Applying a Mechanistic Model to Predict Interacting Effects of Endocrine Active Chemical Exposure and Food Availability on Fish Populations.

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Abstract

The potential environmental impacts of chemical exposures on wildlife are of growing concern. Freshwater ecosystems are vulnerable to chemical effects and wildlife populations, including fish, can be exposed to concentrations known to cause adverse effects at the individual level. Wild fish populations are also often subjected to numerous other stressors simultaneously which in temperate climates often include sustained periods of food limitation. The potential interactive effects of chemical exposures and food limitation on fish populations are however difficult to establish in the field. Mechanistic modelling approaches can be employed to help predict how the physiological effects of chemicals and food limitation on individuals may translate to population-level effects. Here an energy budget-individual-based model was developed and the control (no chemical) model was validated for the three-spined stickleback. Findings from two endocrine active chemical (EAC) case studies, (ethinyloestradiol and trenbolone) were then used to investigate how effects on individual fecundity translated into predicted population-level effects for environmentally relevant exposures. The cumulative effects of chemical exposure and food limitation were included in these analyses. Results show that effects of each EAC on the population were dependent on energy availability, and effects on population abundance were exacerbated by food limitation. Findings suggest that chemical effects and density dependent food competition interact to determine population responses to chemical exposures. Our study illustrates how mechanistic modelling approaches might usefully be applied to account for specific chemical effects, energy budgets and density-dependent competition, to provide a more integrated evaluation of population outcomes in chemical risk assessments.

Key words: Bioenergetics; density dependence; individual-based model; three-spined stickleback; energy budget; endocrine disruptor

1. Introduction

Synthetic chemicals enter freshwater ecosystems directly from domestic and industrial effluents (Petrovic et al. 2002) and from diffuse agricultural runoff (Schulz 2004, Wauchope 1978). Of particular environmental concern are endocrine active chemicals (EACs) which have been reported to cause adverse effects on fish and their populations (Tyler and Jobling, 2008; Kidd et al., 2007; McGee et al., 2012; Geraudie et al., 2017). EACs have been defined in various ways, but put simply they may alter the synthesis, metabolism or function of natural endogenous hormones, causing physiological stress to individuals and subsequent changes in life-cycle processes (Calow, 1991; Beyers, 1999). Exposure to EACs can cause direct effects on the allocation of energy to growth and/or reproduction (Muller et al., 2010).

In addition to chemical exposures, wild fish populations are frequently subjected to a wide array of other environmental stressors often including periods of food limitation, which can vary in intensity spatially and temporally. Since food availability directly affects life-cycle processes and subsequently shapes population dynamics (Beverton and Holt, 1957, Lorenzen and Enberg, 2002), the interactive effects of food limitation and chemical exposures may have interactive and potentially cumulative effects on fish populations (Holmstrup et al., 2010). Investigating the interactive effects of chemical exposures and food limitation on wild populations, however, is extremely challenging.

The development of mechanistic effect models, including population models, for chemical risk assessments has advanced considerably in the last decade (e.g.

see the Chemical Risk Effects Assessment Models Initiative; Grimm et al., 2009) and results from population models are now being considered in a regulatory context as part of effects assessments of EACs on wildlife populations in Europe (EC No 2017/2100 and EC No 2018/6054). Individual-based population models (IBMs) allow for simulations of chemical effects on lifecycle traits directly and also enable consideration of compensatory processes, including density dependent compensation of survival, growth and reproduction. Energy budget models offer the additional benefits of quantifying direct costs (e.g. effects caused by the mode of action of the chemical) and indirect costs (e.g. maintaining homeostasis under cellular stress) of chemical exposure on the partitioning of energy within organisms. Integrated approaches where energy budget models are combined with IBMs are increasingly being applied in order to better link organism level responses to predicted population-level effects (Martin et al., 2013; Johnston et al., 2015; David et al., 2019). Combining these modelling approaches also allows for integration of potential cumulative effects of multiple stressors, including chemical and climatic stressors and food shortages, which is vital for realistic ecological assessments.

To mechanistically model the effects of EAC chemical exposures, links between the molecular initiating events (MIEs) and the whole organism effects need to be established. This enables identification of direct and indirect energetic effects. An adverse outcome pathway (AOP) is a conceptual framework that seeks to link an MIE (e.g. oestrogen receptor agonism) and an adverse outcome at the population-relevant level (Ankley et al. 2010).Qualitative information inferred from AOPs can be used to decipher the energetic mechanisms by which a chemical causes its organism-level effect from specific chemical pathways (Goodchild et al., 2018; Murphy et al., 2018). There has been recent interest in linking AOPs to

bioenergetics and individual-based models (IBMs) to estimate population-level effects of chemical exposures (Forbes and Galic, 2016; Goodchild et al., 2018; Murphy et al., 2018).

Here, a spatially explicit energy budget-IBM for the three-spined stickleback (*Gasterosteus aculeatus*) was developed using an established stickleback IBM (Mintram et al., 2018). Energy budget models account for the partitioning of energy amongst life-cycle processes and use established principles of energy and mass conservation under prevailing environmental conditions, including food availability and temperature (Sousa et al., 2010, Sibly et al., 2013). The model assumes that chemicals impose stress on specific physiological parameters, which have predictable effects on life-cycle processes (e.g. reproduction) following energy allocation principles (Álvarez et al., 2006; Jager and Zimmer 2012; Sibly et al., 2013; Johnston et al., 2014). Using two case study EACs with two different effect mechanisms (17α-ethinylestradiol (EE_2) – an oestrogen receptor agonist and 17β-trenbolone – an androgen receptor agonist) – we assessed for the population-level impacts of a single endpoint (reduction in fecundity) caused by these EACs at environmentally relevant concentrations. Individual-level effects on fecundity for both EACs were obtained from the published, peer-reviewed literature. The key events that link sub-organismal energetic effects to whole-organism effects on fecundity were identified using a combination of established AOPs and chemical-specific mechanistic data obtained from laboratory studies. The aim was to define the most plausible combinations of energetic pathways by which EE_2 and trenbolone cause effects on fecundity and assess how these effects extrapolate to the population-level. The interactive effects of food availability and chemical exposure on stickleback populations were subsequently assessed. Here EE_2 and trenbolone exposures

were simulated in environments with a range of food availabilities in order to explore the influence of different environmental conditions in determining population susceptibility to these EACs. The uncertainty of the approaches used here are fully explored to aid future studies linking mechanistic information (e.g. AOPs) to bioenergetics and population models.

2. Methods

2.1. Study species

The energy budget-IBM was developed for the three-spined stickleback (*Gasterosteus aculeatus*), an extensively studied fish species in the fields of ecology, ecotoxicology and physiology. The laboratory exposure studies supplying individual effects data for EE_2 and trenbolone were based on the fathead minnow (*Pimephales promelas*), a temperate freshwater fish species used widely for regulatory ecotoxicology studies in North America, Canada and Europe (OECD, 1992; OECD 2012; Ankley and Villeneuve, 2006) and with strong breeding strategy commonalities with the stickleback. The fish cross-species extrapolation was necessary because the stickleback is ecologically relevant for European waters, but there is far more ecotoxicological data available for EE_2 and trenbolone for the fathead minnow compared with for the stickleback. Predictions of the relative responsiveness of these two species to either EE_2 or trenbolone can be made through evaluations of the target receptor conservation and receptor target interaction/activation. For the estrogen receptor (ESR1), the stickleback and fathead minnow show 42% amino acid sequence identity across their full receptor length. For the bovine androgen receptor (AR), the amino acid sequence similarity across the full length receptor is 33% (Lalone et al., 2013). More detailed assessment of likely target interaction based on sequence

similarity of the ligand binding domain (LBD) for ESR1 and AR would enable more detailed comparison of the two fish species in terms of their likely susceptibility to EE_2 and trenbolone. Comparing target receptor responses to estrogen between the two fish species similar potencies have been shown for exposure to EE_2 using *in vitro* estrogen receptor transactivation assays (Miyagawa et al., 2014). For androgen, although comparing across different bioassays, specifically an AR competitive receptor binding assay for fathead minnow (Wilson et al. 2004) and an AR-dependent spiggin production in a kidney cell culture for stickleback (Jolly et al. 2006), responses to trenbolone indicate similar AR-dependent potencies.

2.1. Study compounds

17α-ethinylestradiol (EE_2) and 17β-trenbolone were chosen as the two case study toxicants because they are two of the most well studied EACs and they represent two different modes of action.

EE_2 is a synthetic oestrogen commonly used in the contraceptive pill. It is an oestrogen receptor agonist which inhibits endocrine (FSH/LH) and paracrine signalling. Predicted concentrations of EE_2 in typical surface waters are estimated at 0.3 ng L^{-1} but have been reported to reach up to 9 ng L^{-1} in wastewater treatment plant effluents (Hannah et al., 2009); effects in fish have been widely reported within the range of these exposure concentrations. Observed effects on individuals include feminisation of male fish, reduced courtship, impaired growth and reduced reproductive output from females (lower fecundity) (Nash et al., 2004; Parrott and Blunt; 2005; Lange et al. 2008; Zha et al. 2008; Armstrong et al. 2015).

Trenbolone is a synthetic anabolic steroid used as a growth promotor in beef cattle in the US, South America and Australia. It acts as an androgen receptor

agonist and a progesterone receptor agonist. Trenbolone metabolites have been reported in downstream riverine sites in the United States at concentrations up to 50 ng L^{-1} (Durhan et al. 2005). Exposure to trenbolone is known to cause masculinisation of females (Seki et al. 2006), reduced reproductive output in females (Ankley et al. 2003) and weight gain in male and female fish.

2.3. Incorporating energy budgets into an IBM for the stickleback

An energy budget model was incorporated into an IBM for the three-spined stickleback (see; Mintram et al., 2018). The energy budget-IBM is described in detail following the "TRAnsparent and Comprehensive model Evaludation" (TRACE) document (Augusiak et al., 2014; Grimm et al., 2014; Schmolke et al., 2010) and can be found in the Supplementary Information (SI). The model was implemented in the free programming software NetLogo 6.0.1 (Wilensky, 1999) and is available in the SI. A full technical specification for the energy budget model can be found in TRACE section 2.

The purpose of the energy budget-IBM is to investigate the effects of exposure to chemicals on three-spined stickleback populations under realistic field scenarios of spatial and temporal food limitation. The 20 m^2 water body represents the ecological scenario where spatial variation in food availability drives density dependent competition for resources across the landscape. The entities in the model are the spatial units (comprising the landscape) and individual fish which are distributed among four life stages: eggs, larvae, juvenile or adult. Each individual in the model possesses its own energy budget following the methodology of Sibly et al. (2013). In broad terms, the energy budget describes the rates of energy derived from food, and its allocation to the vital processes of maintenance, growth, reproduction and energy storage. The

partitioning of energy among vital processes depends on life stage and gender (Fig. 1). Chemicals induce their physiological effects by altering the allocation of energy to the vital processes listed above (Álvarez et al., 2006; Jager and Zimmer 2012; Sibly et al., 2013; Johnston et al., 2014).

Environmental conditions (water temperature and food density) were modelled based on field data from the South-West of the UK (Turner et al., 2013; Wootton, 1994) and these conditions were used throughout all model simulations (with the exception of model validation; see below).

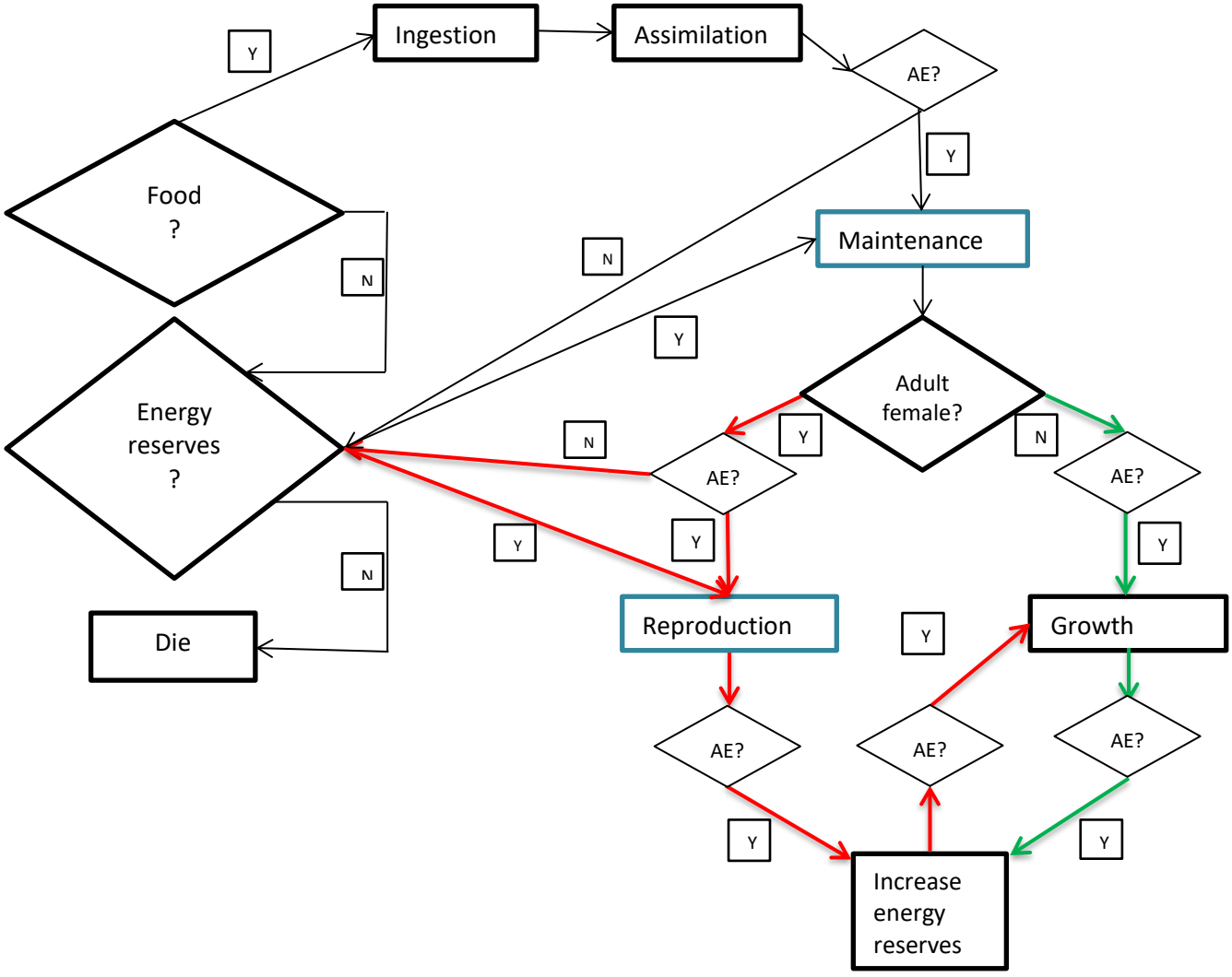

Figure 1. Conceptualisation of the physiological processes in juvenile and adult stickleback which demand allocations of resources (energy). Diamond boxes represent decisions ('AE?' calculates if there is sufficient assimilated energy to

undertake the next process) and square boxes represent life-history processes where purple square boxes are the processes affected by EE_2 and trenbolone exposure. . All juveniles and adults follow the flow of black arrows, adult females follow the flow of red arrows, and juveniles and adult males follow the flow of green arrows. Note that any movement related costs (including the reproductive costs of adult males) are paid for in '*maintenance'* (TRACE, section 2). Individuals use assimilated energy from food to undertake life-history processes. If there is not sufficient assimilated energy to undertake maintenance, or reproduction in adult females, energy is transferred from reserves.

The energy budget model was parameterised using laboratory data from the literature (TRACE, section 2) and no calibration was undertaken. The population model was parameterised using laboratory and field data wherever possible and was calibrated using the mortality sub-model to provide model outputs of stickleback abundances known to occur in the wild outside of the breeding season (see TRACE, section 5). No further calibration of the model was undertaken for model validation (i.e. the model was not forced to fit the observed data); only food densities and temperatures were adapted to match those reported in the lab/field studies.

The primary aim of the validation was to assess how the emergent properties of the individuals (e.g. growth and mortality) and population dynamics (abundances and size distributions) respond realistically to environmental conditions. The quality of validation was assessed quantitatively (R^2 values) and by visually assessing ecological patterns. The energy budget model (excluding chemical exposure) was evaluated at the organism level by comparing modelled outputs of somatic growth rates (body lengths over time and wet weights at 60 days post

hatch (dph) (personal obvservations, full methods and results in TRACE section 5)) and egg production (Wootton, 1972) to laboratory controlled empirical data for stickleback maintained under different levels of food availability (details for these studies can be found in TRACE section 5). The energy budget-IBM was validated by comparing modelled outputs of emergent population dynamics to field population data from two independent stickleback populations with different food resource conditions. This field data has not been used to develop or parameterise the model. The first population, inhabiting the River Rheidol, Wales, represents a favourable habitat for the stickleback with abundant food. Life-history data for this population were obtained from Wootton et al. (1978), Wootton et al. (2005) and Wootton (2007). The second population, located in Lake Frongoch, Wales, represents a less favourable and more variable habitat for the stickleback with food limited throughout the year resulting in individuals of a comparatively smaller body size. Body lengths for this population were obtained from two separate years (1974 and 1977) from Allen and Wootton (1982) and Wootton et al. (1978). For each population, data for water temperatures were obtained from the exact location (Allen and Wootton, 1984) or from a nearby location (Wootton et al., 1978; Turner et al., 2013). Food densities for Lake Frongoch and the River Rheidol were estimated by Allen and Wootton (1982), and Wootton et al. (1980) and Wootton (2007), respectively. Body lengths are presented as total body length and body mass as wet weight. Details of model validation, calibration (predation sub-model and larval mortality) and sensitivity analyses can be found in the TRACE document.

2.4. Parameterisation of EE_2 and trenbolone effects on fecundity in stickleback populations

Effects of EE_2 and trenbolone exposure on fathead minnow fecundity were taken directly from the literature. Armstrong et al. (2015) quantified egg production during a 21day laboratory exposure to EE_2 at concentrations of 0.5, 1.5, and 4 ng L^{-1}, reporting significant reductions in cumulative egg production of 34, 39, and 39%, respectively. Similarly, during a 21 day laboratory exposure to trenbolone, Ankley et al. (2003) reported significant reductions in cumulative egg production of 60% at an exposure to 50 ng L^{-1} and almost complete inhibition of egg production at 0.5, 5 and 50 µg L^{-1}. We simulated constant spatially homogenous exposures of the reported effects at 1.5 ng L^{-1} for EE_2 and 0.05 µg L^{-1} (i.e. 50 ng L^{-1}) for trenbolone to provide environmental relevance, albeit these are at the higher end of reported environmental concentrations for these chemicals (see section 2.1).

2.4.1.Qualifying the energetic pathways associated with the effects of EE_2 and trenbolone on fecundity

The AOPs for oestrogen receptor agonism (EE_2) (AOP-wiki; AOP 29 https://aopwiki.org/aops/29) and androgen receptor agonism (trenbolone) (AOP-wiki; AOP 23 https://aopwiki.org/aops/23) were used to identify the possible energetic pathways associated with the key events linking the MIE to the adverse outcome (reduced fecundity). Additional information is taken from the literature to support these mechanistic links.

2.4.1.1. Oestrogen receptor agonism (EE_2)

EE_2 directly limits energy input to egg production by (i) inhibiting the ovarian growth process and limiting ovulation and (ii) inhibiting female breeding behaviours (Coe et al., 2010) via neurological pathways. These mechanisms result in egg production and/or release being directly inhibited (Coe et al., 2010;

293 Söffker and Tyler, 2012) which are implemented in the model by reducing the
294 'maximum rate of energy allocation to reproduction parameter' (*rm*) in the
295 reproduction sub-model.

296 Increased metabolic costs are incurred following low dose EE_2 exposures
297 primarily via the increased production of vitellogenin (VTG) (Armstrong et al.,
298 2015). To a lesser degree, metabolic costs also include increased production of
299 vitelline envelope protein (Finne et al., 2011) and general sub-cellular costs
300 associated with maintaining homeostasis (Goodchild et al., 2018) and repairing
301 tissue damage (e.g. hepatic and renal damage as a result of increased VTG
302 production (Zha et al., 2008)). Metabolic costs associated with chemical exposure
303 are simulated in the model by increasing the 'taxon-specific normalization
304 constant' (B_0) parameter in the maintenance sub-model.

305 ***2.4.1.2. Androgen receptor agonism (trenbolone)***

306 Trenbolone exposures directly limit energy input to egg production by reducing
307 LH/FSH synthesis, consequently reducing E_2 synthesis and inhibiting VTG
308 production in the hepatocytes. As described above, this mechanism is
309 implemented in the model by reducing the r_m parameter.

310 Increased metabolic costs are additionally incurred as a result of the increasing
311 occurrence/prominence of secondary sexual characteristics (SSCs) (Ankley et
312 al., 2003). In the stickleback notable SSCs include nuptial colouration and eye
313 colouration in males (Barber et al., 2000) and the induction of spiggin (a glue
314 protein synthesised by males to aid nest building) (Katsiadaki et al., 2002). As
315 with EE_2, there will also be sub-cellular metabolic costs associated with
316 maintaining homeostasis (Goodchild et al., 2018). These increased metabolic
317 costs are simulated by increasing the B_0 parameter. Trenbolone exposure as an

anabolic steroid could also incur growth costs via its action on promoting increased somatic muscle mass (Buttery et al., 1984); however evidence suggests that this increased muscle mass is a result of a decrease in total protein degradation with little alteration of total energy retention (Sinnet-Smith et al., 1983; Lobley et al., 2007). Nonetheless, given that reproduction is prioritised prior to growth in the model, we assume that any increases in somatic growth costs do not impact fecundity.

2.4.2. *Quantifying the energetic pathways associated with the effects of EE_2 and trenbolone on fecundity*

Since OECD guidelines for the fish short term reproductive assay (FSTRA, Test No. 229) state that fish should be fed *ad libitum* throughout the holding period and during the exposure phase, the fish in these studies are assumed not to be food limited. Thus, the observed effect on fecundity in these particular studies was explained by a proportional reduction in the r_m parameter in the model. According to the information collated in section 2.4.1, the B_0 parameter must also be increased. Here, we increased the B_0 parameter proportionally to the decrease in the r_m parameter so that excess energy could not be re-allocated to lipid reserves. Since Armstrong et al. (2016) did not report increases in body mass following exposure to EE_2, we conclude that the chemical exposure conditions in this study did not cause obesogenic effects. Trenbolone is known to increase muscle mass (Buttery et al., 1984), but has no known obesogenic effects. However, there are no studies which quantify egg production at limiting food rations during exposure to either chemical, and it is therefore not possible to assess if the increase in the B_0 parameter is greater than the decrease in the r_m parameter. If this was the case, it would be expected that females would lose

343 weight under conditions of food limitation as reserves are used to subsidise egg
344 production (Fig. 1, TRACE section 2). A study exposing mature female
345 largemouth bass (*Micropterus salmoides*) maintained on a diet of 1% body weight
346 to EE_2 reported no significant reductions in body mass despite significant
347 reductions in GSI (Colli-Dula et al., 2014), suggesting that the maintenance costs
348 associated with EE_2 exposure do not exceed the direct endocrine effects on
349 reproduction. Nonetheless, this example is just one study for a single species and
350 so given the uncertainty of this parameter value, a sensitivity analysis was
351 undertaken where the B_0 parameter was increased by 2-, 3- and 4 -fold higher
352 than the values used in the exposure simulations for both chemicals. This
353 analysis allowed us to identify the importance of this uncertainty for model
354 predictions.

355 ***2.5. Simulation experiments***

356 A continuous temporal exposure scenario was used in the model. This is realistic
357 for EE_2 where inputs enter surface waters via wastewater effluent discharges,
358 whereas trenbolone exposures are likely to be more pulsed as they enter via
359 runoff from fields treated with cattle manure. The impact of temporal exposure
360 regime on population-level effects has been investigated previously (Mintram et
361 al., 2018), and we do not therefore repeat those analyses here. To assess for
362 population-level effects of these chemicals, we simulated EE_2 and trenbolone
363 exposures at 1.5 and 50 ng L^{-1}, respectively, continuously for a period of 10 years.
364 The endpoint used in this study is egg production, with the assumption of no
365 adverse effects on non-breeding fish. As such effects are only relevant
366 throughout the breeding season. This is a key assumption for the scenarios
367 modelled here, since both chemicals do cause effects at other life-stages (Länge

et al., 2001; Cripe et al., 2010). However, since the laboratory studies used here only exposed adults, the observed effects on fecundity were a result of female adult exposure rather than cumulative effects from previous life-stages.

Chemical exposure scenarios were simulated in environments representing a range of five food densities, following the same seasonal patterns as those described for the default model (TRACE section 2). Food density conditions are expressed as a proportion of the daily default food density as follows: 0.3, 0.65, 1, 1.35, and 1.7 (see Appendix 2, Fig A1 for a graphical representation). The minimum simulated food densities represent the threshold minimum food density needed to ensure population survival under control (no chemical) conditions. The proceeding food density scenarios scale up linearly so that the middle food density condition represents the default values (section 2.3) and the maximum food densities represent abundant food supplies throughout the year.

Population-level effects were quantified by comparing cumulative egg production and mean population abundances and biomass of exposed and unexposed (no chemical control) stickleback populations on January 1st (i.e. annual pre-spawning census) throughout the 10 year exposure period (year 1 represents the January following the initial exposure).

A preliminary analysis of the model showed that 15 replicate model runs were necessary to generate robust means and standard deviations. Replicate number was considered to be robust once the difference in the average and the standard deviation of the population abundance between model runs became independent of replicate number (± 5%). Thus here, population-level effects were considered detectable if the mean abundance over 10 years exceeded a 5% deviation from the mean control abundance.

3. Results

3.1. Model validation

3.1.1. Organism-level validation

The energy-budget model predicted the empirical observations of somatic growth rates maintained under varying food rations well (body lengths: R^2 = 0.94; wet weights: R^2 = 0.9) (Fig 2). The model had a tendency to under-represent body mass at low food rations and this is possibly because it does not incorporate the adaption of behaviours to conserve energy in low food environments (e.g. Selye, 1973; Dill, 1983). The model also predicted egg production accurately at low (21 mg day^{-1}), medium (32 mg day^{-1}) and high (75 mg day^{-1}) food rations, where ratios of egg production were 1:1.5:3.9 and 1:1.6:3.5 for observed and modelled fish, respectively (details in TRACE, section 5).

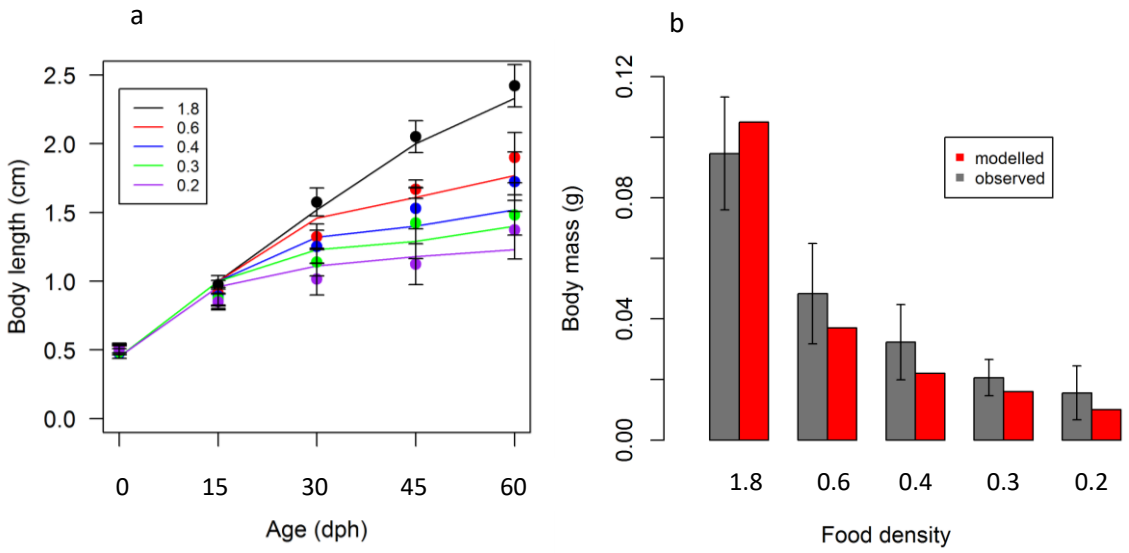

Figure 2. Body lengths over time (a), and final wet weights after 60 days (b), of observed and modelled stickleback for different food allocations. Circles on graph *a* represent observed data, lines represent modelled data and legend (inset)

refers to food amount per individual (mg). Observed data are displayed as mean lengths (± sd), whereas modelled outputs represent a single run.

3.1.2. Population-level validation

Model simulations of stickleback populations in the field showed good predictions of population abundance in the River Rheidol in spring over 11 years (modelled mean = 6.1 fish m^2; observed mean = 4.7 fish m^2) and autumn over 21 years (modelled mean = 17.2 fish m^2; observed mean = 13.5 fish m^2) (Wootton et al., 2005; Wootton, 2007). The model additionally showed good predictions for size distributions of stickleback populations in the River Rheidol during spring (R^2 = 0.43) and autumn (R^2 = 0.74) (Wootton et al., 2005; Wootton, 2007) (Fig 3).

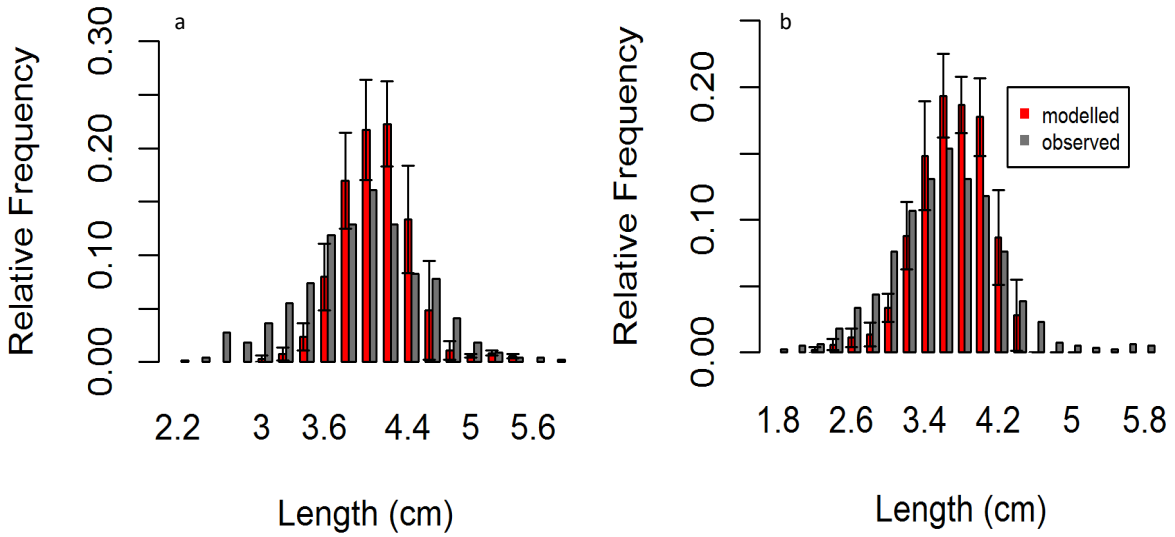

Figure 3. Comparisons of modelled and observed data for population size distributions in spring (Feb/March (a)) and autumn (October (b)). Modelled population size distributions represent mean values from 15 replicate runs (± sd) over 5 years. Observed data were obtained from a stickleback population in the River Rheidol (Wales, UK) after Wootton et al. (2005) and Wootton (2007).

Under favourable environmental conditions in the field, the model showed good predictions for mean body length (R^2 = 0.64) and mass (R^2 = 0.74) from the River

Rheidol over the year from September to August (Fig 4a) (Wootton, 1997). Under less favourable environmental conditions, the model showed good predictions for mean body lengths of stickleback from Lake Frongoch (Fig 4b). The model did not, however, capture much of the variability displayed in the field data. Body lengths from this field population were larger in 1974 than in 1977 demonstrating that there is variance between years. The modelled body lengths tend to sit in between the two years and are approximately 1 cm smaller than the modelled Rheidol population, indicating that the model responds correctly to changes in food availabilities.

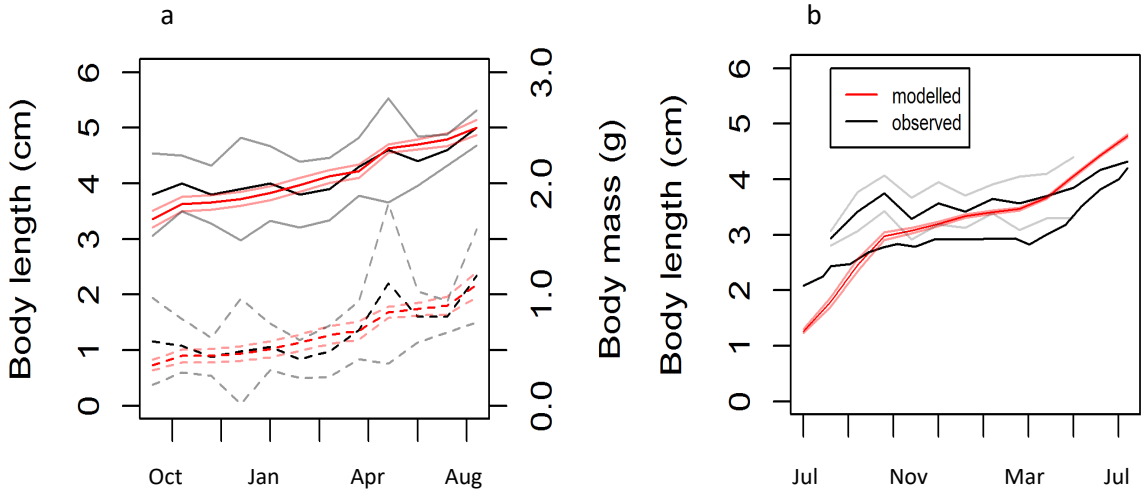

Figure 4. Comparisons of modelled and observed data for mean body length and mass over a year from September to August (a) and mean body length over a year from July to July (b). Modelled data represents mean values from 15 replicate runs (± sd) over 5 years and observed data (mean ± sd (no variance was reported for the 1977 Lake Frongoch population)) were obtained from a stickleback population in the River Rheidol (Wales, UK) after Wootton et al. (1978) (a) and Lake Frongoch (Wales, UK) for two different years after Allen and Wootton (1982) and Wootton et al. (1978) (b).

3.2. Chemical exposure simulation

Continuous exposures to trenbolone (0 and 50ng L^{-1}) and EE_2 (0 and 1.5 ng L^{-1}) resulted in reduced cumulative egg production and population abundance in every food availability scenario, compared with controls (Fig 5). The magnitude of the effect decreased with increasing food availability, with the exception of the two highest food availabilities where the differences were negligible (Table 1). Consistent with the individual-level effects observed in the laboratory, trenbolone exposure resulted in greater effects on egg production and population abundance than EE_2.

Table 1. Effects on egg production, population abundance and population biomass of EE_2 (1.5 ng L-1) and trenbolone (50 ng L-1) for exposures over 10 years. Individual-level effects on fecundity were 39% and 60%, respectively. Data are displayed as the mean percentage change from controls.

	Egg Production		Abundance		Biomass	
Food availability scenario	EE_2	trenbolone	EE_2	trenbolone	EE_2	trenbolone
0.3	- 39	- 65	- 27	- 58	0	- 25
0.65	- 26	- 45	- 18	- 32	+12	+16
1	- 23	- 30	- 15	- 22	- 2	- 8
1.35	- 14	- 32	- 8	- 21	- 2	- 10
1.7	- 14	- 35	- 10	- 22	- 4	- 13

Effects on population biomass for both chemical exposures were consistently less than effects on population abundance. EE_2 did not affect population biomass, with the exception of the second lowest food availability scenario that resulted in

an 11% increase. Trenbolone exposure caused significant decreases in population biomass in all the food availability scenarios, with the exception of the second lowest food availability where a 16% increase was observed. The lowest food availability scenario resulted in the greatest decrease in population biomass during the trenbolone exposure period.

The increase in population biomass seen for the second lowest food availability scenario emerges as a consequence of a reduced population abundance in turn reducing the population density and thus competition for food. Whilst population abundance was reduced, individuals grew to a larger size resulting in a greater overall biomass. This did not occur at the lowest food availability scenario because the food availability was below threshold to allow population recovery to occur; EE_2 and trenbolone exposure in these scenarios lead to population extinction in 2 and 6 of the 15 runs, respectively.

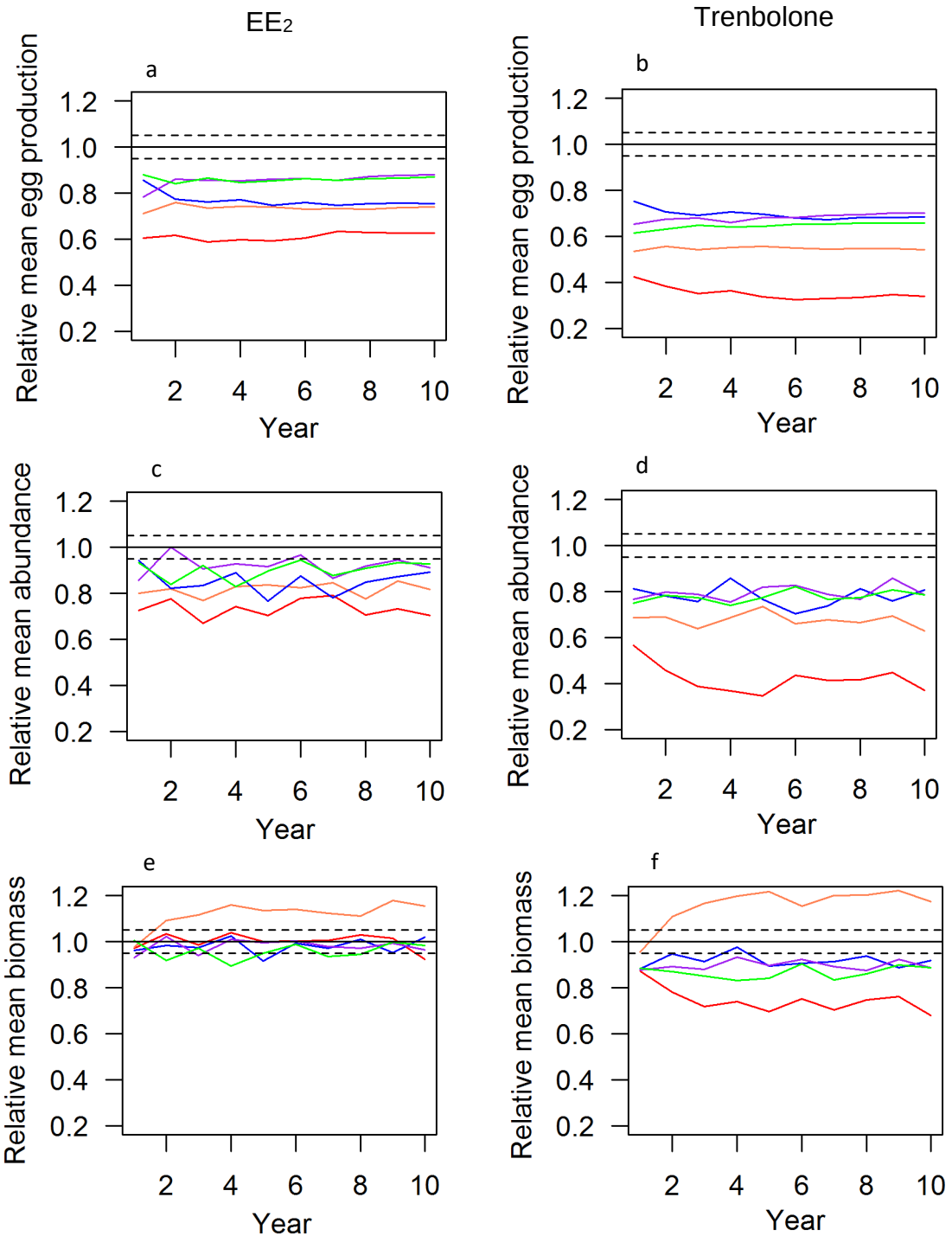

Figure 5. Modelled effects of EE_2 (a,c,e) and trenbolone (b,d,f) exposure on mean cumulative egg production (a,b), mean population abundance (c,d) and mean population biomass (e,f) over 10 years in environments with different food availabilities. The legend refers to the proportion change from the default food availability described in section 2.3. Data are expressed as proportion deviation

481 from control simulations and represent mean values of 15 simulations. Dashed
482 black lines represent the effect threshold of 5%.

483 4. Discussion

484 An existing IBM for the three-spined stickleback (Mintram et al., 2018) was
485 adapted to incorporate individual energy budgets to enable realistic assessments
486 of the effects of chemicals in combination with other stressors (here food
487 availability) on stickleback populations. The control model (no chemical
488 exposure) was validated against organism-level growth and reproduction data
489 and population dynamics data under optimal and limiting food conditions.
490 Application of the model to predict the population-level effects of two potent EACs
491 (EE_2 and trenbolone) demonstrated the exacerbating effects of food limitation on
492 populations exposed to EACs. Our modelled simulations demonstrate the
493 importance of incorporating density dependent food competition into population-
494 level assessments.

495 Our results showed that the effects of EAC exposure on cumulative egg
496 production and population abundance were greater in the low vs high food
497 availability environment. This is because when food was abundant, the increased
498 energetic costs associated with increased B_0 could be met with energy intake and
499 the effect was primarily a result of decreased r_m (as displayed in the laboratory
500 study under high food rations). When food was limited, however, these additional
501 metabolic costs reduced energy allocation to egg production on top of the
502 enforced reduction in r_m. When population abundance was decreased as a result
503 of chemical exposure in all food availability treatments, density dependent
504 competition for food was reduced which resulted in increased individual growth,
505 consequently increasing egg production. This mechanism partly explains why

effects on egg production and population abundance were greater in low food availability environments. The release of density dependent competition also explains why effects on cumulative egg production were consistently greater than effects on population abundance and biomass and why chemical exposures increased population biomass in the low food availability environment. It is notable that modelled effects on populations were usually less than the observed individual-level effects (with the exception of the lowest food availability). These interactions between food availability and chemical exposure effects on populations highlight the importance of explicitly including competition between individuals for energy resources in population models.

The laboratory studies which quantified the effects of EE_2 and trenbolone on individual fecundity did not report exact food rations and it was therefore assumed that the fish were not food limited (OECD, Test No. 229). Given this assumption, it was possible to infer the exact amount by which direct endocrine effects on egg production (r_m) contributed to the observed effect. The model was evaluated to ensure that this pathway resulted in the observed effects on individuals in the laboratory at high food rations. We also know, however, from the AOPs and additional information on metabolic costs, that the maintenance parameter should be increased during the chemical exposures. The only way to infer the exact amount for which the B_0 parameter is increased for each chemical exposure would be to validate model predictions of chemical effects in the laboratory under limiting food rations. Given this, there is some uncertainty when extrapolating the effects of trenbolone and EE_2 to different environmental conditions from those used in the laboratory exposure. However, a sensitivity analysis revealed that model outputs were generally sensitive to this parameter only when it was increased by more than 200% of the value used in the simulations presented here

(Appendix 2). When linking AOPs to bioenergetics models, this will be a recurring limitation because the only way to derive the exact proportions by which each parameter contributes to the adverse outcome is if laboratory studies have quantified effects for multiple food rations (Johnston et al., 2104). This occurs in some cases for invertebrate studies but rarely for fish toxicity tests, for both ethical and practical reasons. Nevertheless, mechanistic models are generally designed to aid in management decisions, rather than providing exact predictions and multiple scenarios can be modelled when uncertainties relating to the chemical mechanism arise. The aim of these simulations was to (i) explore the interaction between environmental conditions and chemical exposures and (ii) explore methods to link mechanistic chemical effect information (e.g. AOPs) with bioenergetics and population models (including highlighting current limitations of this approach).

Recently, a dynamic energy budget (DEB)-IBM was established for the three-spined stickleback (David et al., 2019) and this model was evaluated by comparing outputs to controlled mesocosm data. The accuracy of our presented model could be further assessed against these mesocosm data. Our model however differs from that of David et al. (2019), notably in the methods of incorporating energetics (the use of DEB in the former) and the methods used to incorporate density dependence. David et al. (2019) model density dependence based on the total number/biomass of individuals in the environment, rather than explicitly incorporating food density in a homogeneous landscape. The authors suggest that the DEB-IBM could be improved by modelling spatial variations of the environment so that interactions between individuals (and their environment) can be simulated and resulting density dependence at the population-level predicted. Future work could compare simulated chemical exposure effects on

both models to assess the relevance of different energetics methods and the importance of including food competition explicitly. The possibility for incorporating toxicokinetic-toxicodynamic (TKTD) models could also be explored, to assess for effects on life-cycle traits relating to the chemical uptake, distribution and clearance within the organism.

A limitation of this study was the requirement to use fathead minnow ecotoxicological data in the stickleback population model. Although there are uncertainties associated with this this approach, regulatory risk assessments routinely extrapolate from a very limited number of model species to all fish species in the wild using safety factors. While primary protein target conservation, and target interaction/activation appear to be similar in stickleback and fathead minnow according to *in silico* and *in vitro* tools (see section 2.1), more integrated *in vivo* systems are needed to discern other potential inter-species differences in physiology, behaviour and ecological life history when seeking to make predictions of population-level effects.

Current risk assessments often consider only one stressor at a time because of the difficulties associated with defining, conducting and interpreting multiple stressor studies. In particular, wild fish species in temperate climes are frequently subjected to periods of food limitation (Post and Parkinson, 2001; Shuter et al., 2012); yet the interactive effects of chemical exposures and food limitation are seldom considered explicitly (Holmstrup et al., 2010). Experimental studies have so far displayed some contrasting results, whereby food limitation may exacerbate (Hopkins et al., 2002) or compensate (via the induction of adaptive processes (Hashemi et al., 2008)) chemical exposure effects in individual fish. The results presented suggest that chemical effect assessments need to

583 consider the productivity of the ecosystem, notably food availability when
584 predicting effects at the population-level. General theory proposes that exposing
585 a population to a chemical that impairs population growth when the population is
586 at or approaching carrying capacity can reduce the intensity of intraspecific
587 resource competition, which in turn reduces the impact of the chemical on
588 population abundance (Forbes et al., 2001). This theory has been reported in
589 multiple empirical and modelling studies (e.g. Grant, 1998; Liess 2002; Moe et
590 al., 2002; Hayashi et al., 2009), but is not fully supported by the modelling results
591 displayed here. In contrast, our results suggest a more-than-additive effect where
592 stress caused by exposure to the toxicant can exacerbate density-dependent
593 effects (Forbes et al., 2001).The simulations presented show that effects of EE_2
594 and trenbolone on population abundance (number of individuals) were greater
595 than on population biomass (total mass of the population). Recognising and
596 acknowledging distinctly different responses in population number versus
597 population biomass is crucial for population protection versus preservation of
598 ecosystem functioning. In the latter case, food web modelling (e.g. AQUATOX,
599 Park et al., 2008) is largely concerned with preservation of energy flow and
600 population biomass. It is arguably more challenging to conserve wildlife
601 populations by maintaining their abundance and genetic diversity. It is also
602 difficult to establish the ecological relevance of statistically significant effects on
603 population abundance i.e. establishing if an effect falls within the natural variation
604 for that population (Hamilton et al., 2016). The model shows less variation than
605 data from field populations (largely because environmental conditions are kept
606 the same each year, e.g. Fig 4) so a ‘significant’ reduction in model outputs may
607 not necessarily be ecologically relevant. Nonetheless, even ‘small’ reductions in
608 population abundance as a result of single chemical exposures could be greatly

609 exacerbated by other chemicals (Kortenkamp, 2007), and by physical (e.g.
610 temperature (Gordon, 2003)) and biological (e.g. predation and disease
611 (Rehberger et al., 2017)) stressors. Populations remain the cornerstone of nature
612 conservation and statistically significant reductions in modelled population
613 abundance should therefore be given due consideration in conservative
614 environmental risk assessments (EFSA, 2010).

615 AOPs provide an effective framework for linking molecular initiating events with
616 adverse outcome health outcomes.

617

618 chemicals can cause adverse effects on fecundity by (i) affecting
619 ingestion/assimilation rates, (ii) affecting maintenance costs, (iii) affecting
620 somatic growth costs (including lipid storage) or (iv) directly affecting energy
621 allocation to reproduction (Fig 1). In order to decipher which of these energetic
622 mechanisms are responsible for causing the observed effect for a specific
623 chemical, we used information inferred from AOPs. Linking AOPs to
624 bioenergetics models has been proposed as a promising way to link energetic
625 effects to organismal endpoints (Kramer et al., 2010, Forbes and Galic, 2016;
626 Goodchild et al., 2018; Murphy et al., 2019). It is therefore important to address
627 the current limitations of this approach. In order to link AOPs with energy budgets,
628 assumptions need to be made about the links between energy-regulation
629 processes and the mechanistic pathway of the chemical (Goodchild et al., 2018).
630 As demonstrated in this study, mechanistic data from the literature can be used
631 to help consolidate these energetic links in order to qualify the most plausible
632 mechanisms underlying direct and indirect effects of EACs. In addition,
633 energetics models generally assume linear energy transfer, so that maximum

634 rates of energy allocation for a physiological process (e.g. maintenance) must be
635 met before remaining energy can be allocated to the next process in the
636 energetics sequence hierarchy (e.g. growth for juveniles and reproduction for
637 adult females). This is not necessarily realistic for organisms undergoing
638 physiological stress however as energy trade-offs may occur (e.g. splitting energy
639 allocation between reproduction and growth) (Calow, 1985; Petes et al., 1998;
640 Knops et al., 2001). Trenbolone, for example, is an anabolic steroid known to
641 increase muscle mass and this may contribute to reductions in fecundity as a
642 result of reciprocal energy-trade offs (e.g. between growth and reproduction).
643 Since adult females allocate energy to reproduction prior to growth in the model,
644 the potential for increased muscle mass to contribute to reductions in fecundity
645 was not accounted for in our scenarios.

646 Chemical exposures, and their interactions with other stressors, are threatening
647 the health of freshwater ecosystems and there is a demand for more general (and
648 environmentally relevant) tools to assess multiple stressor scenarios within
649 chemical risk assessments. Here, a fully evaluated model is presented with which
650 the impacts of chemical exposures can be assessed in the context of a fluctuating
651 (and anthropogenically modified) environment for populations of temperate small
652 sized freshwater fish with a short lifespan, relatively low fecundity and a high level
653 of parental investment (i.e. high juvenile survival rate). To investigate how these
654 effects may translate across species with different life-history strategies, further
655 modelling work should focus on comparing chemicals effects across contrasting
656 species. For considerations of ecosystem protection it is necessary also to
657 compare risk across a representative range of species. Integrating mechanistic
658 data, inferred from AOPs and chemical-specific data, into individual-based
659 population modelling can provide an effective and transparent system by which

to link (or at least infer in a more informed manner) adverse effects on organisms to effects on whole populations.

Associated content: The following files are available free of charge.

TRAnsparent and Comprehensive model Evaludation" (TRACE) (PDF)

NetLogo Model

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