbek^{2*}

5 ¹ *College of Life and Environmental Sciences, University of Exeter, Exeter, UK, EX4 4QD*

6 ² *Syngenta, Jealott's Hill International Research Centre, Bracknell, Berkshire, UK, RG42 6EX*

7 ³ *Herbicide Bioscience, Syngenta Ltd, Jealott's Hill International Research Centre, Bracknell,*
8 *RG42 6EY, UK*

9 ⁴ *Centre for Environment, Fisheries and Aquaculture Science, Barrack Road, Weymouth DT4*
10 *8UB*

11 **Corresponding author*

12 **Author details:**

13 Kate S. Mintram km488@exeter.ac.uk

14 A. Ross Brown ross.brown@exeter.ac.uk

15 Samuel K. Maynard samuel.maynard@astrazeneca.com

16 Chun Liu chun.liu@syngenta.com

17 Sarah-Jane Parker sarah-jane.parker@cefas.com

18 Charles R. Tyler c.r.tyler@exeter.ac.uk

19 **Pernille Thorbek* pernille.thorbek@syngenta.com

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23 **Abstract**

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

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

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48 **1. Introduction**

49 Many of the ecological factors which affect the susceptibility of wildlife populations to chemicals are
50 considered in current environmental risk assessment (ERA) schemes via the application of arbitrary
51 (and often conservative) assessment factors. Population resilience, which determines whether effects
52 on individuals translate into effects on the population, is generally not considered, despite substantial
53 evidence for density-dependent regulation of population abundance in a range of wildlife species,
54 including fish (Brook and Bradshaw, 2006; Forbes, 2001; Rose et al., 2001). Population models have
55 the potential to help bridge the gap between individual-level endpoints, obtained from traditional
56 regulatory testing, and population effects (Forbes et al., 2009; Hommen et al., 2010; Thorbek et al.,
57 2010) and to support more realistic ERAs. Matrix models are currently the most common method for
58 analysing the effects of toxicant exposure on fish populations (e.g. Brown et al., 2014; Ibrahim et al.,
59 2014; Miller and Ankley, 2004) due to their minimal data requirements, but they have limited ability
60 to incorporate complex behaviours and density-dependent regulation (Caswell, 2001). Individual-
61 based models (IBMs), on the other hand, enable key life-history traits, behaviours and inter-individual-
62 environment interactions, including density-dependent processes, to be modelled explicitly (Grimm
63 and Railsback, 2005). Understanding the mechanisms of density-dependence allows a more in-depth
64 exploration of the limits to population resilience.

65 Endocrine disrupting chemicals (EDCs) pose a particular challenge in ERA because their effects in fish
66 are often complex and can include subtle behavioural and/or transgenerational effects that have
67 potential for impacting populations (WHO, 2013). The reproductive effects of EDCs on fish are widely
68 reported and they include intersex (the co-occurrence of male and female gonads) (Harris et al., 2011;
69 Jobling et al., 2002; Tetreault et al., 2011) and reduced fecundity (Ankley et al., 2003; Nash et al., 2004;
70 Paulos et al., 2010) and there is evidence that these effects may disrupt whole populations (Jobling et
71 al., 2002; Jobling et al., 1998; Kidd et al., 2007; Schwindt and Winkelmann, 2016; Schwindt et al., 2014).
72 More recently, the potential impacts of EDCs on fish behaviours has received increased attention, with

73 reported effects including significant changes to behaviours such as schooling (Ward et al., 2006; Xia
74 et al., 2010), impairment of predation and predator avoidance behaviours (Weis et al., 2001), and
75 alteration of reproductive behaviours (Brian et al., 2006; Dzieweczynski, 2011; Sebire et al., 2008;
76 Sebire et al., 2011). Although these EDC-induced behavioural impairments are likely to have impacts
77 at the population-level, behavioural effects are not currently considered specifically within regulatory
78 standard risk assessment frameworks. Population models provide a tool to potentially capture these
79 effects (Mintram et al., 2017).

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

98 **2. Methods**

99 *2.1. Models species*

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

111 *2.2. Model description*

112 The model description follows the ODD (Overview, Design Concepts, Details) protocol (Grimm et al.,
113 2006; Grimm et al., 2010). The model was implemented in Netlogo 6.0.1 (Wilensky, 1999) and is
114 available in the Supplementary Information (SI) under the General Public Licence vs 2. The main paper
115 includes the Overview; the Design Concepts and Details sections are presented in the SI.

116 *2.2.1. Purpose*

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

122 2.2.2. *Chosen toxicant*

123 Fenitrothion was chosen as the case study toxicant. It is classed as a 'red list' contaminant and
124 discharges are currently controlled by multiple international directives (Connor et al., 2017).
125 Fenitrothion is now prohibited in the EU (EC No 1107/2009) and its use is restricted in Canada
126 (Directorate, 1995); however, it is still used routinely in the USA, Australia and Africa (Paranjape et al.,
127 2014). In this study, fenitrothion was used as the model EDC because it has been shown to disrupt
128 reproductive behaviours in the stickleback (Sebire et al., 2009).

129 2.2.3. *Entities, state variables and scales*

130 The entities in the model are the spatial units (comprising the landscape) and individual fish. The
131 overall environment is additionally characterised by the breeding season (May to July; Wootton et al.,
132 1978).

133 Spatial units are characterised by the state variables habitat type: open water non-breeding ground,
134 open water breeding ground, vegetated breeding ground; and male ownership: territories (0.063 –
135 0.54 m²) acquired by males in the breeding season are exclusive to one male and cannot overlap. The
136 waterbody scales are user-defined, but in the present study the model system represents a pond
137 measuring 20 m² (10,000 L) divided into 500 patches, each measuring 20 cm (length) * 20 cm (width)
138 * 50 cm (height). These patch dimensions are representative of the likely short-term territory sizes for
139 non-breeding, resident small fish species. The patches have a fixed location with an explicit set of
140 neighbouring cells. Additional abiotic pond conditions are not modelled explicitly; however,
141 temperature and food availability are implicitly incorporated via seasonal growth (Table. 1, Eq. 4).

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

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

152 The time step in the model is one day.

153 **2.2.4.** *Process overview and scheduling*

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

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

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

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

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

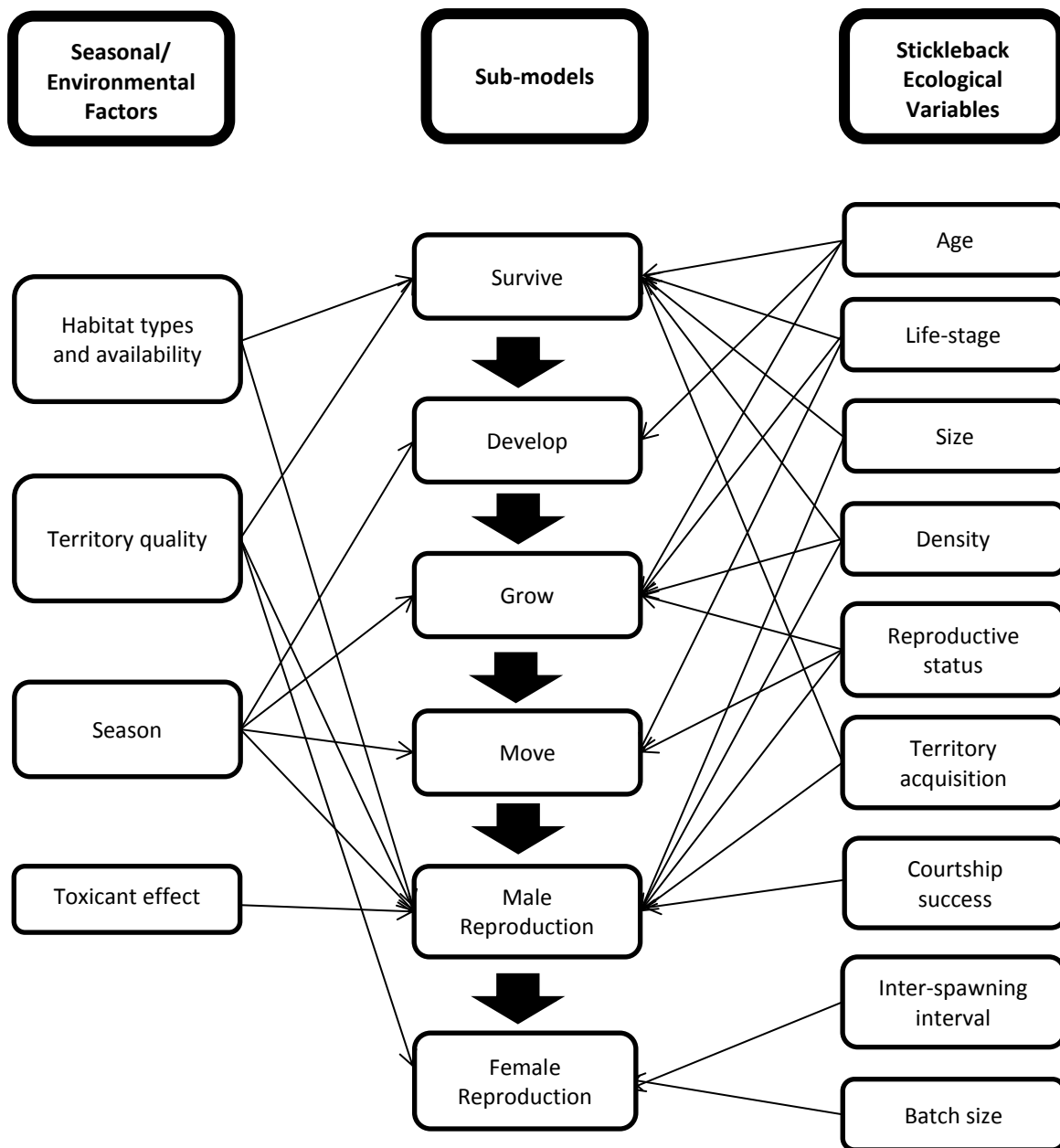
172 **Grow:** Individual growth is dependent upon age, season, and the strength of density-dependent
173 competition from conspecifics. Seasonal growth is an enforced mechanism within the model (Table.
174 1, Eq. 4) which implicitly incorporates seasonal variations in food and temperature to alter growth
175 rates throughout the year. Female fecundity is directly proportional to body length (Wootton, 1979).
176 Body mass determines survival probability for larvae, juveniles, and adults, and the inter-spawning
177 interval of females. Larger males out-compete smaller males for breeding territories and all males lose
178 body weight when exhibiting nesting behaviour to account for additional metabolic costs.

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

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

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

191 **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 ($\mu\text{g l}^{-1}$)	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 Bertalanffy growth function	GR= $L_{inf} \left\{ 1 - \exp \left[\frac{K(t-t_0) + \left(\frac{C \cdot K}{2\pi}\right) \sin 2\pi(t-t_s)}{\left(\frac{C \cdot K}{2\pi}\right) \sin 2\pi(t_0-t_s)} \right] \right\}$	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 (1982b); 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)	<p>If $D > 20$ fish m⁻² $TS = 0.063$</p> <p>If $D < 1.3$ fish m⁻² $TS = 0.54$</p> <p>If $20 > \text{adult male density} > 1.3$ fish m⁻²</p> $TS = aD^b$	<p>TS : Territory-size (m²) a : TS constant D: Male density (fish m⁻²) b : TS exponent</p> <p>a = 0.65 b = -0.80</p>	Van den Assam (1967)
	Eq 7. Courtship success (probability of successfully courting an individual female)	$CS = aLn(TS) + b$	<p>CS : Courtship success probability a : CS constant TS : Territory size (m²) b : CS intercept</p> <p>a = 0.058 b = 0.90</p>	Van den Assam (1967)
	Eq 8. Reproduction rate (viable eggs female ⁻¹ day ⁻¹)	$F = aL^b f$	<p>F : Fecundity (eggs per spawning event) a : Fecundity constant b: Fecundity exponent L : Fish length (cm) f: Fertilisation rate</p> <p>a = 0.82 b = 3.18 f = 0.94</p>	Hagen (1967); Barber and Arnott (2000); Frommen et al. (2008)
	Eq 9. Interspawning interval	<p>If weight ≥ 0.94 ISI = 3</p> <p>If weight ≤ 0.49 ISI = 9</p> <p>if $0.94 > \text{weight} > 0.49$</p> $ISI = aW + b$	<p>ISI : Inter-spawn interval (days) a : ISI constant W : weight (g) b : ISI intercept</p> <p>a = -13.22 b = 15.44</p>	Wootton (1974); Brown-Peterson and Heins, (2009); Wootton et al. (1995)
Survival	Eq 10. Natural mortality	$M_w = M_u W^b$	<p>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</p> <p>M_u = 0.00781 b = -0.43</p>	Lorenzen (1996) W _u parameter changed from annual, as reported, to daily.
	Eq 11. Egg cannibalism	$EC = aD + b$	<p>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</p> <p>a = 0.0037</p>	Whoriskey and FitzGerald (1985)

			b = -0.0036	
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192 * Adapted to allow for a larger maximum length for German validation data (see section 2.3).

193 2.3. Model calibration and validation

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

205 Model validation was undertaken using stickleback population abundance data from the UK
206 (Wootton, 2007; Wootton et al., 2005) and size distribution data from both the UK (Wootton, 2007)
207 and Germany (Whirzinger et al., 2007). The UK data were derived from wild populations of the
208 resident freshwater form sampled in spring (Feb/March) and autumn (October) from a 200 m² river
209 inlet of the River Rheidol (Aberystwyth, UK) between the years of 1972 and 1998. The data from
210 Wirzinger et al. (2007) were size structure data from a stickleback population (unspecified form) in
211 Germany sampled in April and August (2002). Since the field data collected in Germany displayed
212 much larger individuals than the field data collected by Wootton et al. (2007) in the UK, the model
213 could not match the mean fish size for both sets of data. For example, in Germany, the modal fish
214 length was reported to be 4 cm by August compared to the UK population which did not reach 4cm
215 until March. Therefore, for validation against the data collected from Germany, the parameter which
216 determines the absolute maximum length an individual can reach (L_{inf_L} , Table. 1. Eq. 3) was increased

217 in the model to allow fish to grow to a longer length. Validation of the model outputs under default
218 growth settings against the German field data can be found in SI (Fig. A10). The model was allowed to
219 stabilise for 10 years (spin-up) and then data from the subsequent years was used for comparison with
220 the field data. Preliminary analysis had shown that 15 replicate model runs were necessary to get
221 robust means and standard deviation. Replicate number was considered to be robust once the
222 difference in the average and the standard deviation of the population abundance became
223 independent of replicate number ($\pm 5\%$).

224 To compare modelled annual population abundances to field data from Wootton et al. (2005) and
225 Wootton (2007) we calculated the total population abundance each year on the 15th of October and
226 1st March for 21 and 11 years, respectively, to represent the mid values of the field data collection
227 periods. To compare the size distributions, we calculated the proportion of individuals within each size
228 class between 1.8 and 5.8 cm in October and February/March as displayed by (Wootton, 2007), and
229 between 2.5 and 7 cm in April and August as displayed by (Wirzinger et al., 2007) for five years.
230 Modelled size distributions represent the mean frequency of individuals across the whole of each
231 sample period.

232 *2.4. Model sensitivity analysis*

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

242 *2.5. Model application: Effects of fenitrothion on stickleback populations*

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

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254 Effects on individuals were predicted from a concentration-response relationship (Table 1. Eq. 1; Eq.
255 2) parameterised from published data quantifying disruption to male breeding behaviour (courtship
256 and nest building) after exposure to fenitrothion (Sebire et al., 2009). In the study, the average
257 percentage reduction in nests built by exposed males compared to control males was 25%, 65%, and
258 85% after exposure to concentrations of 1, 50, and 200 µg L⁻¹ fenitrothion, respectively. The average
259 percentage of exposed males which failed to display courtship behaviour compared to control males
260 (specifically leading behaviour; the final stage of the courtship display) after exposure to increasing
261 fenitrothion concentrations (1, 50, and 200 µg L⁻¹) were 60%, 90% and 90%, respectively. In the model,
262 we subtract the level of effect calculated from the concentration-response relationship from the
263 default courtship success probability/nesting probability of each individual male. We assume that if a
264 male does not build a nest or court a female, he will not acquire any eggs.

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266 Population-level effects of fenitrothion were investigated by comparing the mean population
267 abundance of control and exposed populations on January 1st each year, as well as cumulative

268 recruitment to each life stage each year. First, the model was allowed to stabilise for 10 years (spin-
269 up period) followed by an exposure period of 10 years, which again was followed by a recovery period
270 of 10 years, when all input parameters in the model were maintained at their default values. The
271 maximum deviation from the mean control population abundance on January 1st over 200 years
272 following a 10 year spin up period was 15%; thus population level effects were considered relevant if
273 population abundances deviated by > 15% of the mean control value on January 1st. Population
274 abundance was recorded in January because this was the time point where the population was most
275 stable and displayed the least annual variability. The population was considered to have recovered
276 once abundances returned within 15 % of the mean control value. We assumed toxicant effects
277 occurred only during exposure, i.e. once exposure was removed there was immediate organism
278 recovery and no delayed effects. For fenitrothion this is not an entirely unreasonable assumption due
279 to the very low accumulation potential and measured clearance time in fish tissues (Fish bio
280 concentration factor (BCF) = 29 L kg⁻¹ ; clearance time (CT₅₀) = 0.19 days) (PPDB 2017).

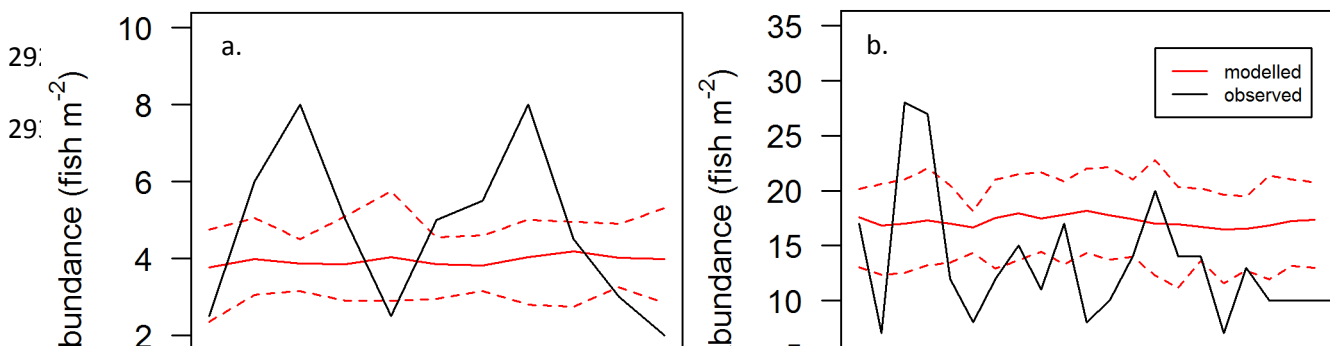
281 3. Results

282 3.1. Validation

283 3.1.1. Population abundance

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

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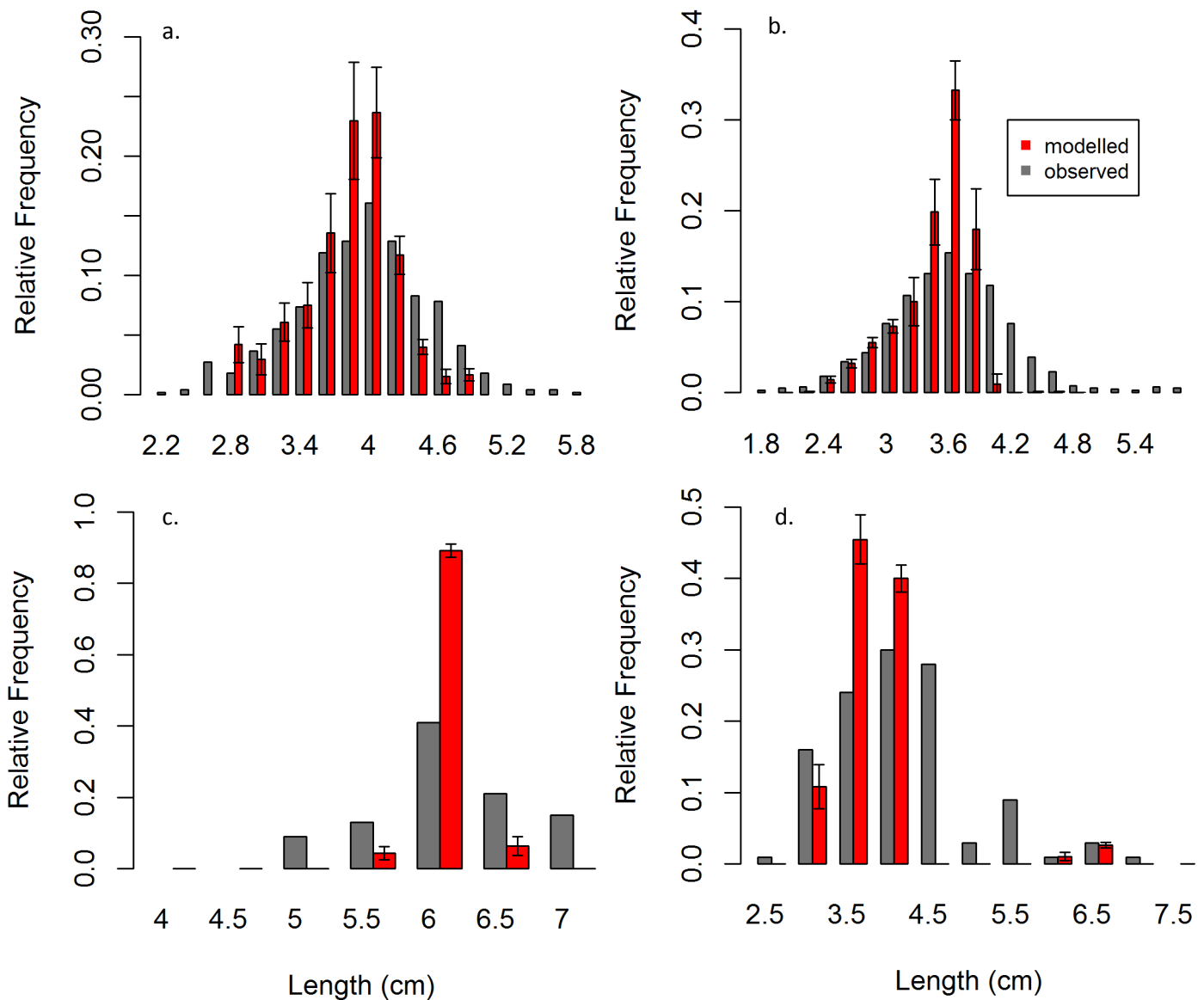
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Fig. 2. *Modelled and observed population abundance in Feb/March (a) and October (b) for 11 and 21 years, respectively. Modelled outputs were recorded on 1st March and 15th October and are displayed as the mean abundance of 15 simulations. Dashed lines 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

320 included in the model scenarios. Additionally, the modelled size distribution in April (Fig. 3c) displayed
 321 the least variation of all modelled size distributions because individuals were approaching their



322 maximum body length, so the range of body lengths was narrowed at this time point.

323 **Fig.3.** Modelled and observed size distribution data for stickleback in the UK in March (a) and
 324 October (b), and in Germany, with an increased maximum length, in April (c) and August (d).

325 Modelled data represents the mean value of 15 simulations (\pm s.d).

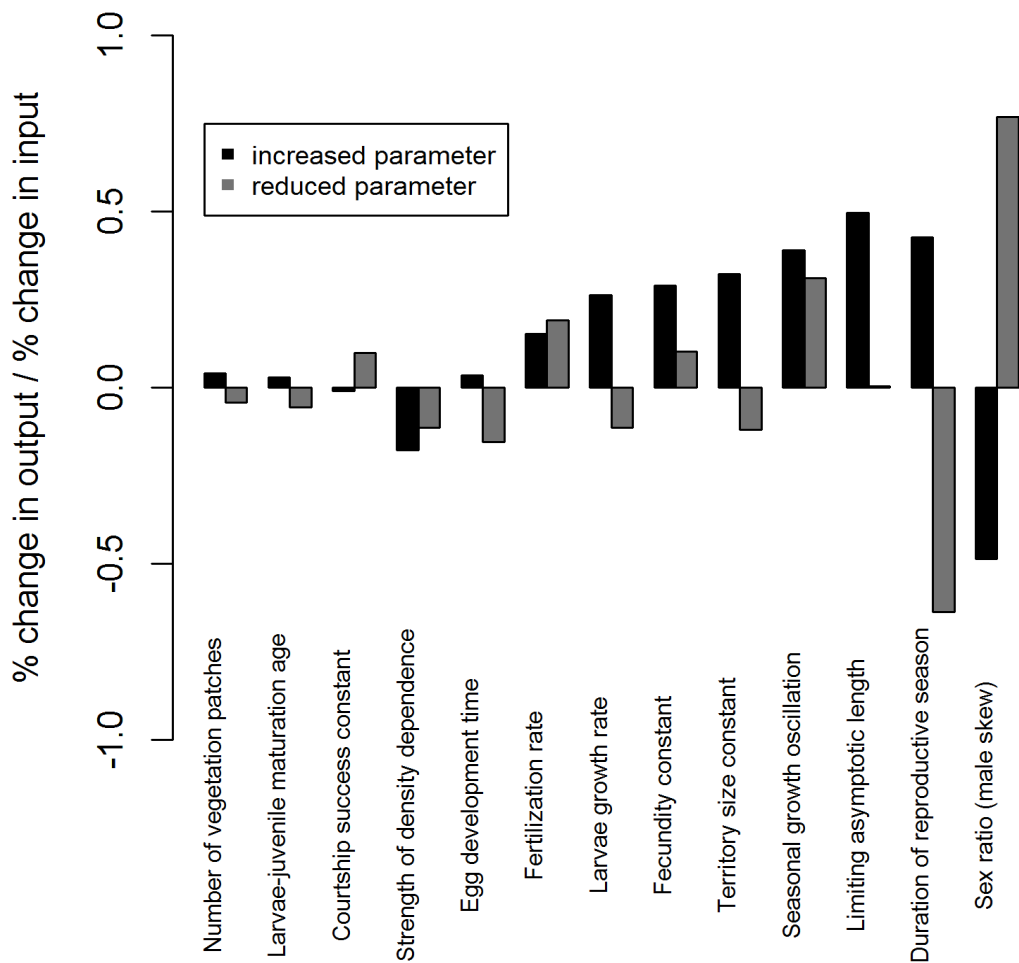
326 3.2. Sensitivity analysis

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

330

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

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



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

345

346 3.3. Population-level effects of fenitrothion-induced disruption of breeding behaviour

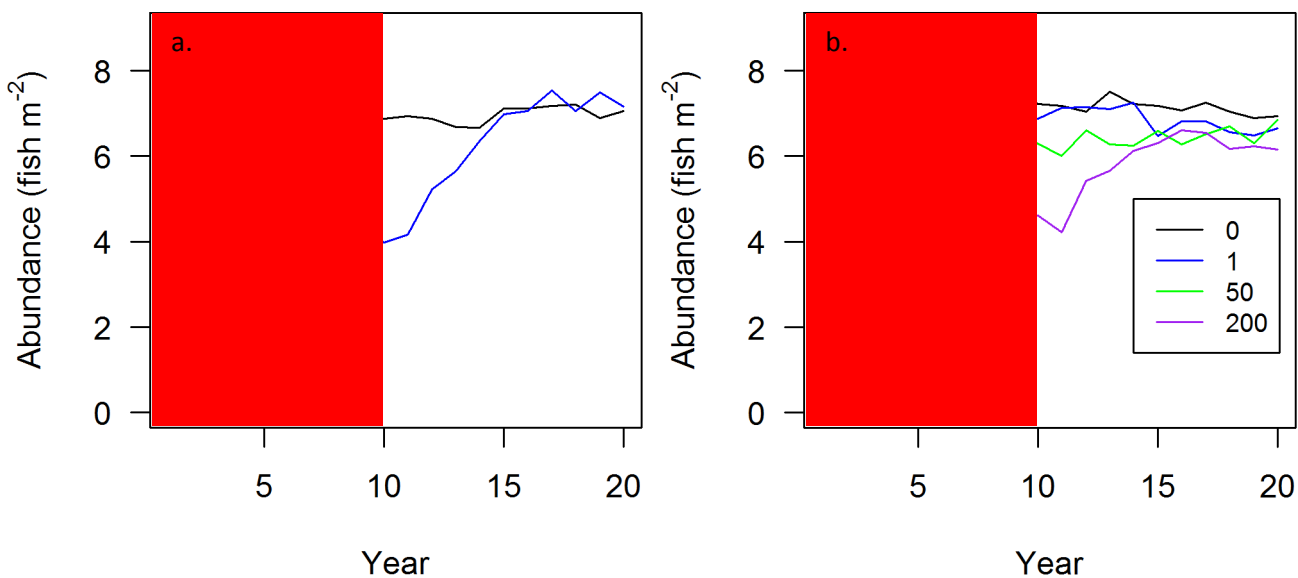
347 In the model, continuous exposure to fenitrothion affected population abundance at all the simulated
348 concentrations spanning 1 to 200 $\mu\text{g L}^{-1}$. A concentration of 1 $\mu\text{g L}^{-1}$ fenitrothion caused a maximum
349 reduction from the mean control population abundance of 43% during the 10 year exposure period.

350 However, the population made a full recovery 4 years after exposure ceased. Exposure to 50 $\mu\text{g L}^{-1}$
351 and 200 $\mu\text{g L}^{-1}$ fenitrothion caused population extinction after 8 and 3 years, respectively (Fig. 5a).

352

353 As expected, the effects of intermittent exposure to fenitrothion were much less detrimental to
354 population abundance than the continuous exposure. Thus, populations subject to intermittent
355 exposure showed a maximum reduction from the mean control population abundance of 9%, 16%,
356 and 41% after exposure to concentrations of 1, 50, and 200 $\mu\text{g L}^{-1}$, respectively. The reduction in
357 population abundance displayed at 1 $\mu\text{g L}^{-1}$ fenitrothion was not considered significant as a reduction
358 of 9% falls within the range of population fluctuations displayed under default settings ($\pm 15\%$).
359 Relevant reductions in population abundance after exposure to 50 $\mu\text{g L}^{-1}$ fenitrothion were transient
360 occurring at years 8 (exposure period) and 11 (recovery period), but population deviations were only
361 1% outside of the range of control fluctuations. Although the reduction in population abundance
362 occurred during the recovery period; the number of juveniles present in January will still be a reflection
363 of the reduced recruitment caused by fenitrothion exposure in the previous breeding season. After
364 exposure to 200 $\mu\text{g L}^{-1}$ fenitrothion, the populations recovered in the 4th year of the recovery phase
365 (Fig. 5b).

366



367

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

370 *fenitrothion concentration ($\mu\text{g L}^{-1}$). Red and white sections divide exposure and recovery time period:*
371 *10 year exposure; 10 year recovery.*

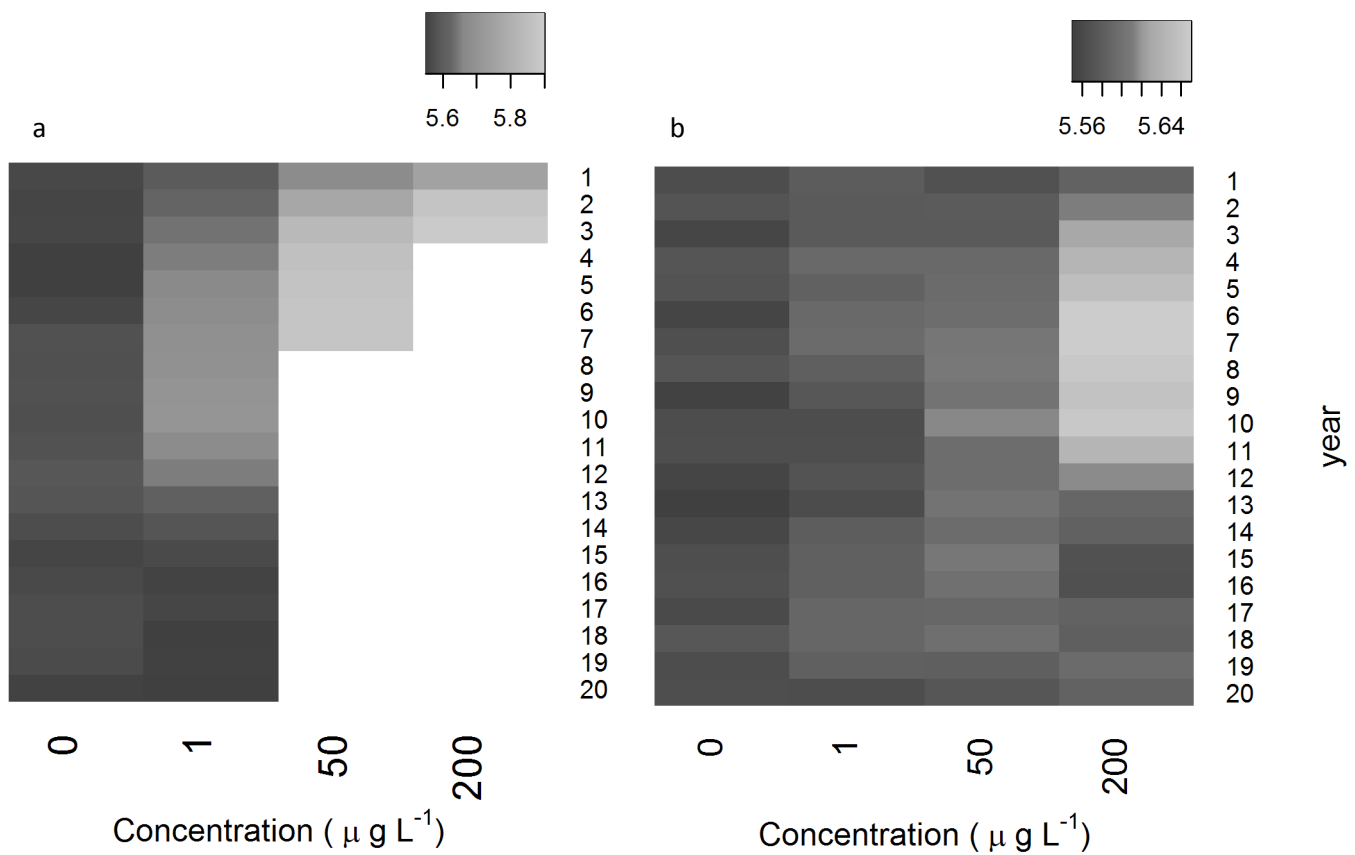
372 The results of the analysis on recruitment to different life stages (data not shown) showed that
373 fenitrothion exposure impacted most on egg numbers and least on adult numbers; for example,
374 intermittent exposure to 1, 50, and 200 $\mu\text{g L}^{-1}$, respectively caused a maximum annual reduction in
375 total egg abundance of 17, 27, and 69% compared to adults, for which the maximum reduction was
376 12, 16, and 36%, relative to control abundances. Further analysis of the model revealed that this
377 pattern emerged as a result of density-dependent compensation when population abundances are
378 low, thus, lower densities of larvae resulted in reduced competition for food, faster juvenile growth
379 rates and a consequent reduction in size-dependent mortality, as well as reduced competition for
380 good quality (sheltered) habitat patches where mortality rates were lower and conditions more
381 optimal for nesting males in the breeding season.

382

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

394

395 Throughout the exposures, the asymptotic length of the control population remained stable (5.55 –
 396 5.59 cm) but increased with increasing concentration of fenitrothion (Fig. 6). Intermittent exposure to
 397 1, 50 and 200 $\mu\text{g L}^{-1}$ resulted in a maximum mean asymptotic length of 5.59, 5.62, and 5.78 cm,
 398 respectively, during the 10 year exposure period. This density-dependent compensation allowed for
 399 some population recovery in between annual exposures and resulted in relatively stable populations
 400 throughout the exposure period, following an initial decline in abundance, as observed in the case of
 401 the two highest exposure concentrations. Comparatively, chronic exposure to 1, 50 and 200 $\mu\text{g L}^{-1}$
 402 caused a maximum asymptotic length of 5.74, 5.89 and 5.89 cm but the extent of the density-
 403 dependent compensation in growth was insufficient to prevent populations going extinct.



404

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

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

410 **4. Discussion**

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

415

416 Overall, the stickleback IBM provided a good fit to the available UK stickleback population data,
417 indicating that the model provides a good representation of an extensively monitored natural system.
418 However, the body size discrepancies between wild sticklebacks in the UK and in Germany meant that
419 the model, with current parameterisation, cannot simultaneously provide a good fit for both
420 populations. We chose to use the UK population data as the main body of validation as the data is
421 more extensive than the data generated from the study sites in Germany. The UK data represents up
422 to 21 years of sampling and records both population abundance and size class distributions for
423 resident freshwater stickleback. In contrast, the data from Germany only recorded size class data in a
424 single year and the fish were not accurately aged, meaning that the size distributions could represent
425 a mixture of 0+ and older cohorts. Additionally, the model is representative of the low-plated resident
426 freshwater form of stickleback and it is possible that the data from the German sites, located close to
427 the sea, includes the genetically different anadromous sticklebacks which grow faster and to a larger
428 maximum size (Wootton, 1984; Schluter, 1995). The larger body lengths displayed in the German
429 population may also be a result of an earlier breeding season and/or differences in abiotic parameters
430 such as temperature (Allen and Wootton 1982*b*), photoperiod (Guderley et al., 2001), or food
431 availability (Allen and Wootton 1982*b*). Importantly, however, the model does reflect seasonal
432 differences in growth observed from both sets of population data. The stickleback is a temperate fish
433 species and seasonal fluctuations in temperature and food availability affect growth rates in the wild,

434 resulting in high growth rates in the summer and low growth in the winter (Allen and Wootton 1982a,
435 Allen and Wootton 1982b). In the model, seasonal growth is one of the key mechanisms driving
436 population dynamics (SI Ap. 12 for more details). We incorporated seasonal growth using an adapted
437 version of the von Bertalanffy equation (where parameters t_s and c enforce seasonal oscillations
438 (Table. 1, Eq. 4)) which predicts temperature-dependent growth accurately according to the UK
439 validation results. A more mechanistic approach to incorporating seasonal growth, such as adding an
440 energy-based element (Martin et al., 2012, Sibly et al., 2013), could better extend the model's
441 application to different latitudes and regions.

442 The sensitivity analysis revealed that the model was most sensitive to alterations in the duration of
443 the breeding season and the operational sex ratio. Stickleback invest a high proportion of energy into
444 nest guarding and egg brooding by males, and females display relatively low fecundity compared to
445 other fish species whose reproduction requires less investment post spawning (Bone and Moore,
446 2008). The model's sensitivity to parameters which directly affect egg recruitment is therefore an
447 emergent property of the stickleback's life history strategy. As a comparison, the zebrafish has a high
448 fecundity and in most latitudes may be able to spawn all year round (Spence et al., 2007). A sensitivity
449 analysis of a zebrafish IBM (Hazlerigg et al., 2014) demonstrated that this model species was more
450 resilient to changes in reproductive parameters, such as a reduction in the duration of the breeding
451 season, than the stickleback IBM. . The comparison between these models demonstrates how life
452 history strategy can pre-determine the capacity for population resilience and therefore the choice of
453 focal species for risk assessment needs to be carefully considered, if these factors are to be taken into
454 account.

455 It is well documented that different EDCs can induce different types of physiological effects on
456 individual fish (e.g. masculinisation and reduced fecundity from androgens (Morthorst et al., 2010);
457 feminisation and reduced fecundity from anti-androgens (Jensen et al., 2004) and oestrogens (Nash
458 et al., 2004); impaired growth rates from thyroid disruptors (Liu et al., 2008)) and behavioural effects

459 have also been widely reported (Dzieweczynski, 2011; Dzieweczynski et al., 2014; Ward et al., 2006;
460 Weis et al., 2001; Xia et al., 2010). There are, however, very few examples of studies on population
461 level-effects of EDCs in fish. A notable example is in the work by Kidd et al. (2007), where a whole
462 Canadian lake (Lake 260) was treated with ethinylestradiol (EE₂) at concentrations between
463 5-6 ng L⁻¹ for a period of 3 years which resulted in the feminization of male fathead minnows and the
464 collapse of the fathead minnow population (Kidd et al., 2007). Breeding behaviours in the fathead
465 minnow have been shown to be disrupted after laboratory exposures to the same concentrations
466 (Majewski et al., 2002), and this may have contributed to the subsequent population crash. The
467 breeding strategy of the fathead minnow is similar to that of the stickleback (e.g. nest guarding by
468 males), and the population-level impacts of disruption to the same behaviours would therefore be
469 expected to be similar for both species. In addition to the physiological and behavioural effects of
470 EDCs, indirect effects may occur via the disruption of food web interactions. These interactions are
471 particularly relevant for pesticide risk assessment since these chemicals target invertebrates and are
472 therefore likely to have adverse effects on the prey of fish species. Specifically, fenitrothion is highly
473 toxic to aquatic invertebrates (PPDB, 2017) and effects have been reported on species which
474 contribute to the diet of the stickleback (Fairchild and Eidt, 1993; Choi et al., 2002). Since the current
475 study aims to predict the effects of a single behavioural endpoint on population abundance, food-web
476 interactions are not considered here. However, disruption of invertebrate communities would likely
477 compromise some of the density dependent compensation observed in the model following
478 fenitrothion exposure.

479 Using the stickleback IBM, we showed that exposure duration, as well as exposure concentration,
480 affected population responses and effect levels were markedly greater in populations subjected to a
481 continuous chronic exposure regime compared to a more realistic intermittent regime. For example,
482 concentrations that only caused negligible effects under pulsed exposure scenarios caused marked
483 decreases or even extinction in continuous exposure scenarios. This is consistent with an empirical
484 study, whereby compensatory responses allowed a fathead minnow population to recover following

485 pulsed exposure to toxicants (Ali et al., 2017). Further analysis of the model (e.g. tracking changes to
486 density dependent parameters) revealed that the compensatory capacity for the investigated effects
487 was driven by density-dependent competition for resources leading to increased growth and survival
488 in early life stages and increased availability of spawning territories for adults. In particular we
489 assessed the extent to which growth (asymptotic length - L_{inf}) was affected by density. In the
490 continuous exposure scenario, the capacity for L_{inf} to increase and compensate population biomass
491 was exhausted at $50\mu\text{ L}^{-1}$ fenitrothion, whereas for the pulsed exposure that did not occur even at a
492 fenitrothion concentration of $200\mu\text{g L}^{-1}$. The compensatory effects of density-dependence also vary
493 between different life-stages. For example, effects were consistently greater for eggs and larvae than
494 for juveniles and adults, and this was particularly evident at the highest exposure concentration.
495 Studies on invertebrates have demonstrated that exposing resource limited populations to toxicants
496 can reduce intra-specific competition and therefore lessen the negative effects of the toxicant (Liess,
497 2002; Moe et al., 2002). However, empirical studies which validate the interaction between resource
498 competition and chemical effects in fish are limited and it is therefore, as yet, difficult to confirm the
499 realism of these modelled results. In addition, since the current model assumes constant
500 environmental conditions (e.g. food availability and temperature), the exposure scenarios simulated
501 here do not consider potential interactive effects that may be associated with extremes in
502 environmental conditions and this should be taken into consideration when interpreting these results.
503 The capacity for compensation in natural systems generally is greatest when a population is close to
504 carrying capacity and populations can recover faster in systems with rich resources (Beverton and
505 Holt, 1957). As a consequence the effect of chemical exposure should be seen in the context of
506 resource availability and considered together with other stressors. Moreover, both stressors and
507 resources fluctuate seasonally, so it is important to understand the environmental context and life
508 history strategies of focal species when extrapolating in risk assessment. With the current level of
509 detail, the stickleback IBM has proved to be useful in risk assessments for assessing the population-

510 level consequences of individual-level endpoints relating to behaviour, growth, survival, and
511 reproduction.

512 **Conclusions**

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

517

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

526

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