

Development and Application of Modelling Approaches for Realistic Assessments on Population Impacts of Endocrine Disruption in Fish.

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'Only within the moment of time represented by the present century has one species - man - acquired significant power to alter the nature of the world.'

Rachel Carson, *Silent Spring*

Abstract

Chemical exposures threaten the health of freshwater ecosystems worldwide. In particular, endocrine disrupting chemicals (EDCs) are of concern because of their ability to cause sub-lethal effects on organisms at low, including environmentally relevant, concentrations. The susceptibility of fish populations to the effects of these chemicals depends on exposure risk, physiological susceptibility, and population resilience. Population models can explicitly incorporate these factors into environmental risk assessments (ERAs) to improve realism and identify potentially vulnerable species. In this thesis, modelling tools were developed and evaluated for the three-spined stickleback (*Gasterosteus aculeatus*) to advance understanding of the ecological relevance of EDC effects.

The thesis begins with a critical review of the current status of EDCs in freshwater ecosystems worldwide and their effects on individual fish and their populations. The potential for different modelling techniques to provide realistic ecological assessments for EDCs is then explored. Individual-based models (IBMs) are used throughout this thesis to provide case study specific chemical assessments. Reproductive endpoints, including disruption of breeding behaviours, are used to extrapolate EDC effects from individuals to the population level. Findings included the importance of considering behavioural endpoints within chemical assessments, since disruption of breeding behaviours caused significant reductions in population abundance. Moreover, it was identified that the breeding strategy of the stickleback makes it particularly vulnerable to chemicals which directly affect reproductive output. The chemical exposure regime and density dependent processes determined whether the population recovered post-exposure.

Empirical experimental exposures were used to investigate the interactive effects of EDC exposure and food limitation on somatic growth in early life stages. An energy budget model was then developed and used to further explore the mechanisms underlying this observed effect. The combined empirical and modelling results suggested that fish can adapt their physiology (by reducing physical activity) to cope with the effects of multiple stressors.

Finally, in order to explicitly incorporate environmental conditions into population level assessments, a model was developed combining the energy budget model with the stickleback IBM. This model allows analyses on direct effects of the EDC as well as the additional metabolic effects associated with the chemical exposure. The effects of two case study EDCs (an oestrogen and an androgen) on individual fecundity were simulated in low and high food availability environments in order to explore how environmental conditions affect population susceptibility. The findings illustrate that the underlying mechanism of the EDC effect and environmental conditions can affect the susceptibility of populations to EDC exposures.

This thesis has developed novel models for use by both researchers and risk assessors for application in realistic population level assessments of chemical risks. The findings presented have important implications for understanding the ecological relevance of EDC exposures for fish populations.

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Chapter 1. Capturing Ecology in Modelling
Approaches Applied to Environmental Risk
Assessment of Endocrine Disrupting
Chemicals in Fish

Abstract

Endocrine disrupting chemicals (EDCs) are widespread in freshwater environments and both laboratory and field based studies have shown reproductive effects in fish at environmentally relevant exposures. Environmental risk assessment (ERA) seeks to protect wildlife populations and prospective assessments rely on extrapolation from individual-level effects established for laboratory fish species to populations of wild fish using arbitrary safety factors. Population susceptibility to chemical effects, however, depends on exposure risk, physiological susceptibility, and population resilience, each of which can differ widely between fish species. Population models have significant potential to address these shortfalls and to include individual variability relating to life-history traits, demographic and density-dependent vital rates, and behaviors which arise from inter-organism and organism-environment interactions. Confidence in population models has recently resulted in the EU Commission stating that results derived from reliable models can be considered when assessing the relevance of adverse effects of EDCs at the population level. This review critically assesses the potential risks posed by EDCs for fish populations, considers the ecological factors influencing these risks and explores the benefits and challenges of applying population modelling (including individual-based modelling) in ERA for EDCs in fish. We conclude that population modelling offers a way forward for incorporating greater environmental relevance in assessing the risks of EDCs for fishes and for identifying key risk factors through sensitivity analysis. Individual-based models (IBMs) allow for the incorporation of physiological and behavioral endpoints relevant to EDC exposure effects, thus capturing both direct and indirect population level effects.

1. Introduction

Endocrine disrupting chemicals (EDCs) represent a class of chemicals with the potential to alter functions of the endocrine system, consequently causing adverse health effects in an intact organism, its progeny, or (sub) populations (Bergman et al., 2012). Entry of EDCs into freshwater environments may occur *via* point source discharges of domestic or industrial effluents and/or from

diffuse land run off from roads and agriculture, and are of increasing environmental concern due to widespread reports of effects on wildlife, including fish (Guillette Jr et al., 1995; Jobling et al., 1998; Matthiessen and Gibbs, 1998; Berg et al., 2016). Reproductive effects in fish resulting from EDC exposure have been reported widely, and they include physiological alterations in gonads resulting in intersex (presence of both male and female structures within the same gonad (Jobling et al., 1998; Tetreault et al., 2011; Jobling et al., 2002)), alterations in reproductive behavior (Weis and Weis, 1974; Mathers et al., 1985; Brown et al., 1987; Saglio and Trijasse, 1998; Bell, 2004) and/or reproductive output (Ankley et al., 2003; Nash et al., 2004; Paulos et al., 2010), each of which can impair individual reproductive success (Jobling et al., 2002; Harris et al., 2011; Tyler et al., 2012; Hamilton et al., 2015). However, it is less clear how these individual effects may impact the sustainability of fish populations in the wild. Studies on one fish species, the roach (*Rutilus rutilus*), in English rivers have shown widespread feminization in males due to exposure to natural and synthetic oestrogens from wastewater treatment works (WwTW) effluent (Jobling et al., 1998; Jobling et al., 2002), but a genetic analysis of populations of wild roach exposed to WwTW effluent in a UK river catchment indicated no effect on the size of the effective breeding populations in those rivers i.e. they were self-sustaining (Hamilton et al., 2014).

Nevertheless, given that physiological effects seen, such as intersex, are considered to be adverse, that they can be induced following controlled exposure to individual EDCs, and the effects of multiple EDCs can, in some instances, be additive (Thorpe et al., 2001; Kortenkamp, 2007; Backhaus and Faust, 2012) it is possible that EDCs may impact at the population level in some fish species.

Current approaches for the environmental risk assessment (ERA) of chemicals, including EDCs, lack certainty for protecting wildlife populations because of differences in species sensitivity, natural variability in population numbers over time, differences in density dependent regulation, and difficulty in defining adverse (unsustainable) population level effects (Hamilton et al., 2016). Typically ERA relies on the application of (often arbitrary) assessment, or uncertainty, factors to extrapolate from laboratory derived no observed effect

concentrations, in model test organisms, to the protection of wild populations. To reduce the reliance on assessment factors, higher tier tests may be conducted for some chemicals in the form of semi-natural single- or multi-species ecosystem studies (micro-/mesocosms). These higher tier studies, however, are expensive, time consuming, can be complex to interpret and often demonstrate low statistical power. Furthermore, micro- and mesocosm experiments may not account adequately for vital ecological processes (e.g. density dependence) and environmental variation (Galic et al., 2010), and very few of these studies have included fish (Giddings et al., 2002).

Typically, fish species are chosen for ERA based on a combination of their physiological sensitivity to chemicals, species-specific information (e.g. genomic resources available), ease of maintenance in aquaria (e.g. fathead minnow, *Pimephales promelas*; zebrafish, *Danio rerio*; rainbow trout, *Oncorhynchus mykiss*) and the ability to measure effects on partial or whole life-cycles in short timescales (e.g. Japanese medaka, *Oryzias latipes*). Some species are used routinely also because of developed biomarker assays that indicate exposure to certain classes of EDCs, for example vitellogenin for oestrogens (all egg laying fish species (Tyler et al., 1996)) and spiggin (a glue-like protein used for nest building in the three-spined stickleback) for assessing (anti) androgenic chemicals (Katsiadaki et al., 2002). Sensitivity of individual fish to an EDC depends on their innate and environmentally mediated physiology, the inherent potency/toxicity of the chemical, the exposure concentration and the timing of the exposure relative to the fishes life-cycle. However, at the population level many other factors influence sensitivity, including fecundity, density dependence, and both abiotic (e.g. water physiochemistry) and biotic (e.g. prey and predators) environmental conditions. Exposure likelihood and population resilience are dependent upon ecological life-history strategy and population level interactions (Van Straalen et al., 1992; Brown et al., 2014). Fish breeding strategies, lifespan and habitat preferences that can affect population resilience are only considered (often arbitrarily) within safety/assessment factors during risk assessments. It is possible that population level processes may mitigate, *via* compensatory density dependence, or exacerbate, *via* depensatory density dependence, the effects of chemical exposure in the wild, but these processes

are difficult to quantify and are not therefore explicitly considered in current ERA schemes.

In this review, we critically assess the potential for adverse impacts on fish populations exposed to EDCs in the wild and the factors affecting their susceptibility. We then assess the applicability (strengths and weaknesses) of individual-based population modelling as a method to provide more integrative assessments of chemical effects in fish within ERA schemes.

2. Exposure to EDCs and potential consequences in fish

EDCs represent a potential threat to aquatic vertebrates, including fish, as they are capable of altering pathways of hormone biosynthesis, metabolism and/or excretion, or binding to and modulating hormone receptors (Swedenborg et al., 2009). The most widely studied EDCs include the environmental (anti)oestrogens, (anti)androgens, aromatase inhibitors, and progestins (Tyler et al., 1998; Hutchinson et al., 2006; Goodhead and Tyler, 2009; Swedenborg et al., 2009). Entry of EDCs into freshwater environments can occur through a wide variety of sources including domestic and industrial waste discharges (Petrovic et al., 2002) and agricultural runoff (Khatun and Mahanta, 2014). A number of naturally occurring EDCs also exist in aquatic environments including, endogenous human hormones (Chang et al., 2009), phytoestrogens (Rearick et al., 2014) and mycotoxins (Molina-Molina et al., 2014). Environmental concentrations of oestrogenic EDCs within sewage effluents and surface waters are widely documented. One of the more potent synthetic oestrogens, 17 α -ethinylestradiol (EE₂), used in the contraceptive pill, has been reported in effluents ranging between < 0.2 ng L⁻¹ (Desbrow et al., 1998) and 42 ng L⁻¹ (Ternes et al., 1999) and in surface waters from below limits of detection of 0.01 ng L⁻¹ (Hintemann et al., 2006) up to concentrations of 273 ng L⁻¹ in some streams in the USA (Kolpin et al., 2002). Hannah et al. (2009), however, reported that predicted environmental concentrations in typical surface waters in Europe and the USA are estimated at 0.2 and 0.3 ng L⁻¹, respectively, and are considered unlikely to exceed 9 ng L⁻¹ in effluent discharges. Reproductive impairments, including feminization of male fish and reduced reproductive success, have been demonstrated in the lab after exposure to concentrations of steroid oestrogens within environmentally relevant ranges (e.g. EE₂ ranging

from $< 1 \text{ ng L}^{-1}$ up to 5 ng L^{-1} (Nash et al., 2004; Parrott and Blunt, 2005; Lange et al., 2008; Zha et al., 2008; Armstrong et al., 2015)) and the incidence and severity of intersex (occurrence of ovo-testis) in male roach sampled from a series of UK Rivers is significantly correlated with predicted concentrations of steroid oestrogens (EE_2 concentrations ranging from 0 to 0.37 ng L^{-1} (Jobling et al., 2005)).

For androgens, the most widely reported effects in fish for environmentally relevant exposures are for the steroid trenbolone, used as a growth promoter in beef cattle in the US, South America and Australia. Aqueous exposure concentrations of trenbolone between $9.2 - 26.2 \text{ ng L}^{-1}$ have been shown to cause male skewed sex ratios and masculinization of female zebrafish (Morthorst et al., 2010). Androgen antagonists appear to be widespread in effluent discharges from UK sewage treatment works with potency of between 21.3 and $1231 \text{ } \mu\text{g L}^{-1}$ flutamide equivalents as assessed using a yeast (anti-) androgen screen (Johnson et al., 2007). Anti-androgenic activity at levels measured in some sewage treatment works effluents have been shown to disrupt reproductive behavior and spiggin production in male stickleback (Sebire et al., 2008) and cause reduced fecundity in fathead minnows (Jensen et al., 2004).

A diverse range of chemicals have been identified that act as aromatase inhibitors (that affect sex hormone biosynthesis) with reproductive effects in fish, including for exposures to environmentally relevant concentrations (e.g. tributyltin (McAllister and Kime, 2003); clotrimazole (Brown et al., 2015)); progestins, synthetic analogs to progesterone (Svensson et al., 2014) have been reported to cause reproductive impairments in fish, including reduced fecundity (Paulos et al., 2010) and masculinization of female fish. Some progestins also act as androgens, (Zeilinger et al., 2009; Runnalls et al., 2013; Svensson et al., 2014) and have been shown to alter secondary sex characteristics (Svensson et al., 2014) in the concentration range measured in some aquatic environments (measured concentration ranges between 1 and 199 ng L^{-1} (Kolpin et al., 2002; Petrovic et al., 2002; Andersson et al., 2005; Viglino et al., 2008; Vulliet et al., 2008; Al-Odaini et al., 2010; Chang et al., 2011; Svensson et al., 2014). Although the reported reproductive effects for all

of these chemicals in individuals have the potential to result in population level effects this has received little empirical study. Furthermore, population level studies have focused almost exclusively on oestrogens.

An experimental study has shown population level effects of EE₂ in a Canadian lake that was dosed at 4–6 ng EE₂ L⁻¹ for 3 years (Kidd et al., 2007). This resulted in delayed ovarian development and the subsequent collapse of a fathead minnow (FHM) fishery. Fathead minnow spawn annually and have a relatively short lifespan of 2-3 years. In contrast there was no evidence for reproductive failure in the pearl dace (*Margariscus margarita*), an annual spawning fish with a lifespan of up to 7 years. This indicates life-history characteristics could be important in determining species risk to EE₂. Evidence for indirect effects of EE₂ were also seen in the Canadian lake study with subsequent declines in the predatory lake trout (*Salvelinus namaycush*) as well as increases in the zooplankton and emerging insects (e.g. Chaoborus) on which FHM prey (Kidd et al., 2014). These findings constitute an ecosystem level effect of EE₂, however, it should be emphasized that the dosing level adopted (4–6 ng EE₂ L⁻¹) is higher than occurs for most undiluted wastewater treatment works (WwTW) effluent discharges (Desbrow et al., 1998; Belfroid et al., 1999; Larsson et al., 1999; Ternes et al., 1999).

Although single chemical exposures give a good indication of potential effects based on the mode of action of that chemical, surface waters generally receive inputs of mixtures of EDCs, resulting in some fish populations being subject to the combined effects of multiple EDCs with potentially additive effects (Silva et al., 2002; Brian et al., 2005; Correia et al., 2007). Furthermore, mixed chemical exposure effect outcomes can differ significantly than for single classes of EDCs. As an example of this, in laboratory based exposures of roach the feminizing effects of a mixture of antiandrogens and EE₂ in combination was far greater than that for either the antiandrogens or EE₂ separately (Lange et al., 2011). The interactive effects of chemicals are now being measured directly in an increasing number of research studies (e.g. exposure to sewage effluents (Lange et al., 2011; Hamilton et al., 2015)) and risk assessment schemes for pesticides now consider the potential cumulative effects of similarly acting compounds (Regulation (2013) No 284/2013). Detecting the effects of low-dose

exposure is another major issue in the study of EDCs. Low-dose effects can be defined as any biological changes which occur at doses lower than those typically used in standard testing protocols (Melnick et al., 2002); consequently, effects at these concentrations are easily overlooked in traditional risk assessments. In order to capture low-dose mixture effects, it has been suggested that regulatory testing needs to incorporate biomarker endpoints rather than traditional dose-response relationships alone, (Kortenkamp, 2008). The US EPA requested the development of a strategy to address the current issues associated with detecting low-dose effects for EDCs (National Academies of Science, Engineering and Medicine, 2017) and which informs regulatory bodies of the appropriate actions, e.g. updating chemical assessments, which should be taken if a chemical is found to incur low-dose effects. Incorporating scenarios for possible low dose effects in modelling for EDC effects has not yet received major attention due to uncertainties into where these effects may occur and for what EDCs.

Collectively, laboratory and (limited) field studies for selected environmental oestrogens suggest that they can impact some wild fish at the individual level with potential for subsequent impacts on the population. Quantifying the effects of EDC exposure at the population level more generally, however, is extremely challenging. Challenges in ERA include major uncertainties in extrapolating effects from a narrow range of model species used within regulatory assessments to the extremely diverse range of existing fish species (~28,000 fish species are known to be extant worldwide (Nelson et al., 2016)) and the lack of accurate data on fish abundance. The latter is lacking generally for freshwater fish and many years of monitoring data are required to be able to determine accurately if a population decline is a result of a natural fluctuation or a stressor response (Hamilton et al., 2016).

3. Assessing population susceptibility

Overall, population susceptibility to chemicals is characterized by the risk of chemical exposure, the physiological sensitivity of individuals within a population, and overall population resilience. In natural populations, species evolve life-history strategies for sustaining a viable population in specific habitats (Spromberg and Birge, 2005, Wootton, 1992) and as a consequence

different species, and different populations of the same species in different geographical regions, may exhibit different susceptibilities to EDCs. This highlights the need for ERA to consider both inter- and intra-species differences in life-history traits.

3.1. *Probability of chemical exposure*

Population level risk of chemical exposure is affected by habitat preferences (e.g. pelagic, demersal), feeding ecology (e.g. bioaccumulation of chemicals through the food chain), and/or migratory behavior (Kirby et al., 2004), as well as factors such as lifespan and fecundity. Overall risk of exposure is determined by the life-history strategies and the susceptibility for effects for all the different life stages combined. Additionally, exposure can be highly variable, both spatially and temporally, depending on the exposure source; inputs of effluent discharges often result in a continuous exposure, compared with agricultural runoff where exposure is largely intermittent (Holt, 2000). The exposure scenario can therefore affect the likelihood and intensity of population exposure.

Using the US EPA's AQUIRE database Baird and Van den Brink (2007) suggested an organism's sensitivity to chemical stress can be predicted from species traits relating to morphology, life-history, physiology, and feeding ecology. Their findings suggested that species possessing predatory behavior and with a long life-cycle were most susceptible to chemical exposure. Similarly, evaluating five different life-history scenarios, Spromberg and Birge (2005) established that the factors most likely to reduce population vulnerability included the following life-history traits: short lifespan, short time to reproductive maturity, parental guarding behavior, and a large number of spawning events. These trait based approaches, however, are not supported by the long-term field study for exposure to EE₂ described above (Kidd et al., 2007) where effects on FHM populations were more significant than for effects on the longer lived pearl dace. Because trait-based assessments do not incorporate vital population level processes (density dependence) or individual variability, they may misrepresent species susceptibility and more integrated approaches, such as population modelling, are likely to be more effective (Brown et al., 2005).

3.2. *Physiological sensitivity*

Sensitivity of individuals to chemical effects within populations varies depending on age, reproductive status, growth rate, and habitat type. Life stage sensitivity will depend on the process affected by the chemical and the temporal exposure profile. The most studied effects of progestins, (anti)androgens, aromatase inhibitors, and oestrogens occur for exposures during sexual maturation, in reproducing adults, and during sexual differentiation (Jobling et al., 2002; Brian et al., 2006; Sebire et al., 2009; Zeilinger et al., 2009; Runnalls et al., 2013; Brown et al., 2014; Svensson et al., 2014). Latent effects for exposures have also been observed; for example, exposure of three spined sticklebacks to ethinylestradiol during early life was observed to subsequently affect breeding behavior in adults (Maunder et al., 2007). Furthermore, longevity of exposure will also impact on potential for effects. As an example, exposure of adult zebrafish to EE₂ (5 ng L⁻¹) for 40 days resulted in no effects on reproductive output, but exposure to the same concentration continuously from embryo to sexual maturity caused complete reproductive failure (there were no egg fertilizations (Nash et al., 2004)). Only in fish full-life cycle (FFLC) tests are the physiological sensitivities to chemicals captured fully. A FFLC test is a requirement for some active ingredients in pesticides (according to EC No 1107/2009 and EC No 283/2013) but they are resource and animal intensive and are rarely used in the routine testing of EDCs (Ankley and Johnson, 2004). It can also be argued that a constant chronic exposure in a FFLC test may represent a worst case scenario as under natural conditions the chemical exposure may fluctuate (be intermittent) and specific life stage behaviors may result in chemical avoidance.

3.3. Population resilience

Population resilience determines the capacity for a population to withstand and recover from disturbances. The regulation of fish population numbers is primarily determined by compensatory density dependent mechanisms (Beverton and Holt, 1957; Ricker, 1987), which result in a slowed population growth at high densities, due to predation, disease and/or increased competition for resources, and conversely an increase in population growth at low densities, due to reduced competition and predation (Rose et al., 2001). Life-history processes are considered to be density dependent if their rates change as a

result of the density (or number) of individuals in a population e.g. individual growth, mortality or reproduction. Population dynamics studies (variation in population numbers over time), indicate that the majority of wildlife populations, including fish, are regulated by density dependent (DD) biotic interactions (Brook and Bradshaw, 2006). This regulation underlies the management of fish populations (Rose et al., 2001) and is exploited throughout fisheries worldwide to permit sustainable yields.

Densatory density dependence, on the other hand, results in a reduced per capita population growth at low densities (Liermann and Hilborn, 2001) as, for example, a result of reduced rates of survival and reproduction (Allee and Alle, 1958; Wood, 1987; Fowler and Baker, 1991). Fish schooling is an example of a densatory mechanism at low densities as it relies on the congregation of numerous fish to increase survival or reproductive success (Marsh and Ribbink, 1986). As such densatory density dependence could exacerbate the effects of chemical exposure at low population densities. As an example, some EDC exposures have been shown to reduce schooling behavior in zebrafish (Xia et al., 2010) and juvenile rainbow trout (Ward et al., 2006); it is therefore possible that densation could reduce population growth rates during EDC exposure, by reducing schooling behavior. Although there is evidence for the occurrence of densation in fish populations (Wood, 1987; Myers et al., 1995), its possible role in exacerbating the effects of chemical exposure has received very little study. This is because densation is difficult to detect as many populations rarely reach such low population levels. Even when they do the effects of demographic and environmental stochasticity may be neutralizing the ability to observe such impacts (Liermann and Hilborn, 2001). The strength of density dependent mechanisms within populations can therefore play a fundamental role in determining the susceptibility versus resilience of a population to chemical exposure.

Forbes et al. (2001) suggested that the mitigating role of compensatory density dependence often leads to reduced level of effects on populations when compared with effects on individual life-cycle traits. As a consequence, it is possible that current extrapolation methods from individuals to population in ERA may be over-protective. Empirical studies on invertebrates have indicated

that exposing a density-limited population (at or approaching carrying capacity) to a toxicant, which reduces survival, growth and/or reproduction, can reduce the intensity of intraspecific competition and/or predation thus compensating for the toxicant-induced reduction in vital rates (e.g. growth, reproduction or survival), and thereby reducing the impact on the population as a whole (Liess, 2002; Moe et al., 2002). It has also been suggested that a toxicant could remove less fit individuals within a population, promoting population growth and population fitness (Calow et al., 1997). Population modelling studies have supported this theory. As an example, Grant (1998), applying life-table response experiments, showed that substantial reductions in some vital rates, as a result of toxicant exposure, were compensated for by density dependence in the copepod *Eurytemora affinis*. Applying matrix models Hayashi et al. (2009) similarly demonstrated that toxic impacts of zinc on populations of the fathead minnow and brook trout (*Salvelinus fontinalis*) depended largely on the strength of density dependence and differences in life histories. However, empirical studies investigating the role of density dependent processes in the population resilience of fish subjected to chemical exposure are lacking and are much needed to help build confidence in the modelled examples. Furthermore, it should be emphasized that chemical resistance in individuals does not necessarily always equate with desired traits for population relevant measures of fitness.

4. Population modelling approaches and incorporating susceptibility and resilience into assessments of EDC effects in fish

Generally, the protection goals for EDCs and other chemicals set out to try to ensure no adverse effects occur for ecosystems and the environment as a whole and the protection of populations is the focus for this (Brown et al., 2016). Models which predict the effects of chemical exposure on individuals can provide highly specific predictions of chemical effects. For example, toxicokinetic / toxicodynamic (TK/TD) models can be used to assess chemical modes of action within individuals; trait-based assessments are useful in identifying species sensitivity based on life-history strategy; energy budget models allow physiological processes, such as metabolic rate, to be incorporated into chemical assessments. However, none of these methods can

provide predictions on how chemical exposure may impact whole populations and are therefore limited as tools when used on their own. Population models, on the other hand, provide tools for extrapolating from individual- to population-level effects, including exploring the importance of interactions between individuals and between individuals and their surrounding environments (Forbes et al., 2009). The choice of model within chemical assessment is dependent upon the specific questions addressed in the risk or hazard assessment schemes and on the level of species-specific detail required, how broad an application or ecological scenario is desired, and the amount of empirical data available (Fig. 1).

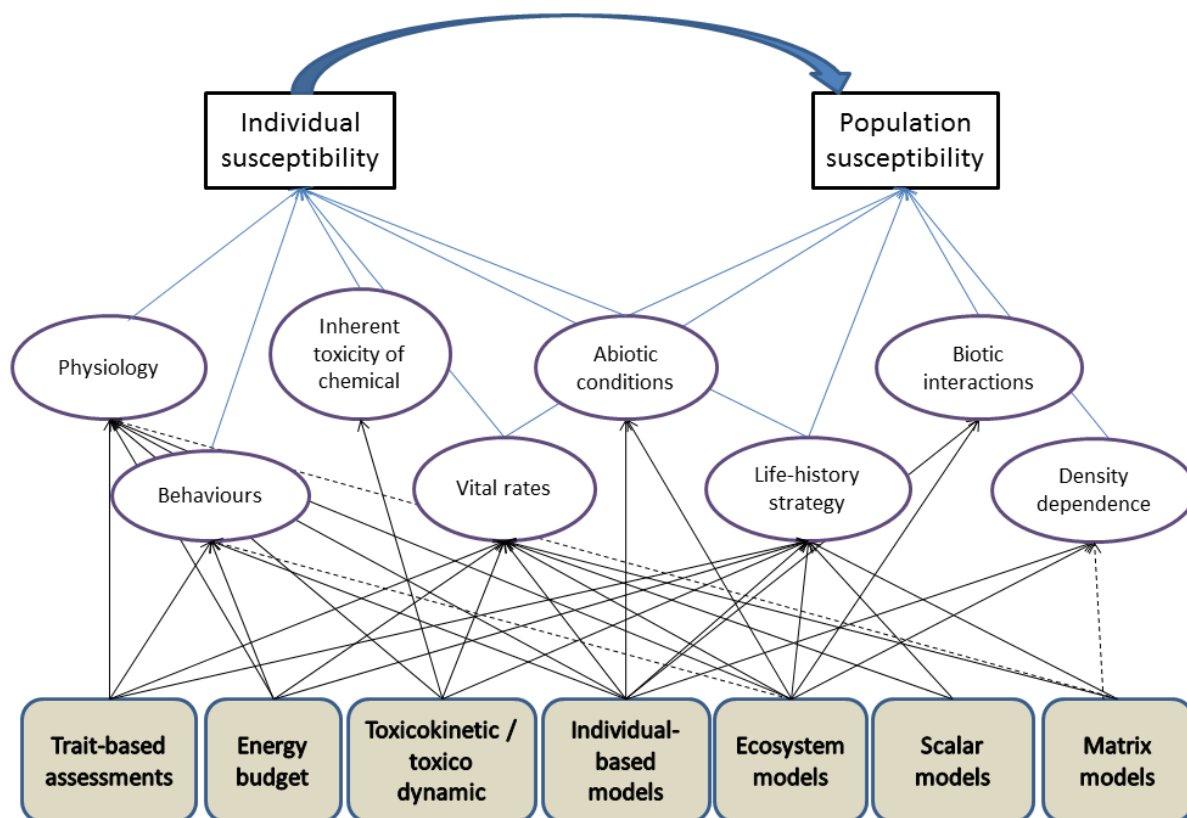


Figure 1. Conceptualisation of the factors / processes which affect individual- and population- level sensitivity to toxicant exposure (blue arrows) and the category of model which incorporates each of these factors/ processes (black arrows). Dotted arrows represent processes which can be incorporated by a model type but are not usually included due to associated complexities.

Scalar modelling has been used in ecology since the 1700s (Malthus 1926). Scalar models have a wide application within fisheries to estimate population recruitment (Ricker, 1954; Beverton and Holt, 1957), growth rates (Von Bertalanffy, 1957) and fecundity (Carlander, 1997), and are relatively easily adapted for use in chemical assessments. However, scalar models provide very simplistic estimates of population processes only because they represent the whole population as a single entity i.e. every process is taken as an average of the whole population. Their interpretation regarding chemical assessments should therefore be approached with caution as they do not include any population level processes and they do not incorporate individual or age/stage based variability.

Age/ stage based (matrix) models are one of the most common methods for analyzing the potential for chemical-induced population level effects, allowing population growth of individual age classes to be quantified using vital rates (fecundity, growth and mortality). Matrix models take vital rates as static values for each age/stage class meaning that they are more integrative than scalar models. They also benefit from their low data requirements and are therefore relatively easy to parameterize. However, similar to scalar models, they remain constrained when incorporating inter-organism and organism-environment interactions, and spatial and temporal variability (Caswell, 2001). These benefits and shortfalls are illustrated in a number of matrix modelling studies (life-table response experiments) which use simple age-based models to assess the potential susceptibility of different fish species to chemicals, including EDCs. Ibrahim et al. (2014), for example, using matrix models provided general predictions of species susceptibility to pesticide risk for a large number of species with relatively low data requirements. The most vulnerable species identified were the minnow, *Phoxinus phoxinus*, the lamprey, *Lampetra planeri* and pike, *Esox Lucius*. These findings however have not been validated empirically. Most studies assessing chemical effects using matrix models have not included validation against field data (Miller and Ankley, 2004; Hayashi et al., 2009; Brown et al., 2014; Ibrahim et al., 2014). Furthermore, matrix models do not generally incorporate density dependent processes or individual variability and thus the level of realism is relatively low.

Individual-based models (IBMs), in contrast, are spatially explicit and benefit from the ability to incorporate ecological processes and life-history strategies including interactions between competing/cooperating individuals within single or interlinking populations. In comparison with mathematical-based/ matrix models, IBMs predict how vital rates (i.e. fecundity, growth, mortality) vary with environmental conditions and interactions with other individuals, allowing the population dynamics to emerge based on these interactions. Therefore, IBMs may provide a better approach to ERA of EDCs, as they allow the impacts of these other factors to be incorporated, and are discussed in detail in the next section.

Ecosystem models include the highest levels of biological organization incorporating interacting species populations, food webs and communities (Galic et al., 2010). They are the most complex and are often the most integrative modelling strategy used in chemical assessment (e.g. AQUATOX (Park et al., 2008)). However, ecosystem models can be limited by their low levels of tractability and few of those developed include uncertainty and sensitivity analysis (Bartell et al., 2003). AQUATOX is perhaps the most comprehensive ecosystem model available and is used regularly in the assessment of chemical effects by the US environmental protection agency (Park et al., 2008). Although ecosystem models benefit from their ability to represent a complete aquatic system and a wide breadth of ecological processes, as a consequence species specific behaviors or traits are often neglected or under-represented. This is particularly important for EDC effect analyses, as many EDCs affect specific behaviors (e.g. breeding behavior) or processes. Furthermore, the time required to develop ecosystem models and large amounts of data required to do so (both biotic and abiotic) will limit the development of new ecosystem models. From the outset it is important to identify the necessary model complexity and specificity required to achieve sufficiently accurate levels of risk as defined by risk managers (Bartell et al., 2003).

4.1. Individual-based models

IBMs are a population and community modelling approach that allow for a high degree of data complexity from individuals and of interactions among

individuals, each of which are treated as unique and discrete entities (DeAngelis and Grimm, 2014). IBMs have been used widely within ecology and conservation since the 1970s and have a good degree of realism, which makes them suitable for use in higher tiers of ERA (Galic et al., 2010). They can also deal with spatial heterogeneity and individual variability (Hölker and Breckling, 2001). Crucially, they enable the integration of a wide range of factors essential for the simulation of realistic population level effects including (1) chemical exposure (*via* spatial tools), (2) physiological processes (they can link directly to TK/TD models) and (3) population resilience emerging from density-dependent interactions between individuals within a population and interactions with their surrounding environment (including chemical contaminants). IBMs are a pragmatic approach towards more complex population modelling, as they bridge the gap between individual effects observed in toxicity studies and the potential consequences on wild populations. The implementation of additional sub-models into IBMs is an approach often adopted to develop greater realism (accuracy). These sub-models include TK/TD models, which allow incorporation of ADME (absorption, distribution, metabolism, and excretion) and internal damage and repair processes into ERA (Liu et al., 2014); fate models, which can be used to predict the fate of chemicals within aquatic water bodies (Focks et al., 2014); and energy budget models, which can simulate mechanistic pathways underlying chemical effects (Sibly et al., 2013).

Population models require guidance and standardization for their development and validation, and communication for their subsequent uptake and acceptance into ERA (Schmolke et al., 2010b). This has been facilitated by a European funded project, CREAM (Mechanistic Effect Models for Ecological Risk Assessment of Chemicals) that has produced several IBMs assessing the effects of various chemicals on a range of taxa (Gabsi et al., 2014; Kułakowska et al., 2014; Liu et al., 2014). However, IBMs which assess chemical effects on fish populations are relatively few compared to other (invertebrate) taxa (Focks et al., 2014; Gabsi et al., 2014; Johnston et al., 2014; Meli et al., 2014). This is likely because life-history data for shorter-lived invertebrates are relatively easy to obtain, making model development and validation more tractable. However, given that population models can now be used to help inform on the

environmental risk and identification of EDCs (EC No 2017/2100 and EC No 2018/6054), in combination with experimental evidence of individual and population level effects in fish (Huestis et al., 1996; Jobling et al., 1998; Kidd et al., 2007), practical challenges for the development of IBMs for fish need to be addressed: i.e. selection of appropriate species and populations, data availability for parameterization and validation.

The relevance and reliability of population models can be established through appropriate model evaluation. Schmolke et al. (2010a) summarized some of the methods of evaluation including: model verification (model outputs compared to data used for parameterization), sensitivity analysis (testing the influence of input parameters on model outputs), and validation (model predictions compared to empirical laboratory and/or field data). Validation is of particular importance because it demonstrates the structural realism of the model as well as the accuracy of parameterization (Schmolke et al., 2010a). However, validation is not always straightforward because empirical data are not always available. In a study which evaluated 62 models dealing with toxicant effects for a range of taxa, Schmolke et al. (2010a) found that only 3% of models were validated against independent empirical data. Validation of fish models is restricted by the fact that there are very few datasets which provide long term information on fish populations and their natural fluctuations, and even less data for chemical effects exposures (Hamilton et al., 2016). In the absence of long-term population dynamics, data validation may be permitted using population census data which provide a snapshot of a population size/ age distribution, as demonstrated by Hazlerigg et al., (2014). This, however, does not account for variation in year class strength. In cases where data on population dynamics do not exist, Augusiak et al., (2014) suggest that a thorough evaluation, including validation of sub-models, can in some cases be sufficient to assess a model's realism in the absence of a full validation. However, this is debatable, and access to population dynamics data for wild fish populations needs to be a key priority when assessing the realism of IBMs.

There is a trade-off between more general models which incorporate a greater range of processes and interactions (i.e. community or ecosystem models), and models which do not necessarily represent a whole system but provide more

specific outputs. Population models are constrained by their ability to represent only single species populations i.e. a discrete population within a defined waterbody or an interconnected meta-population in a larger watershed, resulting in species- and (meta) population- specific outputs. It can be argued therefore that each model is only applicable to a defined set of scenarios and natural environments. Nevertheless, predator-prey interactions (Lorenzen, 1996) and climatic/seasonal variations in habitat selection, growth, and mortality (Railsback and Harvey, 2002) can be factored in. IBMs require a high of level of detail which ultimately results in a more accurate output for a specific exposure scenario compared with ecosystem models. The future development of IBMs should therefore be targeted by focusing on characteristics and life-history strategies influencing ecological sensitivity when considering species selection (Topping, 2014). The choice and specification of IBMs is likely to be guided by the future development of ecological scenarios for ERA within international programs (e.g. those coordinated by the European Federation of Chemical Industries (CEFIC) on-going long-range research initiative (LRI ECO28) and OECD). With regard to assessing the effects of EDC exposure on fish, IBMs currently represent the most viable modelling strategy because of their ability to capture species-specific and emergent effects, resulting from changes to ecological interactions, such as disruption of breeding behavior.

4.1.1. Incorporating behavioral effects into the assessment of EDCs

In the context of fish and EDCs, a strong feature of IBMs is their ability to incorporate aspects of an individual's behavior. However, despite experimental evidence documenting the effects of chemical exposure on fish behavior and possible impacts on individual fitness (Scott and Sloman, 2004; Valenti Jr et al., 2012; Brodin et al., 2014; Dzieweczyński et al., 2014; Klaminder et al., 2014), ERA schemes have not yet begun to explicitly measure the effects of behavioral changes in fish as an endpoint for chemical effects. Territoriality, courtship and guarding of eggs and fry within fish are characteristics seen for a number of species (e.g. three-spined stickleback, *Gasterosteus aculeatus*; fathead minnow, *Pimephales promelas*; sand goby, *Pomatoschistus minutus*) and chemical-induced behavioral impairments of these traits can have significant impacts on young survival rates (Wibe et al., 2002; Brian et al., 2006; Sebire et

al., 2008; Saaristo et al., 2010). This, in turn, could have population level effects, however, the actual relevance or impacts of such effects at the population level have received very little study. This is particularly relevant in the assessment of EDCs because of their reported effects on reproductive behavior (Weis and Weis, 1974; Mathers et al., 1985; Scholz and Gutzeit, 2000; Balch et al., 2004; Bell, 2004; Sebire et al., 2008; Söffker and Tyler, 2012). Similarly, anti-predator behavior is a vital survival trait in virtually every species, and impairments have been documented as a result of EDC exposure, for example in killifish (Weis et al., 2001). Reported declines in fish schooling behavior have also been observed in several fish species after exposure to various EDCs (Ward et al., 2006, Xia et al., 2010) and population declines could emerge from these effects *via* increased predation/ reduced feeding success.

These reported behavioral effects and their potential impacts at the population level are not taken into consideration within ERA schemes because they are both difficult to quantify and interpret. Incorporating behavioral effects into IBMs can be achieved through the incorporation of an energy budget model (Stillman and Goss-Custard, 2010; Sibly et al., 2013), foraging arena theory (Christensen et al., 2005) or, more simply, by using a set of simple physical and biological parameters. For example, basic decision rules and strategies including 'prey perception length' and 'panic distance' have been applied by Vabø and Nøttestad (1997) in an IBM in which they investigated the anti-predator behavior of herring schools. This approach may not be as accurate as an energy budget model approach but it does not require a high density of empirical data. Furthermore, the overall model was found to validate well against wild data regarding the ability to mimic anti-predator strategies e.g. shoaling, splitting. Other approaches for incorporating aspects of behavior into IBMs have included the use of neural networks (a method that applies neurobiological principles of synaptic brain-activity to model behavioral outputs, (Rumelhart et al., 1988; Montana and Davis, 1989) and genetic algorithms (Huse et al., 1999). Simulations run with a model developed by Huse et al. (1999) using this method looked promising, but again validation was not undertaken against empirical data.

Since behavioral effects are both difficult to detect and quantify in the field, IBMs present a tool to extrapolate these effects from laboratory studies to possible effects in the field. An example of an IBM which assesses the behavioral effects of chemical exposure in mammals is described by Liu et al. (2013). The model validated well against field data and was subsequently used to predict the effects of pesticide exposure on the spatial dynamics of the wood mouse (*Apodemus sylvaticus*), with a focus on the effects of varying home range. However, there are currently no published IBMs which incorporate complex behaviors into chemical risk assessments for fish and these are needed for further assessment of EDCs which have been shown to affect behaviors.

5. Conclusions

Environmental risk assessment of chemicals, including EDCs currently fails to account explicitly for factors which affect species and population susceptibility (risk of exposure, innate physiology, population resilience). For example, the assessment of species specific traits and behaviors, and their roles in determining the direct and indirect effects of EDCs on individual organisms and their interactions within populations are currently neglected, despite their potential importance in determining population effects. The need to address these knowledge gaps is emphasized by a growing number of publications reporting on the perturbation of fish behavior by numerous chemicals, including EDCs and the increasing assertion that these behavioral effects can impact significantly on individual and population fitness. Population models, particularly IBMs, offer the possibility of robust testing of these assertions by bridging the uncertainty gap between individual effects observed in laboratory and field studies, and the potential consequent effects on wild populations. Crucially, IBMs can account for species specific traits and behaviors (e.g. breeding behaviors) and simulate inter-organism interactions and organism-environment interactions (including responses to chemical exposure) and can therefore capture both the direct and indirect population level effects of chemical exposures. The main challenges for generating robust models for fish populations include model parameterization and applicability (i.e. striking a balance between site-specific versus generic applicability due to the often

complex and environmentally plastic life-histories of fish) and model validation. We recommend that the development of future models (IBMs or otherwise) should include species representing a range of life-histories and that their selection should be guided by the derivation of ecological scenarios which are relevant to major land use and waterbody types in which chemical exposures and effects are predicted according to current risk assessments. We also advocate better provision and sharing of raw data for fish populations (both reference (control) and impacted populations) or the generation of new data where existing data are lacking; this will be a priority for assessing the realism of existing and future models.

6. Thesis outline

The primary aim of this thesis was to develop and evaluate modelling tools for a temperate freshwater fish species for use within environmental risk assessments to inform management decisions on the regulation of chemicals. Modelling and empirical techniques were used to: (1) explore the factors which affect the susceptibility of individuals and populations to chemical exposure effects; (2) explore the capacity for individuals to adapt to maximise fitness under periods of stress and; (3) assess how environmental conditions interact with the effects of chemical exposures to affect individuals and populations.

For this work, the three-spined stickleback (*Gasterosteus aculeatus*) was selected as the principle study species. The three spined stickleback is the only European fish species recommended as a standard OECD test species and is therefore directly relevant for risk assessments for European waters (OECD, 2012; OECD, 2017). It is abundant in freshwater, brackish and marine waters throughout Europe and other temperate regions, including North America and Canada (Froese and Pauly, 2016; Ostlund-Nilsson et al., 2006; Wootton, 1984). The stickleback is one of the most well-studied fish species within the fields of ecology, physiology, and ecotoxicology, making it an ideal candidate for the development and evaluation of data intensive population models, such as IBMs. Additionally, aspects of the stickleback's life-history (e.g. high level of parental care and low fecundity) make it potentially vulnerable to the effects of EDCs (Sebire et al., 2008; 2009). Throughout this thesis, the resident freshwater form of the three-spined stickleback is used for all empirical and modelling

experiments. A detailed life history of this form of stickleback is described in detail in Chapter 2.

The objectives of this thesis and the investigative approaches adopted for each chapter were as follows:

Chapter 1 (this chapter)

In this chapter a critical analysis on capturing ecology in modelling approaches is presented as applied to environmental risk assessment of endocrine disrupting chemicals in fish and the purpose of the thesis work set out.

Chapter 2

Objective 1: Develop and evaluate an individual-based model for the stickleback for use in chemical risk assessments.

This chapter presents an IBM which was parameterised from life history data (survival, growth and reproduction) obtained from field and laboratory studies on the three spined stickleback, verified against demographic data for wild stickleback populations, and described according to the Overview, Design Concepts, Details ('ODD') protocol (Grimm et al., 2006; 2010). The potential application of the model within regulatory assessments is then demonstrated through an assessment of the population level relevance of chemical-induced disruption of breeding behaviours (i.e. courtship and nest building disruption in male stickleback by the anti-androgenic insecticide, fenitrothion). The Details section of the ODD is placed in the supplementary information of Chapter 2.

Null hypothesis 1: Fenitrothion-induced effects on male breeding behaviours have no effect on stickleback population abundances.

Objective 2: Assess how life-history strategy, risk of exposure and population resilience affect translation of individual-level effects to effects on populations.

In this chapter the roles of extrinsic exposure risk (exposure scenario) and intrinsic population resilience (life history strategy and density dependence) were assessed in determining population susceptibility to chemical exposures. For the former, the same chemical exposures were simulated as described in

Objective 1 under two different exposure regimes (chronic and pulsed) and effects compared on population abundance. For the latter, the density dependent growth parameter was tracked over the exposure period and population recovery post-exposure monitored to assess the role of compensatory mechanisms in determining population resilience. Additionally, how life-history traits affect the sensitivity of stickleback populations to chemical exposure effects was explored using a local sensitivity analysis.

Null hypothesis 2: Chemical exposure regime does not affect the extent of the chemical effect on population abundance.

Null hypothesis 3: Density dependent growth does not compensate for chemical effects on population abundance and the population therefore does not recover post-exposure.

Null hypothesis 4: The model is not sensitive to changes in life-history parameters indicating that the life-history strategy of the stickleback does not affect population susceptibility.

Chapter 3

Objective 3: Assess the interactive effects in early life stage stickleback of chemical exposure and a natural stressor (food limitation) on somatic growth.

A laboratory based empirical *in vivo* study was carried out with early life stage sticklebacks (0-30 days post hatch, dph) to assess how food limitation (simulated by increasing fish stocking density) interacted with the effect of exposure to a somatic growth-limiting synthetic oestrogen, ethinyl estradiol (EE₂).

Null hypothesis 5: Food availability and EE₂ exposure have no interactive effects on somatic growth rates of early life stage stickleback.

Objective 4: Use a combination of empirical laboratory studies and energy budget modelling to assess the potential for physiological adaptations to compensate for the combined effects of chemical exposure and food limitation in early life stage sticklebacks.

In this chapter an energy budget model was developed and used to explore the physiological mechanisms which caused the observed interactive effects of food limitation and EE₂ exposure on somatic growth rates. The model is presented in the supplementary material of this chapter and described according to the ODD protocol. The energy budget model was confronted with multiple hypotheses to explore the potential for physiological adaptations to compensate for multiple stressor effects.

Null hypothesis 6: Physiological adaptations do not compensate for multiple stressor effects (EE₂ and food limitation) in early life-stage stickleback.

Chapters 4 and 5

Objective 5: Develop and evaluate a combined energy budget and individual-based model to allow for more realistic and mechanistic chemical risk assessments for temperate fish species.

In these chapters, a mechanistic population model was developed and evaluated to enable more realistic investigations into chemical exposure effects. Here the energy budget model from Chapter 3 was combined with the IBM from Chapter 2. The model is described following the “TRANSPARENT and Comprehensive model Evaluation” (TRACE) document (Augusiak et al., 2014; Grimm et al., 2014; Schmolke et al., 2010b) described in Chapter 5. The TRACE document provides a standardised format for describing the development and evaluation of models. The model explicitly incorporates food availability and temperature so that chemical exposures can be simulated in the context of realistic environmental conditions.

Objective 6: Apply a mechanistic modelling approach to predict effects of two EDCs (EE₂ and trenbolone) on stickleback populations.

In Chapter 4, the energy budget-IBM described in Chapter 5 was used to simulate the effects of chronic exposure to EE₂ and trenbolone on stickleback populations over a period of 10 years. EE₂ (an oestrogen receptor agonist) and trenbolone (an androgen receptor agonist) affect female fecundity via two different mechanisms. The effects of each chemical on the physiology of

individual animals were extracted from available information in the literature. The following was assessed: (i) if EE₂ and trenbolone exposures caused effects on population dynamics (abundance and biomass) and; (ii) whether the extent by which individual-level effects translate up to the population is affected by the underlying mechanism of the chemical.

Null hypothesis 7: EE₂ and trenbolone exposure do not cause effects on population dynamics.

Null hypothesis 8: The underlying mechanism of the chemical does not affect the extent by which individual-level effects translate up to the population.

Objective 6: Explore how environmental conditions (food availability) affect population susceptibility to chemical exposures.

A second objective of Chapter 4 was to assess the interactive effects of chemical exposure and food availability on population dynamics. To implement this, the same chemical effects described in Objective 5 were modelled in high and low food availability environments. The aim was to facilitate population level ERA under a range of environmentally realistic exposure scenarios, combining multiple stressors.

Null hypothesis 9: Food availability does not affect population susceptibility to chemical exposure effects.

Chapter 6:

In this chapter a critical analysis of the key findings obtained in this thesis are discussed. The implications of these findings for ERA and for fish conservation in general are identified and priorities for future research are recommended.

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Chapter 2. Assessing Population Impacts
of Toxicant-Induced Disruption of Breeding
Behaviours using an Individual-Based
Model for the Three-Spined Stickleback.

Abstract

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

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

1. Introduction

Many of the ecological factors which affect the susceptibility of wildlife populations to chemicals are considered in current environmental risk assessment (ERA) schemes via the application of arbitrary assessment factors.

Population models can be employed to help bridge the gap between individual-level endpoints, obtained from traditional regulatory testing, and population effects (Forbes et al., 2009; Hommen et al., 2010; Thorbek et al., 2010) to support more realistic ERAs. In addition, population models have the potential to provide more targeted and effective protection for populations by identifying the intrinsic factors (i.e. life history traits and density dependence) and extrinsic exposure scenarios which most affect population susceptibility. Matrix models are currently the most common method for analysing the effects of toxicant exposure on fish populations (e.g. Brown et al., 2014; Ibrahim et al., 2014; Miller and Ankley, 2004) due to their minimal data requirements, but they have limited ability to incorporate complex behaviours and density-dependent regulation (Caswell, 2001). Individual-based models (IBMs), on the other hand, enable key life-history traits, behaviours and inter-individual-environment interactions, including density-dependent processes, to be modelled explicitly (Grimm and Railsback, 2005; Sable and Rose, 2008). Understanding the mechanisms of density-dependence allows a more in-depth exploration of the extent of population resilience.

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

risk to populations in the field. Population models provide a tool to assess the ecological relevance of different exposure patterns and ensuing effects (Mintram et al., 2017). Behavioural effects are not currently considered specifically within standard regulatory risk assessment frameworks and population models can help assess whether this is a critical uncertainty in regulatory risk assessments.

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

2. Methods

2.1. *Model species*

The three-spined stickleback (*Gasterosteus aculeatus*) is widespread throughout Europe and other temperate regions across North America, Canada and Asia (Froese and Pauly, 2016; Ostlund-Nilsson et al., 2006; Wootton,

1984). It is one of the most well-studied fish species in ecology and evolutionary biology, and is used regularly as a model species in ecotoxicological studies (Katsiadaki et al., 2007). Sticklebacks are generalist feeders (Sánchez-González et al., 2001) and display a polygamous mating system, in which multiple females are attracted to nests built and guarded by territorial males (Froese and Pauly, 2016; Wootton, 1984). Their lifespan in the wild is usually one year, with the majority of individuals dying after completion of their first breeding season (Allen and Wootton, 1982*b*; Giles, 1987; Wootton et al., 2005). The wealth of ecological and ecotoxicological data sources describing the detailed natural life-history of the stickleback and chemical effects, including on breeding behaviour, make it an ideal species to model for this case study.

2.2. Model description

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

2.2.1. Purpose

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

2.2.2. Chosen toxicant

Fenitrothion was chosen as the case study toxicant. It is listed as a Priority Substance under the EU Water Framework Directive (2000/60/EC; revised list COM (2011) 875 final) and discharges are currently controlled by multiple international directives (Connor et al., 2017). Fenitrothion is now prohibited for use as a pesticide in the EU (EC No 1107/2009) and its use is restricted in Canada (Directorate, 1995); however, it is still used routinely in the USA, Australia and Africa (Paranjape et al., 2014). In this study, fenitrothion was used

as the model EDC because it has been shown to disrupt reproductive behaviours in the stickleback (Sebire et al., 2009).

2.2.3. Entities, state variables and scales

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

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

Individual fish have four life stages: eggs, larvae, juveniles and adults. All sticklebacks are characterised by the state variables age (days post fertilisation (dpf) for eggs and days post hatch (dph) for the remaining life-stages), wet weight (g), and position within the pond, and all life-stages excluding eggs are characterised by length (cm, total length from the snout to the tip of the tail). Juveniles and adults are further characterised by sex (male or female). Adult males possess the state variable breeding status: Boolean; if they establish territories they exhibit nesting behaviour. Additionally, an individual adult male's territory-size (m^2) is determined by total (global) adult male density and the territory-size an individual male holds determines its courtship success probability. Adult females have an inter-spawning interval (days between spawnings; 3 – 9 d) which is determined by body weight (g), and a batch size (eggs per spawning event) which is determined by body length (cm).

The time step in the model is one day.

2.2.4. Process overview and scheduling

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

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

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

Survive: An individual's daily mortality rate is determined by four main factors: developmental mortality (eggs only), senescence (adults only), density-dependent cannibalism (eggs only), and a general mortality rate which represents all other sources of mortality (all life-stages excluding eggs). Survival of breeding males is additionally determined by the habitat-type of the patch occupied for nesting during a breeding cycle.

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

Grow: Individual growth is dependent upon age, season, and the strength of density-dependent competition from conspecifics. Seasonal growth is an enforced mechanism within the model (Table 1, Eq. 4) which implicitly incorporates seasonal variations in food and temperature to alter growth rates

throughout the year. Female fecundity is directly proportional to body length (Wootton, 1979). Body mass determines survival probability for larvae, juveniles, and adults, and the inter-spawning interval of females. Larger males out-compete smaller males for breeding territories and all males lose body weight when exhibiting nesting behaviour to account for additional metabolic costs.

Move: Individuals move in search of vegetated habitat patches. At the beginning of the breeding season, adult males move to acquire nesting sites and establish territories, whilst adult females move in search of a mate. Males prefer to nest on a vegetated patch which they occupy for the duration of a breeding cycle; however, the habitat type of the patches making up the remainder of the territory may be a combination of habitat types.

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

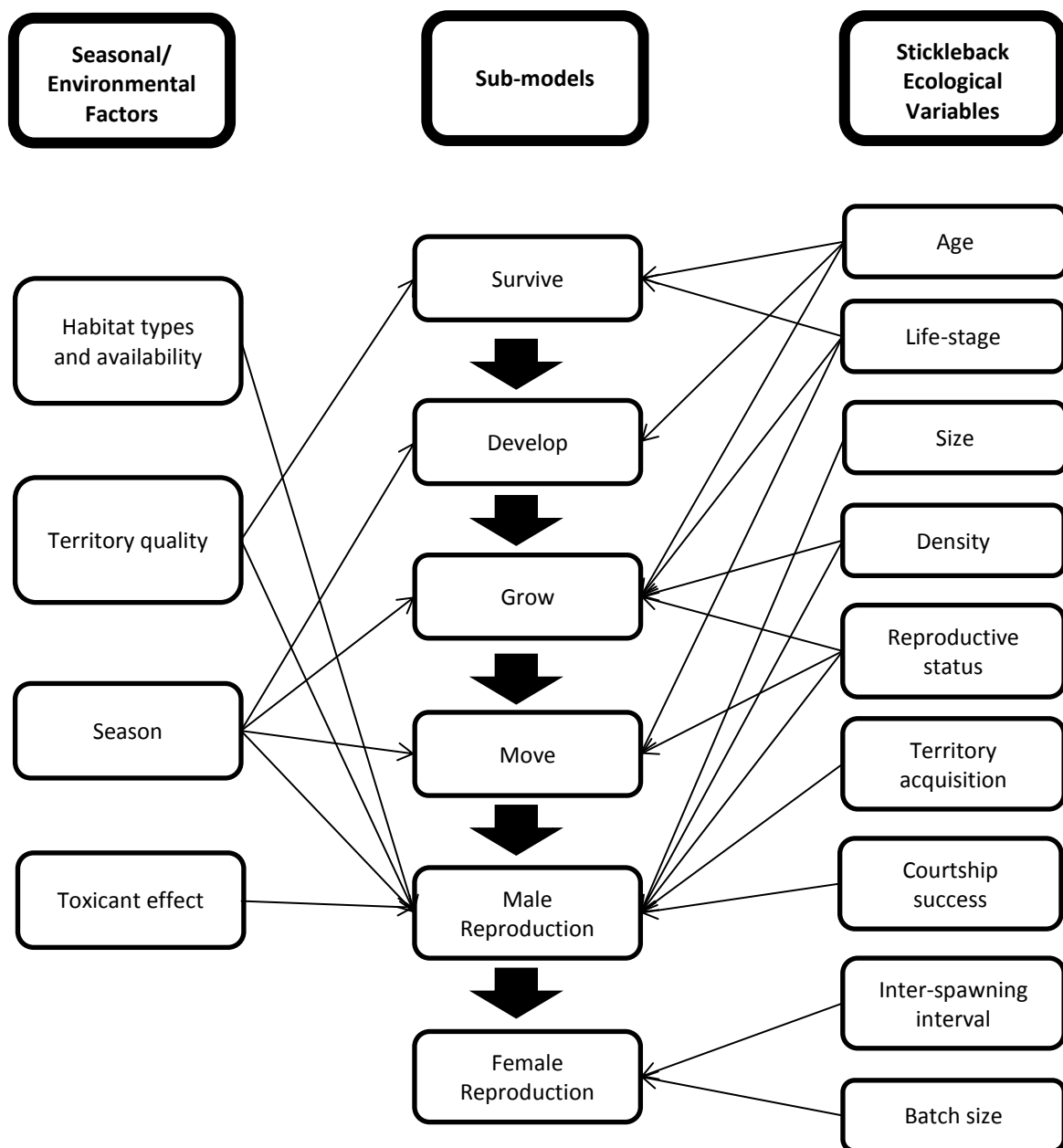


Figure 1. Conceptualisation of the key processes (sub-models) undertaken by the stickleback and the ecological and environmental variables which influence them. Small arrows indicate interactions and large arrows indicate the order of processes.

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

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

	relationship for nest building disruption.		<p>C_w: Water concentration ($\mu\text{g L}^{-1}$)</p> <p>a : ND intercept</p> <p>b : ND gradient</p> <p>a = -0.42</p> <p>b = 0.40</p>	
	Eq 2. Fenitrothion dose-response relationship for courtship behaviour disruption	$CD = \left(\frac{1}{1 + e^{d + C_w \cdot f}} \right)$	<p>CD: Courtship disruption probability</p> <p>C: Water concentration ($\mu\text{g L}^{-1}$)</p> <p>d : CD intercept</p> <p>f : CD gradient</p> <p>d = -1.01</p> <p>f = 0.36</p>	Sebire et al. (2009)
Growth	Eq 3. Body length – biomass density relationship	$L_{inf_B} = L_{inf_L} - Gr \cdot B$	<p>L_{inf_B}: Asymptotic length at a given population biomass density (cm)</p> <p>L_{inf_L}: Limiting asymptotic length as biomass density approaches zero (cm)</p> <p>Gr: Strength of density-dependence ($\text{cm m}^{-2} \text{g}^{-1}$)</p> <p>B: Population biomass density</p>	Lorenzen and Enberg (2002); Cefas Animal Production Unit (APU) data (2013-2015)

			(g wet weight m ⁻²) L _{inf} _L = 5.9 * Gr = 0.09	
Eq 4. Seasonal von Bertalanfy growth function	GR = $L_{inf} \left\{ -exp \left[\frac{K(t-t_0) + \left(\frac{C \cdot K}{2\pi}\right) \sin 2\pi(t-t_s) - \left(\frac{C \cdot K}{2\pi}\right) \sin 2\pi(t_0-t_s)}{L} \right] \right\}$	L: Length (cm) 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 L: Length(cm) 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: Wet weight (g) a : Weight constant b: exponent	Froese and Pauly (2016)	

			a = 0.0068 b = 3.28	
Reproduction	Eq 6. Territory size	If $D > 20$ fish m^{-2} TS = 0.063 If $D < 1.3$ fish m^{-2} TS = 0.54 If $20 > \text{adult male density} > 1.3$ fish m^{-2} $TS = aD^b$	TS : Territory size (m^2) a : TS constant D: Male density (fish m^{-2}) b : TS exponent a = 0.65 b = -0.80	Van den Assam (1967)
	Eq 7. Courtship success (probability of successfully courting a female)	$CS = aLn(TS) + b$	CS : Courtship success probability a : CS constant TS : Territory size (m^2) b : CS intercept a = 0.058 b = 0.90	Van den Assam (1967)
	Eq 8. Reproduction rate	$F = aL^b f$	F : Fecundity (eggs per spawning event) a : Fecundity constant b: Fecundity exponent L : Fish length (cm) f: Fertilisation rate a = 0.82 b = 3.18 f = 0.935	Hagen (1967); Barber and Arnott (2000); Frommen et al. (2008)
	Eq 9. Inter-spawning interval	If wet weight ≥ 0.94 ISI = 3 If wet weight ≤ 0.49	ISI : Inter-spawning interval (days)	Wootton (1974); Brown-

		<p>ISI = 9</p> <p>if $0.94 > \text{wet weight} > 0.49$</p> $ISI = aW + b$	<p>a : ISI constant</p> <p>W : Wet weight (g)</p> <p>b : ISI intercept</p> <p>a = -13.22</p> <p>b = 15.44</p>	<p>Peterson and Heins (2009); Wootton et al. (1995)</p>
Survival	Eq 10. Natural mortality	$M_w = M_u W^b$	<p>M_w: Natural mortality probability at wet weight W</p> <p>M_u: Natural mortality probability at unit wet weight (1 g)</p> <p>W: Wet weight (g)</p> <p>b: Allometric scaling factor</p> <p>$M_u = 0.00781$</p> <p>b = -0.43</p>	<p>Lorenzen (1996)</p> <p>W_u parameter changed from annual, as reported, to daily.</p>
	Eq 11. Egg cannibalism	$EC = aD + b$	<p>EC: Egg cannibalism probability</p> <p>a : EC constant</p> <p>D : Global adult and juvenile (fish length ≥ 1.5 cm) density (fish m^{-2})</p> <p>b : EC intercept</p> <p>a = 0.0049</p> <p>b = -0.0133</p>	<p>Whoriskey and FitzGerald (1985)</p>

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

2.3. Model calibration and validation

The model was calibrated using the growth sub-model. The density-dependent growth algorithm used in the model was taken from Lorenzen and Enberg (2002), and is based on the assumption that as fish density (measured as biomass (g wet weight m⁻²)) increases (i.e. approaches carrying capacity), growth rates of juveniles and adults decrease as a result of exploitative competition. There is no wild stickleback population data quantifying density-dependent growth in the wild, therefore the Gr parameter (strength of density-dependence, Table 1, Eq. 3) was calibrated to provide model outputs of stickleback abundances known to occur in the wild outside of the breeding season (2 – 28 fish m⁻² (Krokhin, 1970; Reimchen, 1990; Reimchen, 1994; Whoriskey and FitzGerald, 1985; Wootton and Smith, 2000)) and to produce an adult length of 4.5 cm at the start of the breeding season (Froese and Pauly, 2016, Paepke, 1983). Calibration was achieved iteratively by visually assessing patterns of population abundances and body lengths.

Model validation was undertaken using stickleback population abundance data from the UK (Wootton, 2007; Wootton et al., 2005) and size distribution data from both the UK (Wootton, 2007) and Germany (Whirzinger et al., 2007). The UK data were derived from wild populations of the resident freshwater form sampled in spring (Feb/March) and autumn (October) from a 200 m² river inlet of the River Rheidol (Aberystwyth, UK). Abundance data was sampled every year over 11 and 21 years in spring and autumn, respectively, and size distribution data was sampled once a year over 5 years. The data from Wirzinger et al. (2007) were size structure data from a stickleback population (unspecified form) in Germany sampled over a single year in April and August. Since the field data collected in Germany displayed much larger individuals than the field data collected by Wootton et al. (2007) in the UK, the model could not match the mean fish size for both sets of data. For example, in Germany, the modal fish length was reported to be 4 cm by August compared to the UK population which did not reach 4cm until March. Therefore, for validation against the data collected from Germany, the parameter which determines the absolute maximum length an individual can reach ($L_{inf}L$, Table 1. Eq. 3) was increased in the model to allow fish to grow to a longer length. Validation of the model outputs under default growth settings against the German field data can

be found in SI (Fig. A10). The model was allowed to stabilise for 10 years (spin-up) and subsequent years were used for comparison with the field data. Preliminary analysis had shown that 15 replicate model runs were necessary to get robust means and standard deviation. Replicate number was considered to be robust once the difference in the average and the standard deviation of the population abundance became independent of replicate number ($\pm 5\%$).

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

2.4. Model sensitivity analysis

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

2.5. Model application: Effects of fenitrothion on stickleback populations

The potential population level impacts of disrupted male breeding behaviours following exposure to the anti-androgenic pesticide fenitrothion were explored

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

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

Population level effects of fenitrothion were investigated by comparing the mean population abundance of control and exposed populations on January 1st each year, as well as cumulative recruitment to each life stage each year. First, the model was allowed to stabilise for 10 years (spin-up period) followed by an exposure period of 10 years, which again was followed by a recovery period of 10 years, when all input parameters in the model were maintained at their default values. The maximum deviation from the mean control population abundance on January 1st over 200 years following a 10 year spin up period

was 15%; thus population level effects were considered relevant if population abundances deviated by > 15% of the mean control value on January 1st. Population abundance was recorded in January because this was the time point where the population was most stable and displayed the least annual variability. The population was considered to have recovered once abundances returned within 15 % of the mean control value. We assumed toxicant effects occurred only during exposure, i.e. once exposure was removed there was immediate organism recovery and no delayed effects. For fenitrothion this is not an entirely unreasonable assumption due to the very low accumulation potential and measured clearance time in fish tissues (Fish bio concentration factor (BCF) = 29 L kg⁻¹; clearance time (CT50) = 0.19 days) (PPDB 2017).

3. Results

3.1. Validation

3.1.1. Population abundance

The population abundances predicted by the model had a good match to those recorded in the field in both spring and autumn (Fig. 2); thus the average abundances were similar (modelled: 4.0 and 16.9; observed: 4.7 and 13.3 fish m⁻² in Feb/March and October, respectively). However, the between year variability in the field data was higher than in the model outputs. The mechanism behind this discrepancy was likely due, in part, to the fact that in the field environmental conditions, such as temperature and food availability, varied between years, whereas the model was run with same conditions each year.

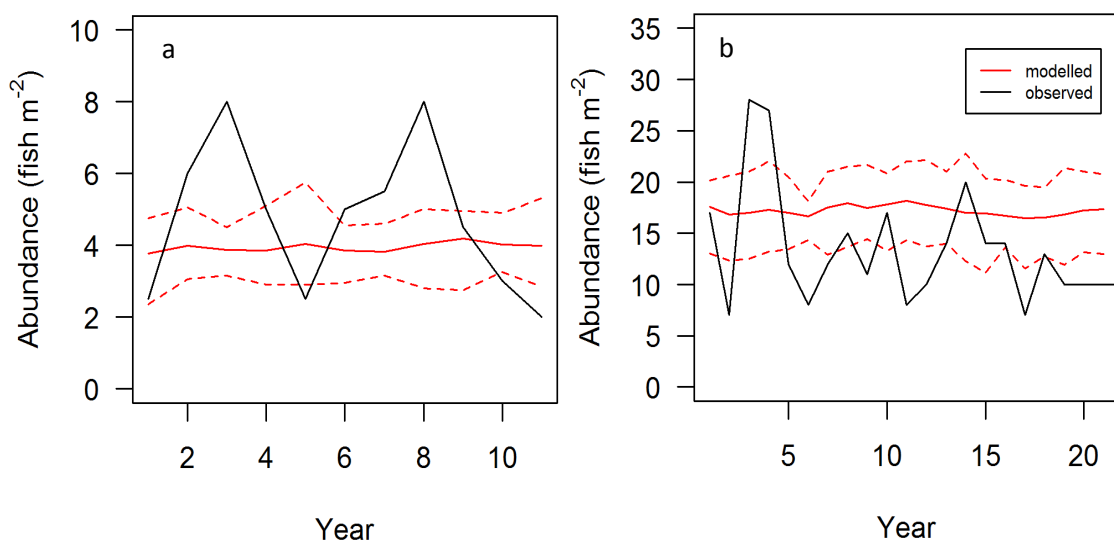


Figure 2. Modelled and observed population abundance in Feb/March (a) and October (b) for 11 and 21 years, respectively. Modelled outputs were recorded

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).

3.1.2. Population size distribution

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

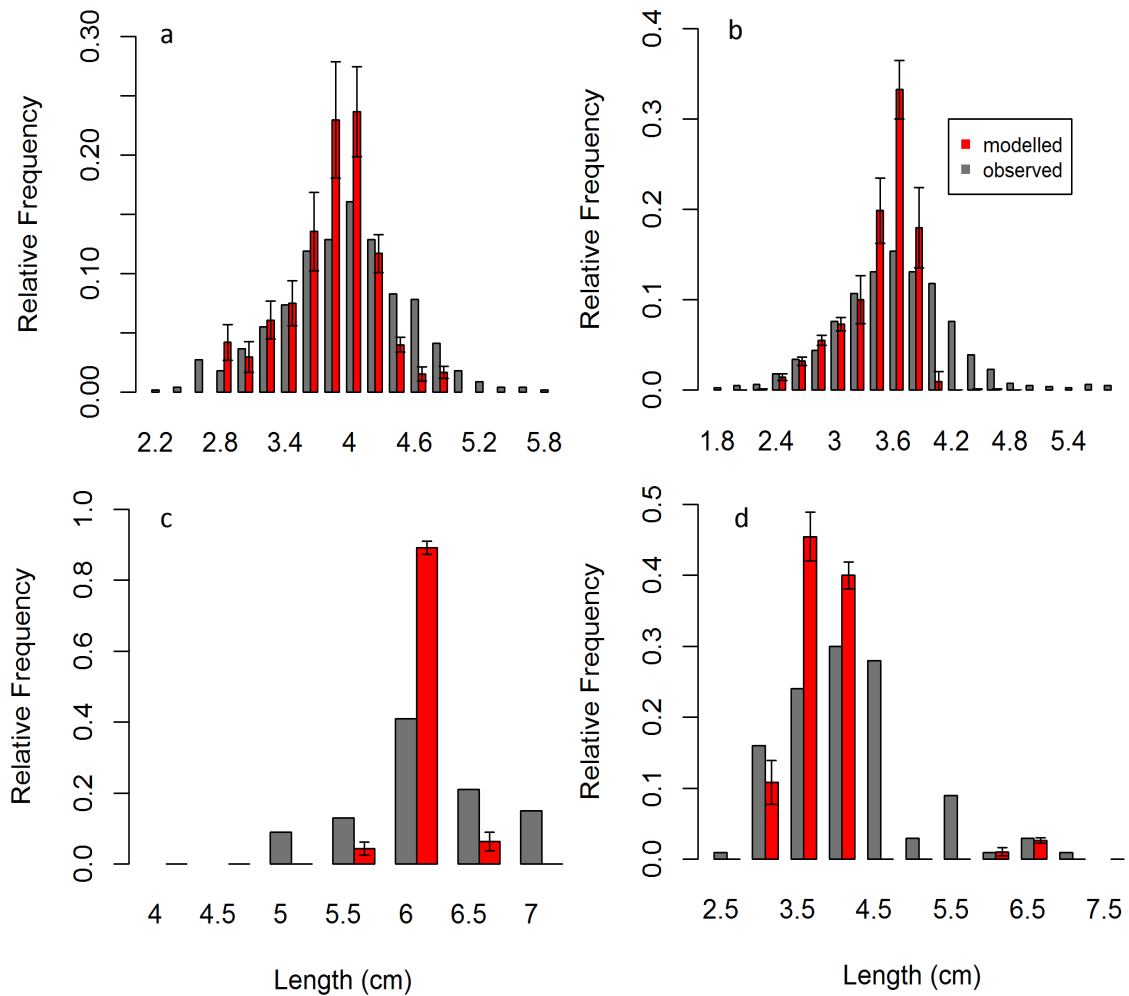


Figure 3. Modelled and observed size distribution data for stickleback in the UK in March (a) and October (b), and in Germany, with an increased maximum length, in April (c) and August (d). Modelled data represents the mean value of 15 simulations over 5 years (\pm s.d). Observed data after Wootton et al. (2007) represents mean values over 5 years (a,b); observed data after Wirzinger et al. (2007) represents data from a single year (c,d).

3.2. Sensitivity analysis

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

The model was most sensitive to changes in the duration of the reproductive season and changes to sex ratio. Sticklebacks have a relatively low fecundity and are limited to a three month breeding season in the model; consequently the population has a low buffering capacity to changes in key reproductive parameters, particularly those which directly affect the total number of eggs

spawned. Therefore, despite the reduced levels of density dependent competition following lower annual egg recruitment, the breeding strategy of the stickleback did not allow the population to recover fully from a reduction in the duration of the breeding season or a skewed sex ratio. The model was less sensitive to changes in life-stage development time, growth, or other reproduction parameters indicating effective regulation of population numbers via density-dependent growth, competition for mates and nest sites, and survival.

Results of the full local sensitivity analysis can be found in SI (Table A2).

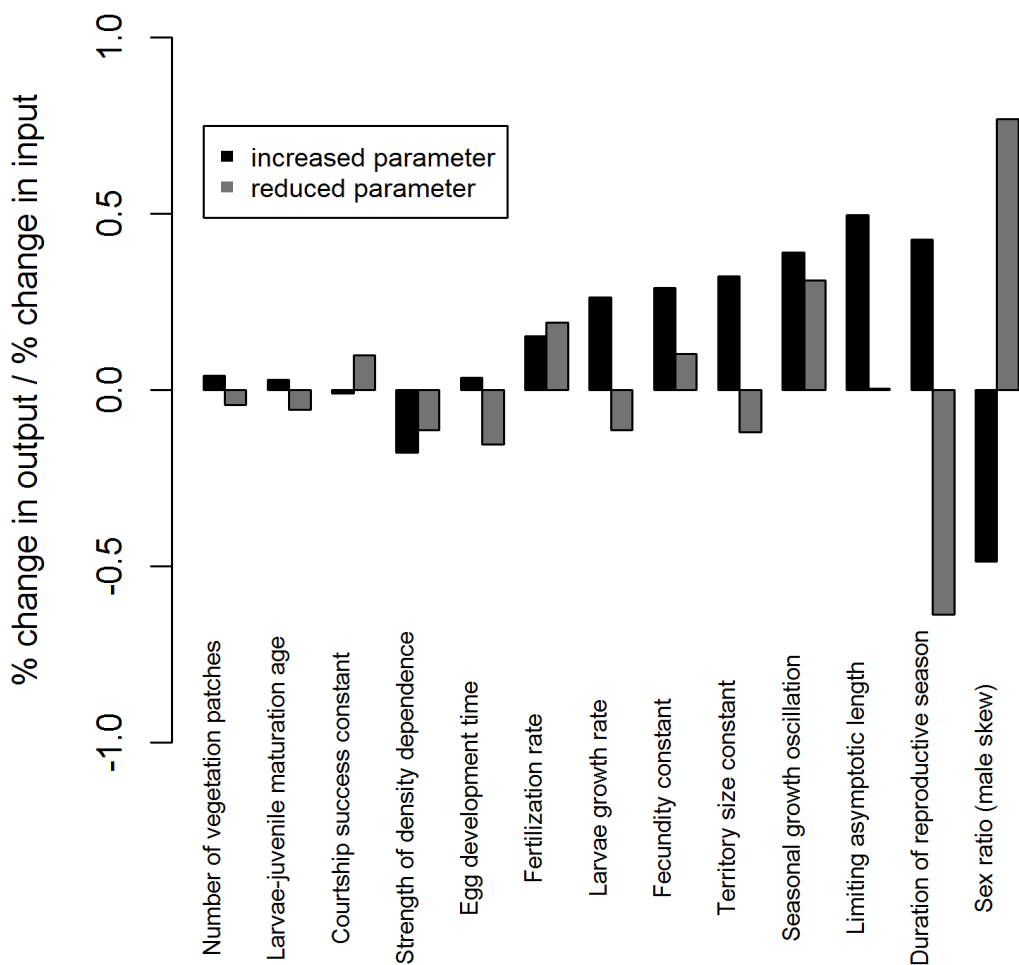


Figure 4. Local sensitivity analysis of key parameters within the model displayed as the ratio of the percentage change in output population abundance and the percentage by which each input parameter was increased/decreased (mean value of 15 simulations).

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

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

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

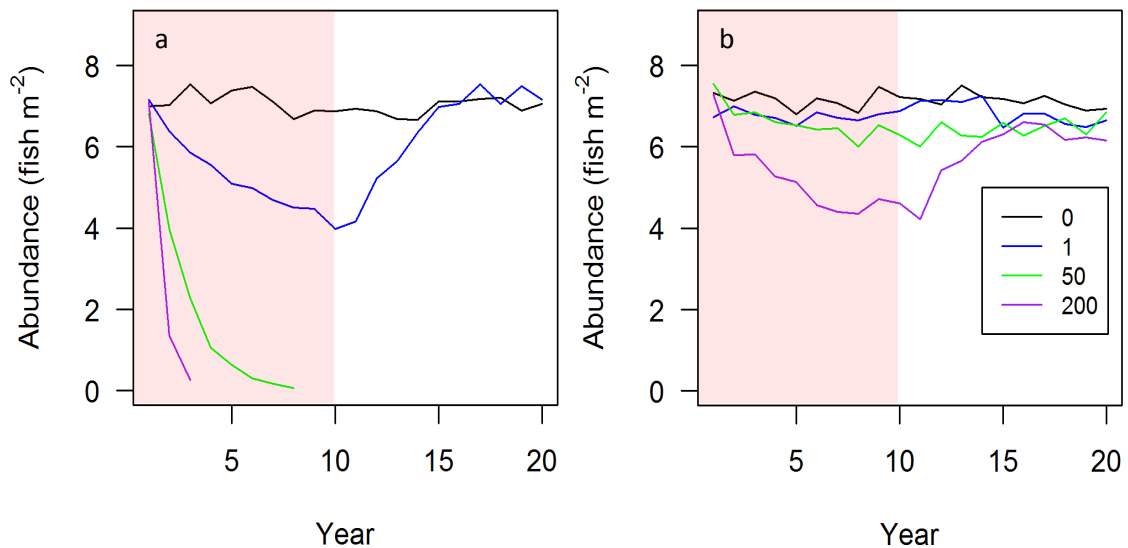


Figure 5. Mean modelled annual total population abundance (mean value of 15 simulations) on the 1st January of each year for (a) continuous and (b) intermittent exposures to fenitrothion. Legend refers to fenitrothion concentration ($\mu\text{g L}^{-1}$). Red (10 years of exposure) and white (10 years of recovery) sections divide exposure and recovery time periods.

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

In order to assess the role of density-dependent processes in the model in more detail, we assessed how the asymptotic length parameter, which determines maximum body length, oscillated with annual changes in population abundances. Asymptotic length is a good measure of the strength of density-dependence because in the model, growth is affected by competition and this is implemented by increasing the asymptotic length as the population biomass of fish decreases (see SI, Details section). Thus, the less biomass in the system, the larger the individuals can grow. Larger individuals are less susceptible to

size-dependent mortality (Lorenzen, 1996) and larger females produce more eggs; this mechanism can, to some degree, therefore compensate for low population abundance. However, the asymptotic length parameter is additionally used here as an indicator for the strength of the effects of population abundance on density-dependent life history processes, including growth, mortality (egg cannibalism) and reproduction (competition for territories).

Throughout the exposures, the asymptotic length of the control population remained stable (5.55 – 5.59 cm) but increased with increasing concentration of fenitrothion indicating release from density dependence (Fig. 6). Intermittent exposure to 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, respectively, during the 10 year exposure period. This density-dependent compensation allowed for some population recovery in between annual exposures and resulted in relatively stable populations throughout the exposure period, following an initial decline in abundance, as observed in the case of the two highest exposure concentrations. Comparatively, chronic exposure to 1, 50 and 200 $\mu\text{g L}^{-1}$ caused a maximum asymptotic length of 5.74, 5.89 and 5.89 cm but the extent of the density-dependent compensation was insufficient to prevent populations going extinct. As an example of the subsequent effects of these fluctuations in asymptotic length on other life history processes, females with a body length of 5.89 cm produce 20% more eggs than females with a body length of 5.57 cm (Table 1, Eq. 8).

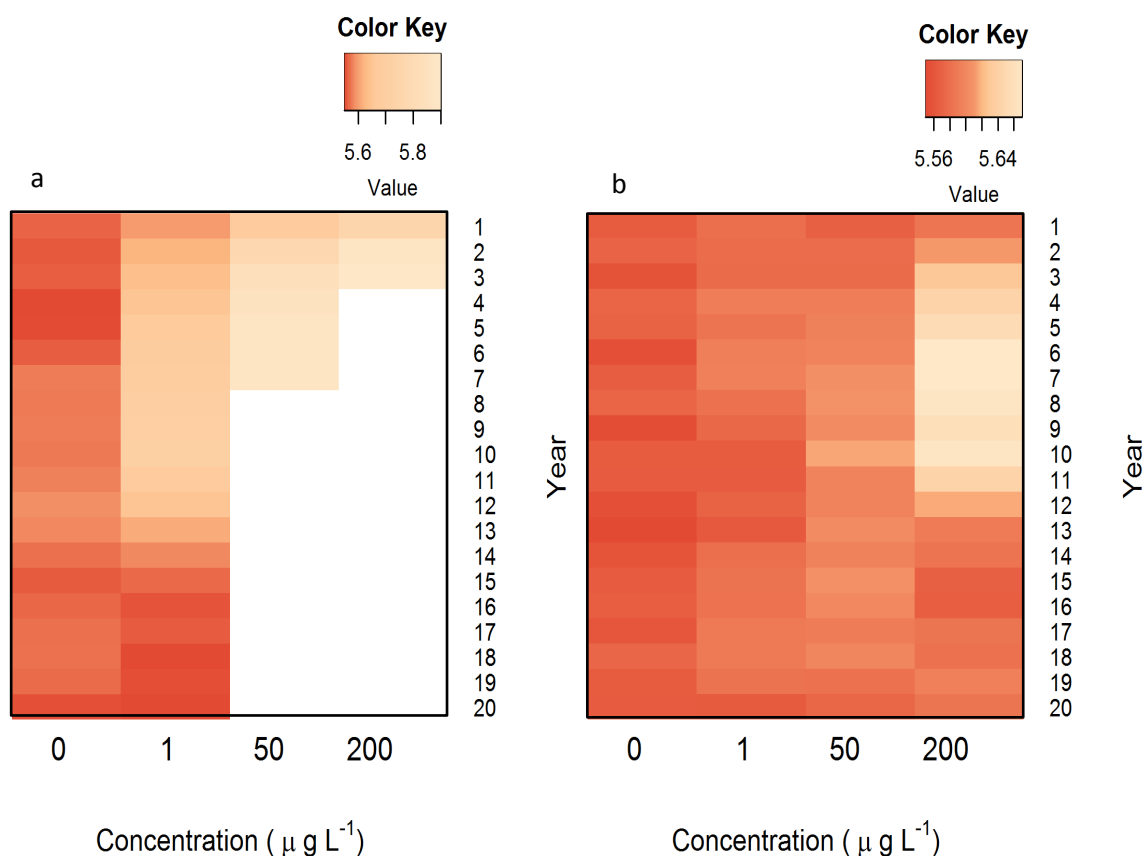


Figure 6. Annual fluctuations in asymptotic length (cm) after exposure to 1, 50, and 200 $\mu\text{g L}^{-1}$ fenitrothion under an intermittent (a) and a chronic (b) exposure regime (10 year exposure; 10 year recovery). Red represents low asymptotic length, cream high asymptotic length and release of density dependence and white space marks population extinction. The colour scale is adapted for each graph and is expressed in the colour keys.

4. Discussion

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

Overall, the stickleback IBM provided a good fit to the available UK stickleback population data, indicating that the model provides a good representation of an extensively monitored natural system. However, the body size discrepancies between wild sticklebacks in the UK and in Germany meant that the model, with current parameterisation, cannot simultaneously provide a good fit for both populations. We chose to use the UK population data as the main body of

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

The sensitivity analysis revealed that the model was most sensitive to alterations in the duration of the breeding season and the operational sex ratio. Stickleback invest a high proportion of energy into nest guarding and egg brooding by males, and females display relatively low fecundity compared to other fish species whose reproduction requires less investment post spawning (Bone and Moore, 2008). The model's sensitivity to parameters which directly affect egg recruitment is therefore an emergent property of the stickleback's life

history strategy. As a comparison, the zebrafish has a high fecundity and in most latitudes may be able to spawn all year round (Spence et al., 2007). A sensitivity analysis of a zebrafish IBM (Hazlerigg et al., 2014) demonstrated that this model species was more resilient to changes in reproductive parameters, such as a reduction in the duration of the breeding season, than the stickleback IBM. The comparison between these models demonstrates how life history strategy can pre-determine the capacity for population resilience and therefore the choice of focal species for risk assessment needs to be carefully considered, if these factors are to be taken into account.

It is well documented that different EDCs can induce different types of physiological effects on individual fish (e.g. masculinisation and reduced fecundity from androgens (Morthorst et al., 2010); feminisation and reduced fecundity from anti-androgens (Jensen et al., 2004) and oestrogens (Nash et al., 2004); impaired growth rates from thyroid disruptors (Liu et al., 2008)) and behavioural effects have also been widely reported (Dzieweczynski, 2011; Dzieweczynski et al., 2014; Ward et al., 2006; Weis et al., 2001; Xia et al., 2010). There are, however, very few examples of studies on population level-effects of EDCs in fish. A notable example is in the work by Kidd et al. (2007), where a whole Canadian lake (Lake 260) was treated with ethinylestradiol (EE₂) at concentrations between 5-6 ng L⁻¹ for a period of 3 years which resulted in the feminization of male fathead minnows and the collapse of the fathead minnow population (Kidd et al., 2007). Breeding behaviours in the fathead minnow have been shown to be disrupted after laboratory exposures to the same concentrations (Majewski et al., 2002), and this may have contributed to the subsequent population crash. The breeding strategy of the fathead minnow is similar to that of the stickleback (e.g. nest guarding by males), and the population level impacts of disruption to the same behaviours would therefore be expected to be similar for both species. Fenitrothion is likely to be toxic to aquatic invertebrates at the two highest concentrations used in the current study (*Daphnia magna* EC₅₀ 8.5 µg L⁻¹ (PPDB, 2017)), so in addition to the physiological and behavioural effects of EDCs, indirect effects may occur via the disruption of food web interactions i.e. reducing invertebrate prey (Fairchild and Eidt, 1993; Choi et al., 2002). Such interactions would constitute multiple stressors since the population would be affected both by the EDC and by food

shortages. Here, we focussed on the impact of EDC induced-behavioural effects on population abundance; however, the potential interactive effects of food shortages and toxicant exposure could impact the level of effect demonstrated by the model. For example, synergistic interactions have been reported for food shortages and toxicant exposure in individual fish (Jørgensen et al., 1999; Hopkins et al., 2002) but the extent to which these interactive effects translate up to effects on populations is uncertain. Furthermore, the population and ecological dynamics of prey species affected by the chemical will be uncertain. In the model, although seasonal variability in environmental conditions is included, annual environmental conditions are constant which means that the interaction between extremes in biotic and abiotic conditions and chemical exposure cannot currently be assessed. In order to explore the interactions between multiple stressors the present model could be expanded with an energy budget to explicitly represent food availability; however any multiple stressor scenarios introduced are likely to be associated with significant assumptions and uncertainty.

Using the stickleback IBM, we showed that exposure duration, as well as exposure concentration, affected population responses and effect levels were markedly greater in populations subjected to a continuous chronic exposure regime compared to a more realistic intermittent regime. For example, concentrations that only caused negligible effects under pulsed exposure scenarios caused marked decreases or even extinction in continuous exposure scenarios. This is consistent with an empirical study, whereby compensatory responses allowed a fathead minnow population to recover following pulsed exposure to toxicants (Ali et al., 2017). Further analysis of the model (e.g. tracking changes to density dependent parameters) revealed that the compensatory capacity for the investigated effects was driven by density-dependent competition for resources leading to increased growth and survival in early life stages and increased availability of spawning territories for adults. In particular we assessed the extent to which growth (asymptotic length - L_{inf}) was affected by density. In the continuous exposure scenario, the capacity for L_{inf} to increase and compensate population biomass was exhausted at $50\mu\text{ L}^{-1}$ fenitrothion, whereas for the pulsed exposure that did not occur even at a fenitrothion concentration of $200\mu\text{g L}^{-1}$. The compensatory effects of density-

dependence also vary between different life-stages. For example, effects were consistently greater for eggs and larvae than for juveniles and adults, and this was particularly evident at the highest exposure concentration. Studies on invertebrates have demonstrated that exposing resource limited populations to toxicants can reduce intra-specific competition and therefore lessen the negative effects of the toxicant (Liess, 2002; Moe et al., 2002). However, empirical studies which validate the interaction between resource competition and chemical effects in fish populations are limited and it is therefore, as yet, difficult to confirm the realism of these modelled results.

The capacity for compensation in natural systems generally is greatest when a population is close to carrying capacity and populations can recover faster in systems with rich resources (Beverton and Holt, 1957). As a consequence the effect of chemical exposure should be seen in the context of resource availability and considered together with other stressors. Moreover, both stressors and resources fluctuate seasonally, so it is important to understand the environmental context and life history strategies of focal species when extrapolating in risk assessment. With the current level of detail, the stickleback IBM has proved to be useful in risk assessments for assessing the population level consequences of individual-level endpoints relating to behaviour, growth, survival, and reproduction.

5. Conclusions

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

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

EDC or other chemical effects on individuals are likely to be ecologically relevant at the population level.

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Chapter 2. Supplementary Information

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

Part 1: Model description (Design concepts and Details)

Design concepts

Basic Principles

Emergence

Adaptation

Sensing

Interaction

Stochasticity

Observation

Details

Initialisation

Sub-models

Toxicant-effect

Survival

Development

Growth

Movement

Reproduction

Part 2: Appendices documenting all data used for sub-model formulation and parameterisation

Appendix 1: Parameterisation of toxicant effect

Appendix 2: Parameterisation of hatch success

Appendix 3: Parameterisation of mortality

Appendix 4: Standard length (sl): fork length (fl): total length (tl) conversion factors

Appendix 5: Parameterisation of growth

Appendix 6: Parameterisation of length: weight relationship

Appendix 7: Parameterisation of fecundity

Appendix 8: Parameterisation of male breeding behaviour

Part 3: Appendices documenting model analysis

Appendix 9: Model verification

Appendix 10: Additional information on sensitivity analysis

Appendix 11: Additional information on model validation

Appendix 12: Additional information on seasonal growth

Part 4: References

Part 1: Model description

Design concepts

Basic principles: The model represents a system by which population dynamics are regulated by ecological processes such as density-dependent growth, mortality and reproduction. Density-dependent processes result in a slowed population growth at high densities due to increased competition for resources and/or predation and disease and conversely an increase in population growth at low densities due to reduced competition and predation (Rose et al, 2001). We have included density dependence for stickleback growth, reproduction (male territory size and competition for optimal breeding areas (van de Assam, 1967)) and egg cannibalism (Whoriskey and FitzGerald, 1985) parameterised from empirical data or calibrated (growth, see main paper, section 2.3).

Reproductive behaviour (courtship and egg guarding) and territoriality determine reproductive success, where larger males dominate in a territory limited environment and males guarding larger territories are more likely to attract spawning females (van den Assam, 1967). Additionally, habitat selection affects an individual's susceptibility to predation as well as determining male reproductive success during the breeding season.

Each of these basic principles is fundamental in determining the resilience of a population to toxicant exposure.

Emergence: Population dynamics (age and size structure, spatial distribution and abundance of fish) emerge from the adaptive traits, behaviours and interactions between individuals (survival, growth and reproduction) and their environment (including toxicant exposure).

Adaptation: Several behaviours in the model are implicitly adaptive. Reproductive behaviour is the primary adaptive trait driving reproductive success. Female choice is determined by male traits (nest building, courtship, territory size, and nest quality), and male post-fertilisation care determines offspring survival. Juvenile and adult movement is implicitly adaptive. Juveniles move to vegetated habitats to minimise predation risk. Adult males will search for optimal breeding grounds to establish territories which reduce predation risk and increase the chance that a female will spawn in his nest.

Sensing: Sticklebacks sense the presence of conspecifics in the local (territorial males and spawning females) and global (total biomass, g) - all adults and juveniles) environment. Adults sense the presence of eggs and/or larvae in the nest and adjust their territorial behaviour accordingly. Juveniles and adults sense patch conditions (habitat type/ quality) and adjust their movement (move to find a higher quality patch) and behaviour (e.g. establish territory, spawn) accordingly.

Interaction: Direct interaction occurs during breeding where a paired male and female carry out both communicative signalling (courtship,) and physical contact (fertilisation). These interactions are modelled implicitly. Each male will continue interacting with its fertilised eggs and larvae until the larvae leave the nest. Indirect interactions occur via density-dependent growth, survival, and reproduction, including male territoriality during the breeding season. Competition for food, mates and nest sites affect an individual's growth, survival and reproductive success.

Stochasticity: Habitat types are distributed randomly within the system. At initialisation, age, position, and sex are the results of stochastic processes. Stochasticity is implemented at the beginning of the breeding season to stagger sexual maturity in adults.

Observation: Total abundance, number of individuals at each life stage, and population biomass (g) are recorded daily. Population abundance and size (length) structure of the population are used to indicate toxicant effect.

Details

Initialisation

The model begins on January 1st with only juveniles present within the system. An initial density of 160 juveniles (8 fish per m⁻²) was chosen as an estimated abundance in January based on the available literature (Krokhin, 1970; Reimchen, 1990; Reimchen, 1994; Whoriskey and FitzGerald, 1985; Wootton and Smith, 2000). Age is drawn from a uniform distribution (i.e. set randomly) between 142 and 232 days post hatch (dph) based on a three month breeding season between May and July, assuming that the first spawning occurs on May 2nd and accounting for fertilisations 1 day after the season ends (accounting for

males which build their nests on July 30th). Length of an individual is set according to his age using the seasonal von Bertalanffy growth function (Table 1, Eq. 4) and wet weight was set using the allometric length: weight equation (Table 1, Eq. 5). The position of individuals within the pond was allocated based on a preference for complex vegetated habitat, as displayed in wild populations (Candolin and Selin, 2012). Juveniles are randomly allocated to a vegetated patch; as densities have been observed at up to 60 fish m⁻² in the wild (Whoriskey and FitzGerald, 1985), there is assumed to be no exclusion of juveniles from vegetated patches outside of the breeding season with the densities reached here.

The pond patches are setup with 10% vegetation cover following a wild pond which was mapped by Whoriskey and Fitzgerald (1987). All of these vegetated patches are considered potential territories for males during the breeding season. The remaining patches are set as open water all of which are set as potential breeding-grounds; however this can be changed by the user. The configuration of patches is set randomly.

Sub-models

This section focusses on the assumptions, data and empirical knowledge that the sub-models are based on. All equations and algorithms, including parameters, their definition, default values and references, can be found in Table 1. Details of parameterisation methods can be found in Part 2.

Toxicant effect:

The model was allowed to stabilise for 10 years before the anti-androgenic toxicant, fenitrothion, was applied. Fenitrothion exposure results in impairments to nest building and courtship behaviour in male sticklebacks. Literature laboratory data quantifying behavioural impairments after exposure to 0, 1, 50 and 200 µg L⁻¹ fenitrothion was used to parameterise this sub-model (Sebire et al., 2009), whereby the number of individuals displaying both nest building behaviour and courtship displays decreased with increasing concentration. The level of effect was determined by fitting data to a classic logit dose-response curve (Appendix 1, Fig. A1). To quantify impairments to courtship behaviour, the laboratory study quantified disruption of zigzag displays, bites, and leading of the female back to his nest as independent endpoints. We use male leading

behaviour of the female to the males nest as the behavioural endpoint which determines courtship success because this is the final stage of the courtship display (van den Assem, 1967). In the model, we assume that if a male does not exhibit leading behaviour or acquire a nest, then a female will not spawn. We therefore directly relate the probability that a male will display leading behaviour to his courtship success probability by subtracting the level of behavioural impairment from the default courtship success probability. Thus, the endpoints used in this sub-model are (1) probability of nest building impairments and (2) probability of leading behaviour impairments relative to control exposures. Fenitrothion was applied under two exposure regimes: constant, where the population was exposed continuously for 10 years, and intermittent, where the population was exposed for 10 days at the peak of the breeding season (10th – 20th of June) each year for 10 years. The toxicant effect ceases once exposure stops.

Survival:

Four different types of mortality are included in the model; developmental mortality (eggs only), senescence (adults only), density dependent cannibalism (eggs only), and a general mortality rate (representing predation, background mortality and overwintering mortality; all life-stages excluding eggs).

Developmental mortality relates to natural mortality caused by mutations, incorrect egg development and infections. Laboratory data on natural mortality from the literature and from a study undertaken by the authors in the absence of predation showed a mean (\pm standard deviation) hatching success of 91.9% \pm 6.6%. Eggs are additionally subjected to density dependent egg cannibalism (Whoriskey and FitzGerald, 1985), whereby daily mortality probability depends on global juvenile and adult density with body length \geq 1.5 cm (Table 1, Eq. 11). In the model, mouth gape size prevents juveniles with a body length $<$ 1.5 cm from cannibalising eggs. Since there are no studies quantifying mouth gape size in stickleback, a body length \geq 1.5 cm was considered to be a sensible estimate, based on observations in the laboratory (personal observation), to represent individuals capable of cannibalism. The cannibalism data provided did not differentiate between concealed sites or open sites or between filial and non-filial cannibalism and therefore we do not consider these factors in the model. Egg predation by other species is assumed to be minimal because of

the paternal guarding investment undertaken by the male stickleback. It is therefore assumed in the model that non-cannibalistic egg predation only occurs if the guarding male dies prior to completing his breeding cycle.

Larval, juvenile and adult mortality is set according to the natural mortality at unit weight equation (Table 1, Eq. 10) where an increase in wet weight results in a reduced daily mortality rate (Lorenzen, 1996). This equation implicitly incorporates all forms of mortality including predation, background mortality and overwintering mortality, and follows the general theory that as fish get older, and larger, mortality decreases (Wootton, 2002). To account for different rates of predation in different habitat types, correction factors of 1.5 and 0.5 are applied to adult males which have established nests on open and vegetated patches, respectively, to ensure that adult males which guard nests in open areas have a higher chance of predation than those nesting in vegetation. The ratio of vegetated and open-water patches within the whole territory does not affect predation because it is assumed that the male spends the majority of a breeding cycle guarding the patch on which the nest is located (Wootton, 1984), using the remainder of his territory only to chase away predators and attract females during the courtship phase. Additionally, since the configuration of patches is set randomly throughout the grid at initialisation (i.e. no deliberate clustering of habitat types), there is no substantial variation around the ratio of vegetated vs open-water patches between male territories. These correction factors implicitly apply to eggs and larvae occupying open or vegetated nests because once the guarding male dies, his eggs and larvae are assumed to be predated. Correction factors are not applied to juveniles, adult females or adult males which are not nesting because of their limited time occupying open water patches.

Senescence occurs in adults only. In the wild, the majority of sticklebacks die after completing their first breeding season, with very few surviving beyond 12 – 16 months (Allen and Wootton, 1982*b*; Wootton et al., 2005). In the model, each fish has a maximum lifespan of 450 days (Allen and Wootton, 1982*b*).

Development:

Each stickleback ages 1 day each time step. Eggs hatch into larvae after 6 – 8 days post fertilisation (dpf) and absorption of the yolk sack is complete after 4

dph (Swarup, 1958). Larvae length is set to 0.453 cm (pers. obs). Larvae develop into juveniles once they are feeding independently at 4 dph and differentiate into males or females based on a 1:1 sex ratio. Juveniles develop into adults on the first day of the breeding season in the following from year from which they hatched (Allen and Wootton, 1982*b*).

In the model, eggs are represented as super-individuals (Scheffer et al., 1995) for efficiency purposes and are defined as 'egg-batches'.

Growth:

Eggs do not grow. For other life stages fish length (total length, cm) is used as the primary element of growth in the model. Larvae have a constant growth rate of 0.033 cm/ day (pers. obs). Larvae are not affected by density dependence because they rely on their yolk sack for food and are therefore not affected by competition for food. Juveniles and adults outside of the breeding season grow according to a version of the von-Bertalanffy growth function (VBGF) which incorporates seasonal growth (Table 1, Eq. 4). The VBGF determines an individual's length for a given age and results in decreasing growth rates as fish get older and larger. However, it is known that stickleback growth is also dependent on food supply and temperature (Allen and Wootton, 1982*a*) and seasonal oscillations in growth have been displayed in the wild (Allen and Wootton, 1982*b*). The VBGF has therefore been adapted to incorporate these seasonal oscillations using a sine wave (Somers, 1988; Hoenig and Choudary-Hanumara, 1982). The standard VBGF model (see Appendix 5) was parameterised using data collected from both wild (referring to larvae in the nest: Allen and Wootton, 1982*b*) and laboratory reared fish (juvenile to adult: Snyder, 1991; Cefas Animal Production Unit (APU) data 2013-2015) and the parameters defining the seasonal oscillations (C and t_s) were parameterised independently using wild length-time data (Allen and Wootton, 1982*b*). Since the t parameter in the seasonal VBGF refers to age, rather than date, the equation does not consider that fish spawned in May will have a longer period of higher growth rates compared to those spawned in July. To account for this, we adjusted the t_s parameter, which determines the start of the seasonal oscillation, based on the date that the individual was spawned so that all fish reach the winter period of growth at the same time independent of their age.

Density dependent growth affects all juveniles, as well as adults outside of the breeding season, and is incorporated using the linear asymptotic body length – biomass density relationship (Table 1, Eq. 3). This is based on the assumption that asymptotic length (L_{inf_B}) is a linear declining function of population biomass density (Lorenzen, and Enberg, 2002); so as fish density (measured as biomass, $g\ m^{-2}$) increases (i.e. approaches carrying capacity), growth rates of juveniles and adults decrease. Since eggs and larvae remain in the nest and therefore do not compete for resources such as food, these life-stages are not included in the population biomass density. Parameters for this equation were estimated independently. The L_{inf_L} (asymptotic length when population biomass is 0) parameter was parameterised using data collected from laboratory reared fish (Cefas APU data 2013-2015), and the Gr (strength of density dependence) parameter was calibrated (see main paper, section 2.3). Density dependence is implemented as a function of global juvenile and adult, as these life-stages are capable of moving throughout the whole system.

Growth is adjusted for adults in the breeding season. Adults do not increase in length once they are sexually active until the last day of the breeding season (or the last day of a final breeding cycle for males) (Wootton et al., 1978, Dufresne et al., 1990). Females maintain a constant wet weight throughout this time (weight fluctuations after each individual spawning are not modelled). During a breeding cycle, males do not leave the nest to feed and expend energy aerating and defending their nests resulting in a daily weight loss of 0.725% of body wet weight (Dufresne et al., 1990). Due to searching efforts and failed attempts at nesting, non-breeding sexually mature males will also lose 0.585% of body wet weight per day (Dufresne et al., 1990). Since body length is a function of age (Table 1, Eq. 4), weights and lengths of adults are adjusted after the breeding season to account for the lack of growth during the breeding season and prevent a disproportional increase in body length/ mass over a single day once growth commences again.

An individual's wet weight (all life stages except for eggs) is derived from its length using the allometric length: weight equation (Froese and Pauly, 2016).

Movement:

Eggs do not move and larvae are restricted to movement within the nest. Stickleback are found at densities up to 60 fish m⁻² in the wild (Whoriskey and Fitzgerald, 1985); it is therefore presumed that there is no exclusion of juveniles from a patch at the densities found here relating to the density of fish within that patch. However, an exception is during the breeding season when juveniles with a length ≥ 1.5 cm are excluded from male territories as they are capable of cannibalising eggs and therefore pose a threat to guarding males (see *survival*). Each juvenile will move to a different vegetated patch each day if any are available. Although they are adapted to life in open water (Van den Assem., 1967), it has been demonstrated that stickleback will choose sheltered habitats over open spaces if any are available (Candolin and Selin, 2012). The volume of the modelled pond allows juveniles and adults to have global knowledge of their environment and can therefore move to any desired patch within the system. Eggs and larvae possess only local knowledge of the patch that they occupy.

Adult males and females which are not sexually active move to unowned vegetated patches if any are available. Adult movement while breeding is described in '*reproduction*'.

Reproduction:

This process is executed by adult stickleback in the breeding season. To add stochasticity, all adults have an equal chance of becoming sexually mature each day within the first 30 days of the breeding season (Wootton, 1984). Once a male has nested once, his chance of nesting again is based only on competition for territory space. Once a female is initially ready to spawn, her next spawning is determined by her inter-spawning interval and competition for receptive males. In the model, the breeding season begins on the 1st May and finishes on the 30th July; however, a male can defend an already established territory into August.

Larger males display a competitive advantage over smaller males when choosing territory sites (Rowland, 1989; Dufresne et al., 1990; Kraak et al., 2000). Each day, the largest males which have reached sexual maturity acquire nests on available vegetated patches and establish territories around these patches according to their territory size (Table 1, Eq. 6). If there are no

vegetated patches available, males will establish territories around open water patches. Thus, a male may establish a nest on a single vegetated patch, but the territory surrounding this nest may be a mixture of vegetated and open water patches. This is consistent with evidence from wild stickleback demonstrating that individuals show a preference for concealed nest sites over open water nest sites (Black, 1971; Moodie, 1972; Kynard, 1978; Hagen, 1967; Sargent and Gebler, 1980; Krakk et al., 2000). In the presence of competition for territories, whereby there are more sexually mature individuals than there are available territories, the smallest males will be outcompeted and will settle on an unoccupied patch and will not nest for that day. However, since male sticklebacks ferociously guard their territories, it is presumed that once a male has established a nest, he cannot be excluded from that nest by a larger male. Competition therefore only relates to males which do not have established nests. A male which acquires a territory is defined as a nesting male.

It is clear from the literature that male size plays an important role in determining which individuals are able to establish territories in a space limited environment (semi-field study: Dufresne et al., 1990, laboratory study: Rowland, 1989). However, empirical evidence to suggest that body size plays a significant role in determining an individual's territory size is limited. Kynard (1978) found no correlation between male standard length and the number of eggs obtained in the nest in a wild population, indicating that larger males do not necessarily obtain larger territories. This is suggested to be a result of male stickleback being smaller than females and maintaining a relatively uniform size within populations (Rowland, 1989). A hypothesis presented by Rohwer (1978) stated that in species where filial cannibalism is abundant, such as the stickleback, females should hypothetically choose the smallest males as they require fewer eggs per unit time. Comparatively, a laboratory controlled study demonstrated the largest males to defend the largest territories (Candolin and Voigt, 2001). Unfortunately data for the latter study was unavailable, and considering the contrasting information demonstrated it was decided that within the model, male size will not affect territory size.

The modelled breeding season lasts 3 months (based on a UK population (Wootton et al., 1978) – this may vary for other climates) during which a single male can complete as many breeding cycles as possible until he is

outcompeted or dies (Kynard, 1978; Wootton, 1984). Each breeding cycle lasts 14 days and consists of a nest building phase (day 0); a courtship phase (days 1 - 4) which determines if a female will spawn; and a parental phase (nest guarding and fanning of embryos and fry: days 2 - 14) (Wootton, 1984; Kynard, 1978; van den Assem, 1967). Males do not leave the single patch upon which they have established their nest for the duration of a breeding cycle. Although in reality individuals would leave the nest for brief periods of time to defend the territory, this is not explicitly modelled. A male can acquire a maximum of 5 clutches in days 1 - 4 of each breeding cycle, after which point he will reject any more females into his territory (van den Assem, 1967; Wootton, 1984). If a male fails to acquire a clutch within this period, the cycle is set back to 1 (courtship phase). This is to avoid males guarding a territory for a full breeding cycle without being able to acquire any egg clutches after day 4. The owner will abandon his territory at day 14 of his cycle and begin searching for another space as previously described (Kynard et al., 1978). In wild populations, the time spent on parental care of fry is often variable and can range from abandonment prior to the development of free-swimming fry (van den Assem, 1967; Kynard, 1978), to continuing the parental phase until the fry leave the nest independently (Kynard, 1978). In the model, this depends on which day of the breeding cycle the clutch was acquired. We assumed that if a male is removed from his territory through mortality, all of the eggs and larvae in his nest will die as they are unable to survive without aeration or guarding from predators.

Once a female is ready to spawn, she will first search for an available nest in a vegetated patch (Kraak et al., 1999); if none are available she will search for an open water nest. In the wild, although only one female can visit a nest at a given time, more than one female can visit each day (van den Assem, 1967; Wootton, 1984). In the model, two females can visit a nest at one given time to account for the one day time step. An available territory is therefore defined as containing one male guarding no more than 4 clutches in days 1 – 4 of his breeding cycle, with no more than 1 female already present. If there are no available nests, females will move to an unoccupied vegetated patch if any are available and search again the following day. The probability that she spawns in the nest of the owner of that territory is determined by the courtship success

probability of the owner (Table 1, Eq. 7). As in the wild, the model allows gaps between territories so non-nesting or spawning individuals continue to forage and shelter (van den Assem, 1967).

Female fecundity is a function of body length, where larger females will produce a larger clutch of eggs (Table 1, Eq. 8). An average fertilisation rate of 0.935 is used for all females, as the literature indicates no changes in fertilisation rate with age or size (Frommen et al., 2008; Barber and Arnott, 2000). Once she spawns, the time until she is next able to spawn is determined by her inter-spawning interval (ISI); this is a function of body mass whereby larger females will have a shorter ISI (Wootton, 1974). Females with a wet weight ≤ 0.49 g have an ISI of 9 days, females with a wet weight ≥ 3 g have an ISI of 3 days and females between these wet weights have an increasing ISI with weight between 3 and 9 days (Brown-Peterson and Heins, 2009; Wootton et al., 1995). These ISI's were defined based on an empirical study undertaken by Wootton (1973).

Part 2: Appendices documenting all data used for sub-model formulation and parameterisation

Appendix 1: Parameterisation of toxicant effect sub-model

The level of effect on the nest building and courtship behaviour of individuals after exposure to fenitrothion was modelled using dose-response curves generated from laboratory data found in the literature (Sebire et al., 2009, Fig. A1). The empirical study used two batches of stickleback, generating two independent data points for each concentration (0, 1, 50 and 250 $\mu\text{g l}^{-1}$) for each endpoint. Nesting impairment refers to the frequency of males in each batch which did not build a nest, and leading behaviour impairment refers to the frequency of males which did not undertake leading behaviour. Least squares were used to fit log concentration ($\mu\text{g l}^{-1}$) vs nesting impairment/ leading behaviour impairment corrected for controls ($0\mu\text{g l}^{-1}$) to the logit form of the dose-response curve (Table 1, Eq. 2).

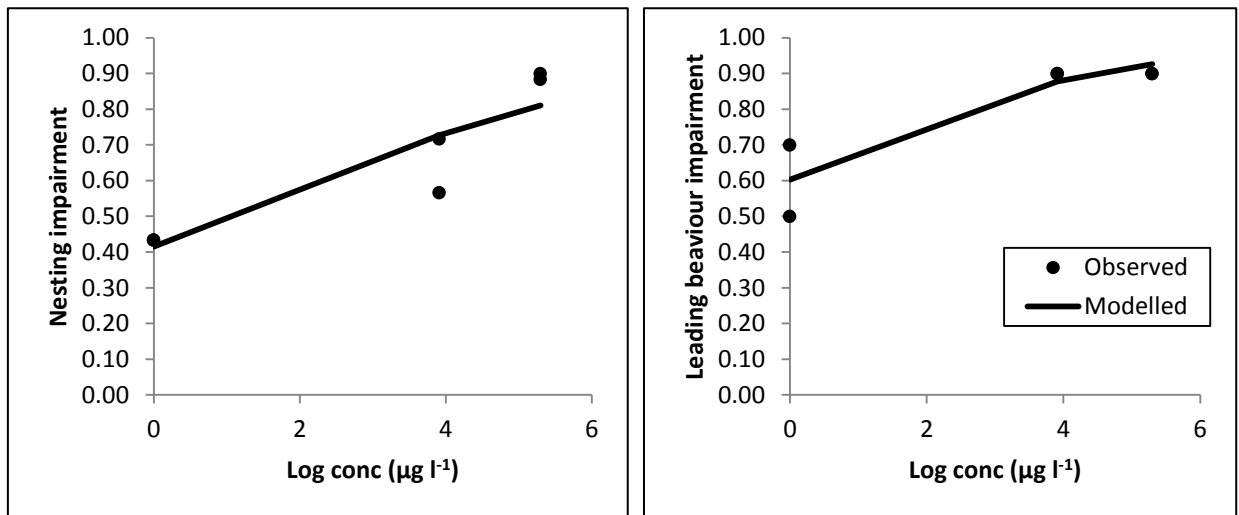


Figure A1. Dose-response curves for fenitrothion-induced effects on nest building impairments (frequency of males which did not build nests, left) and leading behaviour impairments (frequency males which did not display leading behaviour, right) relative to controls ($0\mu\text{g l}^{-1}$).

Appendix 2: Parameterisation of hatch success

Natural mortality (infections, mutations, incorrect egg development) was determined from three laboratory controlled studies (Hagen, 1967; Candolin et al., 2008; pers. obs) ($n = 12$, Table A1). Natural mortality was not measured in response to parent fish length or age. A constant hatching success of 91.9%, which gives a daily mortality probability of 0.013, was therefore used in the model.

A semi-wild density dependent cannibalism study undertaken by Whoriskey and Fitzgerald (1985) provided data to quantify egg mortality caused by cannibalism from conspecifics. The model was fit to the linear equation,

$$EC = aD + b$$

where a denotes the egg cannibalism constant, D represents adult and juvenile density with body length ≥ 1.5 cm (fish m^{-2}), and b denotes the intercept. The original data reported total egg mortality over an average of 7 days, which was converted into daily mortality for the model. The model was fit to 4 data points (means) using least squares. Cannibalism increases with the density of juveniles and adults (Fig. A2). A minimum daily rate of egg cannibalism of 0.0073 was set to account for filial cannibalism at low densities; this was the lowest recorded in the study by Whoriskey and FitzGerald (1985).

Table A1. Hatching success measurements.

Source	hatching success %
Hagen, 1967	82
Hagen, 1967	95
Hagen, 1967	75
Hagen, 1967	93
Hagen, 1967	94
Hagen, 1967	96
Hagen, 1967	92
Hagen, 1967	98
Hagen, 1967	96
Hagen, 1967	82
Hagen, 1967	98
Hagen, 1967	92
Hagen, 1967	90
Hagen, 1967	97
Hagen, 1967	82
Hagen, 1967	100
Hagen, 1967	93
Hagen, 1967	97
Hagen, 1967	95
Hagen, 1967	100
Hagen, 1967	89
Hagen, 1967	92
Candolin et al. 2008	82
pers.obvs.	94.5

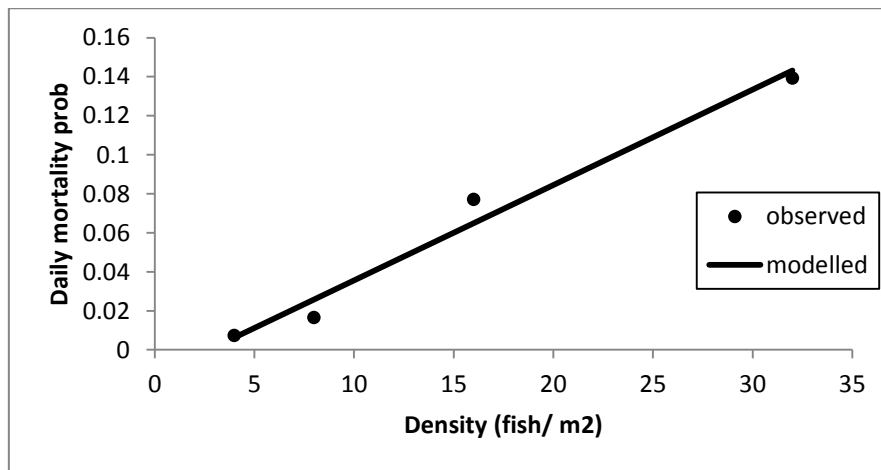


Figure A2. Data from a density dependent egg cannibalism study fitted to a linear model with parameter values $a = 0.0043$ and $b = -0.0133$.

Appendix 3: Parameterisation of mortality

Juvenile and adult mortality is modelled using the natural mortality at unit weight algorithm

$$M_w = M_u W^b$$

where M_w is the natural mortality rate at wet weight W , M_u is the natural mortality rate at unit wet weight, and b is the allometric scaling factor. The model was parameterised by Lorenzen (1996) using the complete Thail estimator, a robust non-parametric regression model, from 103 data points derived from the published literature and specific to fish in temperate regions.

Appendix 4: Standard length (sl): fork length (fl): total length (tl) conversion factors.

Total length (from the tip of the snout to tip of the tail, cm) was chosen as the primary measurement used within the model.

Conversion factors were obtained from Froese and Pauly (2016) with SL: TL 1.150, TL:SL 0.879 and FL:TL 1.000. All lengths are measured in cm.

Appendix 5: Parameterisation of Growth

Density independent growth in juveniles and adults is modelled using a version of the von Bertalanffy growth function (VBGF) (von Bertalanffy, 1938) which is adapted to incorporate seasonal growth (Somers, 1988; Hoenig and Choudary Hanumara, 1982)

$$L_{inf} \left\{ 1 - \exp^{-\left[K(t-t_0) + \left(\frac{C \cdot K}{2\pi} \right) \sin 2\pi(t-t_s) - \left(\frac{C \cdot K}{2\pi} \right) \sin 2\pi(t_0-t_s) \right]} \right\}$$

where L_{inf} (cm) represents asymptotic length, K represents a growth rate constant (cm year^{-1}), t_0 represents the hypothetical age at which length equals zero, t_s defines the start of the convex segment of a sinusoid oscillation with respect to $t = 0$, and C expresses the relative amplitude of the seasonal oscillation. Unfortunately, wild population data which directly links age to length is limited to a study undertaken by Allen and Wootton (1982*b*), whereby mean lengths of stickleback sampled from a river inlet throughout a single year were reported. However because the data behind the figure displayed in the publication was unavailable, the fish were not aged, and there are no reported mean lengths below 1.7 cm, this data could not be used to accurately produce estimates of growth rates throughout the whole lifecycle of the stickleback. Nonetheless, the data displayed by Allen and Wootton (1982*b*) provided a good overall trend in age-length data in the wild and was therefore used to parameterise parameters C and t_s (the parameters which determine the seasonal oscillation, Fig. A3) by fitting the data to the seasonal VBGF using least squares.

The remaining parameters were parameterised using the standard VBGF, where length is determined for a given age

$$L_{inf} \{ 1 - \exp - (K[t - t_0]) \}$$

The standard VBGF was fitted to 613 datapoints derived from laboratory reared fish (Snyder, 1991; Cefas APU data, 2013-2015) and wild larvae (Allen and Wootton, 1982*b*). Least squares were used to fit the VBGF to the data (Fig. A4). Because the model may at some points cause negative growth, and a decrease in fish length is not possible, individuals stay the same length over the negative growth period in the IBM. The t_s parameter is additionally adapted depending on when in the breeding season the fish were spawned to ensure that the seasonal oscillations occur at the same time for all fish independent of the date they were spawned. The t_s value obtained from the parameterisation of wild data was used for the middle of the breeding season (15th June). Either side of this, the t_s value was either increased or decreased by 0.5 with each day that deviated from this date. This value was determined by graphically analysing

growth rates so that individual fish cease growth for winter and begin to grow again for summer at around the same date, independent of their age.

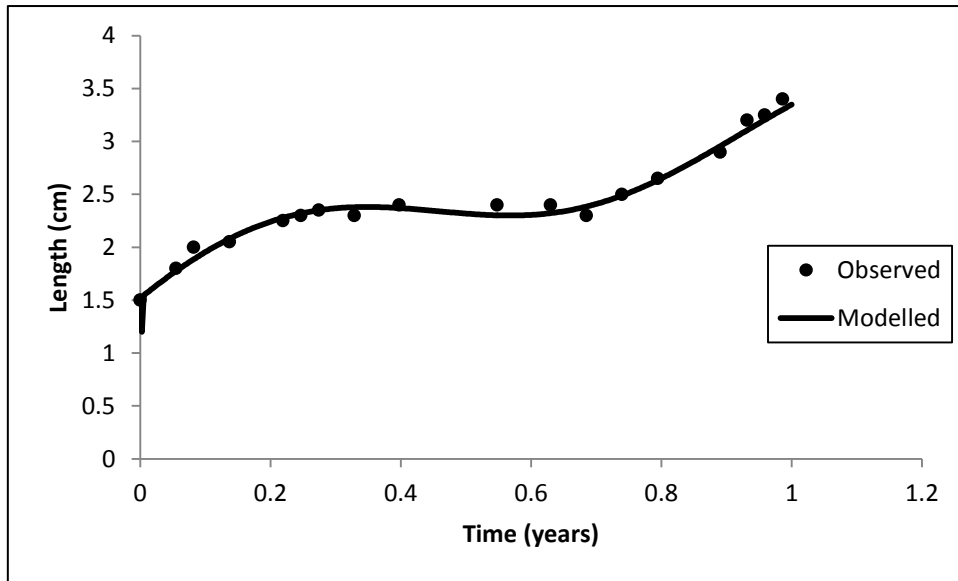


Figure A3. Seasonal VBGF fit to wild stickleback length (cm) vs time (years) data to determine the start and amplitude of the seasonal growth oscillation (parameters C and t_s), with parameter values $L_{inf} = 6.33$, $K = 1.96$, $t_0 = -0.02$, $t_s = -0.042$, $C = 1.30$.

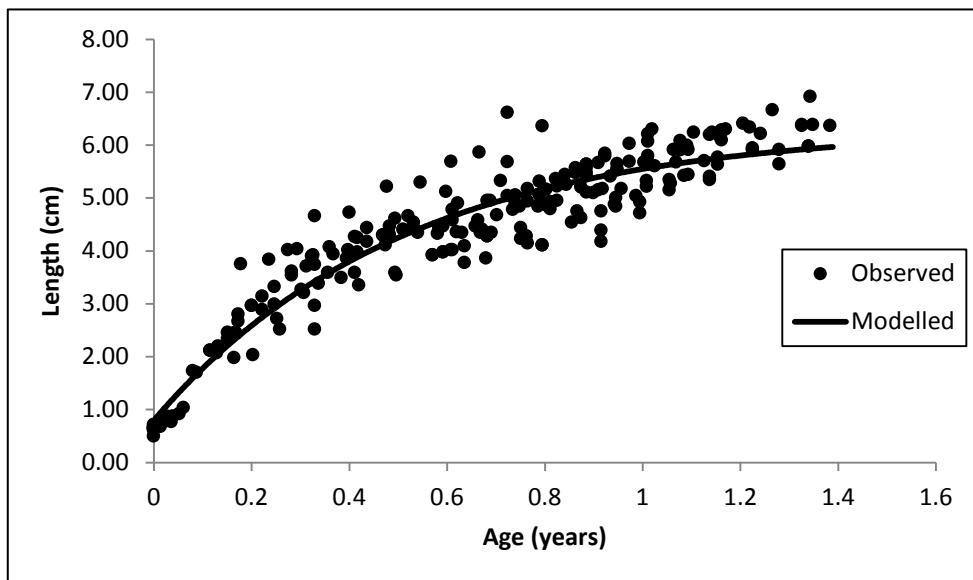


Figure A4. Standard VBGF fit to age (dph) and length (cm) data collected from 613 wild and laboratory reared stickleback, with parameter values $L_{inf} = 6.33$, $K = 1.96$, $t_0 = -0.02$.

Density dependence was implemented using the body length – biomass density relationship

$$L_{inf_B} = L_{inf_L} - Gr \cdot B$$

where L_{inf_B} denotes the asymptotic length at a certain biomass, L_{inf_L} (cm) denotes the limiting asymptotic length (L_{inf} when population biomass = 0, cm), Gr denotes the strength of density dependence ($\text{cm m}^{-2} \text{g}^{-1}$) and B biomass (g). Both L_{inf_L} and Gr are constants; the B parameter (biomass density (g)) is the variable which allows the parameter L_{inf_B} (asymptotic length at a certain biomass density, cm) to fluctuate i.e. the maximum length an individual can reach is increasingly limited as biomass increases. In the IBM, the asymptotic body length – biomass density equation is implemented prior to the VBGF so that the L_{inf_B} parameter (Table 1, Eq. 3) can replace the L_{inf} parameter (Table 1, Eq. 4) and density dependence can be implemented. Each parameter was parameterised independently.

L_{inf_L} (cm) represents the hypothetical length which an individual would reach when biomass density = 0 (i.e. in the absence of competition). This was parameterised by fitting laboratory data, where ages ranged from 355 - 505 dph (Cefas APU data, 2013-2015), to the standard VBGF using least squares. This range of ages was chosen because individuals have reached their maximum length under optimum conditions with minimum competition for resources.

Gr ($\text{cm m}^{-2} \text{g}^{-1}$) represents the growth response to changes in biomass density (Lorenzen and Enberg, 2002). Unfortunately, there is no long-term data for stickleback which quantifies the average biomass density of a population. The Gr parameter in the model was therefore calibrated by comparing the population density modelled by different Gr values with population densities and adult lengths estimated from wild populations (2 – 28 fish m^{-2} ; (Krokhin, 1970; Reimchen, 1990; Reimchen, 1994; Whoriskey and FitzGerald, 1985; Wootton and Smith, 2000); 4.5 cm at the start of the breeding season (Froese and Pauly, 2016; Paepke, 1984)). A Gr value of 0.09 resulted in population densities within the ranges observed.

Appendix 6: Parameterisation of length: weight relationship

Wet weights and lengths of 29,975 unsexed sticklebacks were fit to the allometric equation,

$$W = aL^b$$

by Froese and Pauly (2016) using type 1 linear regression of Log W vs Log L .

Appendix 7: Parameterisation of fecundity

Fecundity of different sized females was modelled according to the equation,

$$F = aL^b$$

where a denotes the fecundity constant, L denotes fish length (cm), and b denotes the fecundity exponent. The model was fit to data from 39 sticklebacks (Hagen, 1967) using least squares (Fig A5).

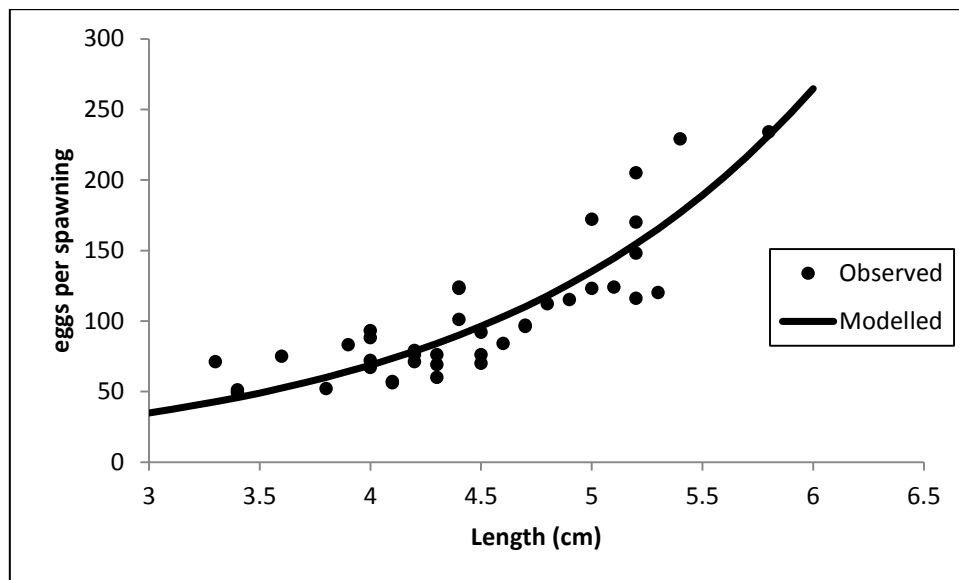


Figure A5. Observed and modelled fecundity (eggs per spawning) for stickleback based on body length (cm), with parameter values $a = 0.82$ and $b = 3.18$.

Appendix 8: Parameterisation of male breeding behaviour

Male territory size was modelled according to the power function equation

$$TS = aD^b$$

where a denotes the territory size constant, b denotes the territory size exponent and D represents fish density (fish m^{-2}). The model was fit to 5 data points from a laboratory controlled experiment (van den Assem, 1967) using

non-linear least squares (Fig. A6). Minimum and maximum territory sizes in the model are set based on the ranges observed in this study (0.063 – 0.54 m²).

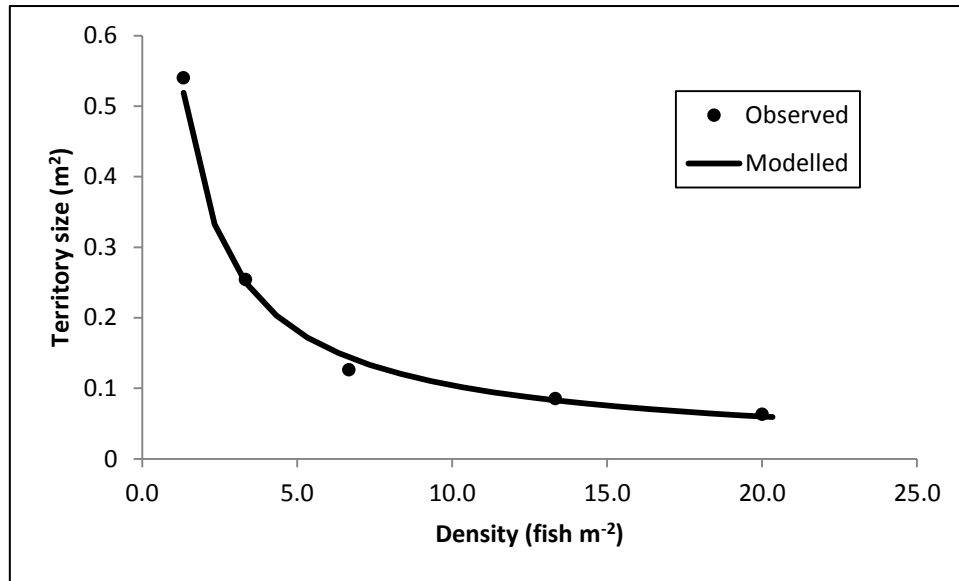


Figure A6. Observed and modelled data for male territory size as a function of global fish density, with parameter values $a = 0.65$ and $b = -0.80$.

Courtship success is a function of territory size and was modelled according to the logarithmic equation

$$CS = a \ln(TS) + b$$

where a denotes the courtship constant, TS (m²) denotes territory size and b denotes the courtship intercept. The model was fit to 6 datapoints from a laboratory controlled experiment (van den Assem, 1967) using least squares (Fig. A7).

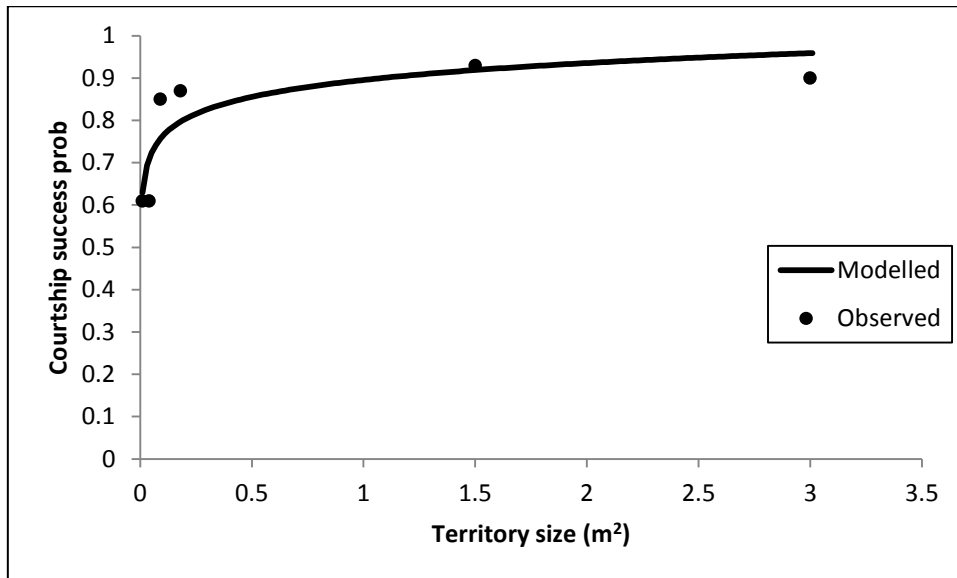


Figure A7. Courtship success probability modelled as a function of territory size (m²) using the logarithmic equation, with parameter values $a = 0.058$ and $b = 0.90$.

Part 3: Appendices documenting model analysis

Appendix 9: Model verification

Model verification was undertaken following the guidelines from Grimm and Railsback (2005) to ensure that the model corresponded with the original conceptual model. Initially, all equations and algorithms in the model were calculated in Excel (Microsoft Corp., USA) and the outputs were checked against those from the NetLogo implementation of the model. All global and state variables were thoroughly checked throughout simulations. Patterns and processes, including size-class distribution; individual movement patterns; total abundance; and number of nesting males at a given time point, were monitored carefully over a period of 10 years using the graphical outputs in the NetLogo programme. The scheduling of sub models and individual processes was checked to ensure that the sequencing of events matched those described in the conceptual model. Finally, the NetLogo code was checked by Chun Liu who was previously involved in the model development to ensure that the model ODD matched the programming code and to check for bugs. All testing of the final version of the model resulted in the expected outputs, providing confidence that the model has been implemented correctly.

Appendix 10: Additional information on sensitivity analysis

Table A2. Sensitivity of population abundance to changes in parameter/algorithm values for each sub-model.

Sub-model tested	Parameters tested	Parameter change	% change in abundance	
			-	+
Initialization	Number of vegetated patches	10%	0.4	-1.2
		25%	1.0	-1.0
Survival	Egg mortality constant	10%	-1.5	0.6
		10%	0.8	3.3
	Vegetation mortality correction factor length (cm) of juveniles	10%	-0.3	-0.8
		10%	0.4	2.0
		25%	1.1	-2.4
Development	capable of egg cannibalism	10%	-1.4	0.7
		1 d	-2.2	0.52
	Larvae-juvenile maturation age	10%	7.1	-5.1
Growth	Sex ratio (male skew)	10%	4.4	2.5
		25%	-2.9	-4.6
	dependence	10%	-2.1	2.1

Reproduction	Limiting asymptotic length (L_{inf_L})	10%	0.04	4.7
	Age where length = 0 (T_0)	10%	-2.1	-0.5
	Start of convex segment of oscillation (T_s)	10%	3.0	3.8
	Amplitude of oscillation (C)	10%	0.7	1.1
	Weight constant	10%	2.3	4.9
	Weight exponent	10%	-0.2	-1.7
	Larvae growth rate	10%	-1.1	2.6
	Duration of reproductive season	30d	-26.5	12.4
	Fertilization rate	10%	1.9	1.5
	Fecundity constant	10%	1.0	1.8
	Fecundity exponent	10%	3.3	0.4
	ISI constant	10%	2.1	2.4
	ISI exponent	10%	-3.8	-2.0
	min ISI	10%	-3.3	-1.9
	max ISI	10%	-0.1	1.0
	Territory constant	10%	-1.2	3.1
	Territory exponent	10%	-0.7	-2.7
	Courtship success constant	10%	1.0	0.1
	Courtship	10%	0.8	-0.7

success exponent			
Daily % weight loss while nesting	10%	0.7	-0.7

Appendix 11: Additional information on model validation

Under default settings, the model could not match both the UK field data and German field data due to size differences in the two populations (see main paper, section 2.3). The asymptotic length parameter (L_{inf_L} , Table 1, Eq. 3) was therefore adapted to produce model outputs that matched the German field data (main paper, Fig. 4). Prior to adapting the growth parameter, modelled size class outputs followed the same patterns of growth as the field population, but individuals were notably smaller (Fig. A8).

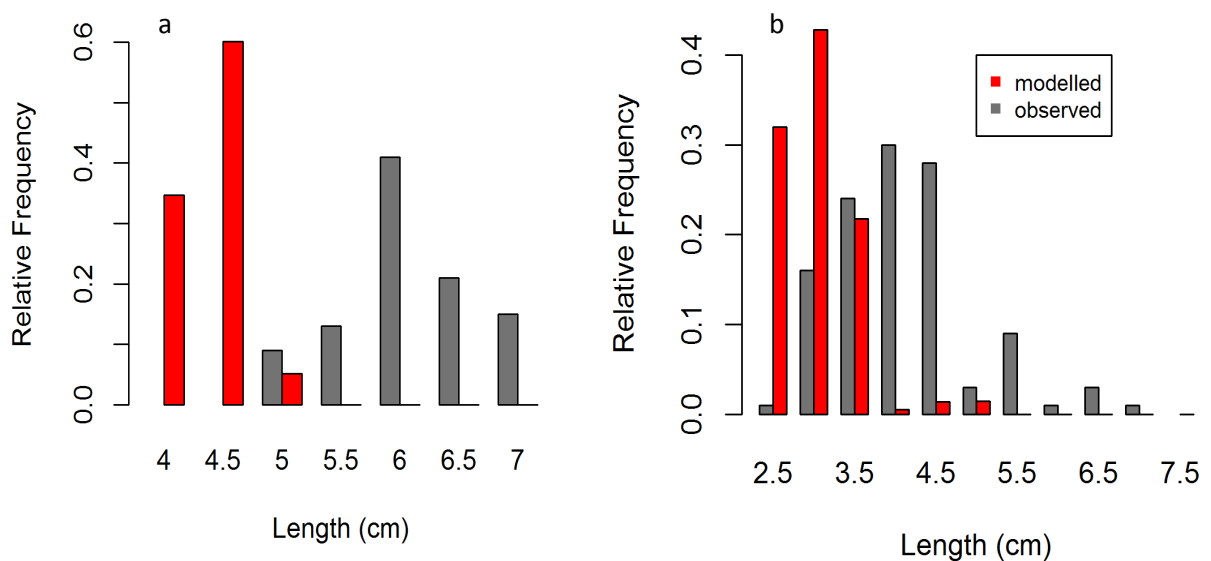


Figure A8. Modelled and observed size-class data for stickleback in April (a) and August (b). Modelled data represents the mean value of 15 simulations under default parameters.

Appendix 12: Additional information on seasonal growth

Initially, the model was developed without seasonal growth so that fish grew gradually throughout the year to reach a maximum length according to the von Bertalanffy equation (von Bertalanffy, 1938). However, model outputs displayed marked discrepancies when compared to field data (Wootton et al., 2005,

2007). Modelled individuals grew slower than the field population over the first summer of life resulting in a higher proportion of smaller fish in October. Additionally, because individuals are growing gradually throughout the year, including over winter, the fish reached their maximum size more quickly, resulting in larger fish by Feb/March. In the absence of seasonal growth, the fish reached the same maximum length as those modelled with seasonal growth by the start of the breeding season; however, the lengths did not match well for the different seasons. We used pattern orientated modelling (Grimm et al., 2005) by going back to the conceptual model and adding 'season' as an environmental factor (Fig. A1). Implementing seasonal growth resulted in an improved model validation to the UK field data (main paper, Fig. 3).

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Chapter 3. Interactive Effects of Food
Limitation and Environmental Oestrogen
Exposure in the Three-Spined Stickleback
(*Gasterosteus aculeatus*).

Abstract

Freshwater fish in natural environments are frequently subject to multiple physiological stressors that include periods of food limitation and toxicant exposures. These stressors could have significant implications for population dynamics, but such interactive effects have received little study and are difficult to simulate in the laboratory. Here, we combined laboratory based experimental work and mechanistic modelling to explore the interactive effects of food limitation and exposure to the potent environmental oestrogen, 17 α -ethinylestradiol (EE₂). We exposed the three-spined stickleback to EE₂ at a concentration shown previously to reduce somatic growth in juvenile fish, and assessed how reduced food availability affected the extent of the EE₂-induced growth reduction. Results from our *in vivo* study showed that body length at 20 days post hatch (dph) and growth conversion efficiency (i.e. growth rate per mg of food provisioned) between 0 and 20 dph were reduced in the EE₂ treatments where fish were maintained under high food availability. This growth effect was, however, not discernible in fish maintained under low food availability. We employed an energy budget model to test the hypothesis that the lack of an EE₂ induced effect in food-limited fish was due to energy budget reallocation in physiologically stressed fish. We hypothesised that this reallocation was due to the adaption of active metabolism (i.e. reductions in physical movement) compensating further reductions in growth rates caused by the chemical. Empirical and modelled results indicate that fish can modulate their physical activity to support their ability to cope with the effects of physiological stressors. We present an integrated mechanistic approach for assessing multiple stressor effects in fish. The energy budget model presented has wide utility as a tool to investigate the resilience of organisms to the effects of realistic multiple stressor exposure scenarios.

1. Introduction

Animal populations in the wild are subject to a wide range of natural stressors, including periods of food limitation, which affect individual vital rates and shape population dynamics (Beverton and Holt, 1957, Lorenzen and Enberg, 2002). Freshwater fish populations are additionally vulnerable to the effects of anthropogenic chemical exposures, e.g. from diffuse agricultural runoff (Schulz,

2004; Wauchope, 1978) and/ or direct inputs of domestic and industrial effluents (Petrovic et al., 2002). Under optimal conditions, organisms assimilate energy from ingested food and, in general terms, allocate it to maintenance, growth and reproduction (Kooijman and Kooijman, 2010; Sibly et al., 2013). Exposure to both chemicals and natural stressors has the potential to cause physiological changes which alter the allocation of energy between these processes (Sibly and Calow, 1989). Furthermore, potential interactions between these stressors make it difficult to predict the outcomes of toxicant exposure in natural environments (reviewed in Holmstrup et al., (2010)).

Chemical contaminants are considered as a source of physiological stress to fish (Fry, 1947; Warren, 1971; Beamish et al., 1975; Calow, 1991; Beyers et al., 1999; Jager and Klok, 2010). Often this stress incurs energetic costs, which have to be provisioned in order to re-align any resulting physiological disruption (Hutchinson et al., 2006) and/or repair the affected systems (e.g. tissue damage) (Beyers et al., 1999). This reallocation of available energy in response to physiological stress is often referred to as the 'general adaptation syndrome' (Selye, 1973). The syndrome describes how organisms adapt their physiology in an attempt to maintain homeostasis in the presence of a stressor, and this reallocation of energy is likely to be reflected by reduced growth rates particularly in early life-stage fish. The adaption of growth rates in response to chemical exposure has been observed empirically in laboratory fish (Beyers et al., 1999; Smolders et al., 2003; Tsai et al., 2006). However, when energy is further limited under periods of food stress, so that the energy available for somatic growth is already limited, organisms may need to adapt their physiology further to cope with the combined effects of chemical and food stress. This reallocation of energy, as well as the direct toxicological effects of the chemical exposure, are likely to have implications for individual fitness relating to growth, reproduction and survival (Forbes and Calow, 1997; Kooijman and Kooijman, 2010; Sibly et al., 2013) and may have subsequent ecological consequences.

The urgency to develop novel exposure tools to assess interactions between multiple stressors has been widely recognised by risk assessors (Backhaus et al., 2013; Løkke et al., 2013; Beyer et al., 2014; van den Brink et al., 2016). To address this, conventional methods for risk assessments of single stressors can be adapted to deal with the specific challenges posed by multiple stressors

(Callahan and Sexton, 2007; Løkke et al., 2013). Traditional *in vivo* studies which focus on whole organism effects can provide useful information on the adverse effects of multiple stressors on individual fitness; however, they do not give any information on the mechanisms underlying the observed interactive effects of the stressors which limits our ability to extrapolate the results to other organisms or other combinations of stressors (Sokolova, 2013). To effectively address this challenge, an integrated approach can be used to provide a link between the physiological mechanism and the whole organism effect in order to predict the ecological consequences of multiple stressors (Sokolova, 2013). Energy budget models which quantify the partitioning of energy amongst life-cycle processes can be used to predict mechanistic pathways underlying chemical effects (e.g. Calow and Sibly, 1990; Beyers et al., 1999; Maltby, 1999; Johnston et al., 2015; Goodchild et al., 2018) but their potential for use in interpreting multiple stressor responses has been little explored. Combining empirical studies with mechanistic modelling has the potential to provide detailed assessments of multiple stressor effects without relying on complex and expensive experimental designs.

Here, the three-spined stickleback (*Gasterosteus aculeatus*) — a temperate fish species with a global distribution, and used widely for environmental and evolutionary studies — was exposed to a potent synthetic oestrogen (17 α -ethinylestradiol (EE₂)) known to cause reductions in growth rates in juvenile fish (Lange et al., 2000; Papoulias et al., 2000; Shved et al., 2008; Van den Belt et al., 2003; Zha et al., 2008). The interactive effects of food availability and EE₂ exposure on growth were then assessed. EE₂ is an oestrogen receptor agonist and its effects on early life stage fish range from intersex and gonadal impairments (e.g. Kidd et al., 2007) to behavioural disruption (Brian et al., 2006; Coe et al., 2008). This study, however, focusses on somatic growth as an endpoint because (i) both EE₂ and food limitation are known to affect growth rates in early-life stage fish (ii) growth rates relate directly to individual fitness and (iii) body length can be quantified non-invasively over time. This study is not designed to be a risk assessment of EE₂; rather, EE₂ is used as a model chemical so that the interactive effects of a natural and a chemical stressor on growth can be assessed. We furthermore developed an energy budget model to explore possible mechanisms behind the observed food limitation and toxicant

induced growth effects. The overall aims of this investigation were (i) to assess the interactive effects of a natural stressor (food limitation) and a chemical exposure and (ii) to understand the potential for fish to adapt physiologically (in terms of energy budget partitioning) to cope with different levels of combined stress in early life.

2. Methods

2.1. Empirical studies

2.1.1. EE₂ exposure

A stock solution of 17 α -ethinylestradiol (EE₂, Sigma Aldrich, purity \geq 98%, CAS 57-63-6) at a nominal concentration of 100 $\mu\text{g L}^{-1}$ (0.1% ethanol (EtOH)) was added to a mixing tank at a rate of 0.0264 L hr⁻¹ with de-chlorinated water at a rate of 26.4 L hr⁻¹ using peristaltic pumps to give a final EE₂ tanks concentration of 100 ng L⁻¹. This concentration of EE₂ was chosen based on a range finding study undertaken by the authors, where final body lengths were significantly reduced ($p < 0.001$) without causing any observed developmental abnormalities in fish fed to satiation and exposed to 100 ng EE₂ L⁻¹ from 0 to 21 days. Four replicate 2 L aquaria per treatment regime were dosed from the mixing tank at a rate of 2 L hr⁻¹. An identical set-up was used to dose solvent control (final EtOH concentration 0.0001%) and water control treatments (n=4 aquaria for each control treatment).

2.1.2. Animals and husbandry

The chemical exposure study was conducted between June 2017 and August 2017 at the University of Exeter. Embryos were obtained via *in vitro* fertilisations using a stock colony of fourth generation (F4) three-spined stickleback (originally obtained from a large wild population at Houghton Springs, Blandford, Dorset) and subsequently maintained under the following environmental conditions: 10 \pm 1 °C, 8L: 16D (light: dark hours) photoperiod with regular feeding to satiation, and simulated seasonal spawning conditions (from April to September): 15 \pm 1 °C, 18L: 6D hours photo period.

Throughout the chemical exposure study, fish were maintained in flow through aquaria containing 2 L of water, with flow rates remaining constant for all tanks throughout the exposure (2 L hr⁻¹). Fish were kept at a temperature of 15 \pm 2° C

and a photoperiod of 8L: 16D. Additional water quality parameters were: pH 7.7 \pm 0.5; ammonia $<0.01 \text{ mg L}^{-1}$; dissolved oxygen $> 80\%$. All fish were fed live *Artemia salinus* nauplii (enriched with HUFA, ZM Systems, UK) twice daily from hatch with feed rations referring to dry weight of viable *Artemia*. Any food remaining in the aquaria was siphoned out at the end of each day to maintain high levels of water quality.

2.1.3. Ethical statement

All experimental procedures with fish were conducted in accordance with UK Home Office regulations for the use of animals in scientific procedures (Animals (Scientific Procedures) Act (ASPA), 1986) and followed local ethical review guidelines and approval processes. Water quality parameters including temperature, pH, ammonia, and dissolved oxygen were assessed daily.

2.1.4. EE₂ exposure - food ration interaction study (Study 1)

Newly hatched stickleback larvae (0 days post hatch - dph) were transferred into aquaria containing 2 L of water at densities of 5 (Low Density, LD), 15 (Medium Density, MD), and 30 (High Density, HD) larvae per aquarium. Twelve aquaria per density were set up in a random block design, with four aquaria per density assigned to each treatment (100 ng L⁻¹ EE₂; solvent control (0.0001% EtOH); water control). Fish were fed at a constant diet of 4.5 mg food per tank per day from hatch until termination of the experiment. Food was allocated on a per tank basis so that food became increasingly limiting with increasing density and fish size. Every 10 days, 5 fish from each aquarium were measured for total body length (from tip of snout to tip of the tail) from images taken using a digital camera (Canon 100D with a 70 mm macro lens) where the fish were placed in a glass vessel with a measurement grid placed below it. Images were analysed using proprietary imaging software (imageJ, Rasband, 1997–2006) to obtain accurate body length measurements (to the nearest 0.1 mm). Mortalities were recorded daily. The exposure was completed at 30 dph and all fish were euthanised in $> 250 \text{ mg L}^{-1}$ benzocaine, followed by physical destruction of the brain, and measured for total body length (cm) and wet weight (mg).

2.1.5. Statistical analysis

All data were tested for normality using Shapiro-Wilks normality tests. Spearman's rank correlations were used to investigate general trends between fish density and final body lengths and wet weights in control and EE₂ treated fish. The effect of EE₂ on total body length at each time point was compared with solvent controls for all fish density treatments using t-tests, since there were no statistical differences between water and solvent controls ($p > 0.05$ using t-tests). To normalise for any differential mortality within treatments, growth conversion efficiencies were calculated. We define growth conversion efficiency as somatic growth relative to energy (food) availability per individual (expressed as $\text{cm day}^{-1} \text{mg food}^{-1}$). Growth conversion efficiencies of EE₂ treatments at each individual density were measured for each of three interim 10 day periods (i.e. 0–10, 10–20 and 20–30 dph). The measurements were compared to solvent controls using t-tests. Pearson's correlation coefficients were used to investigate general trends between food availability and growth conversion efficiency for control and EE₂ exposure treatments. Significant differences are reported at the 5% significance level. Comparisons refer to EE₂-treated fish and solvent controls.

2.1.6. Model validation study (Study 2)

The energy budget model described below in section 2.2.1 was validated using empirical data from an independent pilot study conducted prior to the EE₂ exposure study. This pilot study was undertaken between June 2016 and August 2016. The aim of this pilot study was to (i) determine appropriate stocking densities and food rations for the exposure study and (ii) to generate data for validation of the energy budget model.

Fish embryos were obtained from the earlier (F3) generation of stickleback held at the University of Exeter; otherwise all animal and husbandry conditions were identical to those described in section 2.1.2.

Sticklebacks were maintained at densities of 3, 10, 15, 20 and 30 fish per 2 L aquaria from 0–60 dph in de-chlorinated water. All aquaria were given a constant diet of 5.5 mg food per aquaria each day from hatch until termination of the experiment, so that food became progressively limiting as density and fish size increased. Body lengths of fish were quantified every 15 days throughout the experiment using the methods described in section 1.1.3. As

part of this pilot study, the authors additionally confirmed that space limitation did not affect growth rates and any differences in body length between density treatments emerged solely from the varying levels of food competition. Full methods and results of the space limitation study can be found in the supporting information (SI).

2.2. Model

2.2.1. Model description

A detailed model description of the energy budget model following the ODD protocol (Overview, Design concepts, Details) can be found in the SI. The model simulates the development and growth of an individual fish from embryo to 60 dph in a 2 L body of water (equivalent to the 2L aquaria used in our study), represented by a 2 L patch. It is built as an individual based model (IBM) in NetLogo 6.0.1 (Wilensky, 1999) to facilitate future integration into an existing population model (Mintram et al., 2018). The structure of the model follows Sibly et al., (2013).

All life-history processes are modelled implicitly via energetic costs. The individual begins as an egg and develops into a hatched eleuthero-embryo (referred here-in as a larva) following a temperature-dependent incubation period. The energy reserves of eggs and larvae are implicit as we assume these life stages have sufficient energy reserves in the yolk sac to cover maintenance, growth and development costs. Subsequent juvenile stages feed exogenously. Over each daily time step, juvenile fish ingest and assimilate food according to the food density and energy content of the patch. First, they allocate energy to cover maintenance costs (including movement) followed by somatic growth costs. If there is surplus energy after the costs of maintenance and somatic growth, this is stored as lipid reserves. If energy assimilated from food is not sufficient to cover maintenance costs, juveniles use energy from their reserves.

Maintenance costs are determined by the active metabolic rate (AMR = basal metabolism and cost of swimming activity) for juveniles. Under periods of food limitation, however, AMR is adapted so that juveniles increase movement in an attempt to search for food up to a critical point, after which energy is conserved and metabolic rate (via movement) is decreased (Wieser, 1991). This strategy

has been reported in early life stages of several fish species (Méndez and Wieser, 1993, Wieser et al., 1992), including stickleback (Beukema, 1968). This adaptive management of movement costs under periods of food limitation is a key concept within the model and is referred to here-in as adaptive foraging behaviour.

Two versions of the model were constructed to facilitate model analysis: (1) 'Non-adaptive foraging', where AMR remains constant, at twice the BMR (Fry, 1947; Meakins and Walkey, 1975; Tytler and Calow, 1985), throughout all simulations and is thus independent of food availability; and (2) 'Adaptive foraging', where AMR is dependent on food ration as described in the model description. Model 2 is the default model and is used for all analyses (e.g. validation and sensitivity analysis) unless stated otherwise.

2.2.2. Sensitivity analysis

A local sensitivity analysis was performed, where each of the input parameter values in the model were altered one at a time by $\pm 10\%$. The effects of the altered parameter values were assessed by comparing modelled outputs (total body length and wet weight) from control and altered simulations at 60 dph.

2.2.3. Model validation

Model simulations were compared to data from Study 2 (section 2.1.6) by comparing body lengths over time where food densities (mg food fish^{-1}) matched those used in in Study 2 (exact food densities used in the model can be found in SI). We assume that food was evenly ingested by each fish in the tank. This gives a good indication of the reliability of the parameters obtained for ingestion, assimilation, maintenance, and growth.

Additionally, since adaptive foraging behaviour is a key concept in the model (see section 2.2.1) we assessed the importance of including this behaviour. To do this, we compared outputs of body lengths and body mass from the two versions of the model described in section 2.2.1 (i.e. with and without adaptive foraging behaviour) to the validation data from Study 2 using the Akaike Information Criterion (AIC, for methods see Motulsky and Christopoulos, 2004).

2.2.4. Simulation experiments

We used the energy budget model to explore hypotheses generated by the results observed in the EE₂ exposure – food ration interaction study (Study 1). These hypotheses, as well as the details describing the model's application in testing these hypotheses, are described in section 3.3.

3. Results

3.1. EE₂ exposure – food ration interaction study (Study 1)

3.1.1. Mortality

Mean mortality rates did not exceed 15% for any of the control or exposure treatments. In the Low Density (LD) treatment, three out of four EE₂ exposure replicates had one mortality per aquarium and since food ration was maintained constant over time, these mortalities were accounted for by assessing growth conversion efficiencies.

3.1.2. Body lengths

Final body lengths increased with increasing food availability in both the control and EE₂ treatments (control: Spearman rank correlation (S) = -0.716, $p < 0.001$; 100 ng EE₂ L⁻¹: S = -0.723, $p < 0.001$) (Fig. 1).

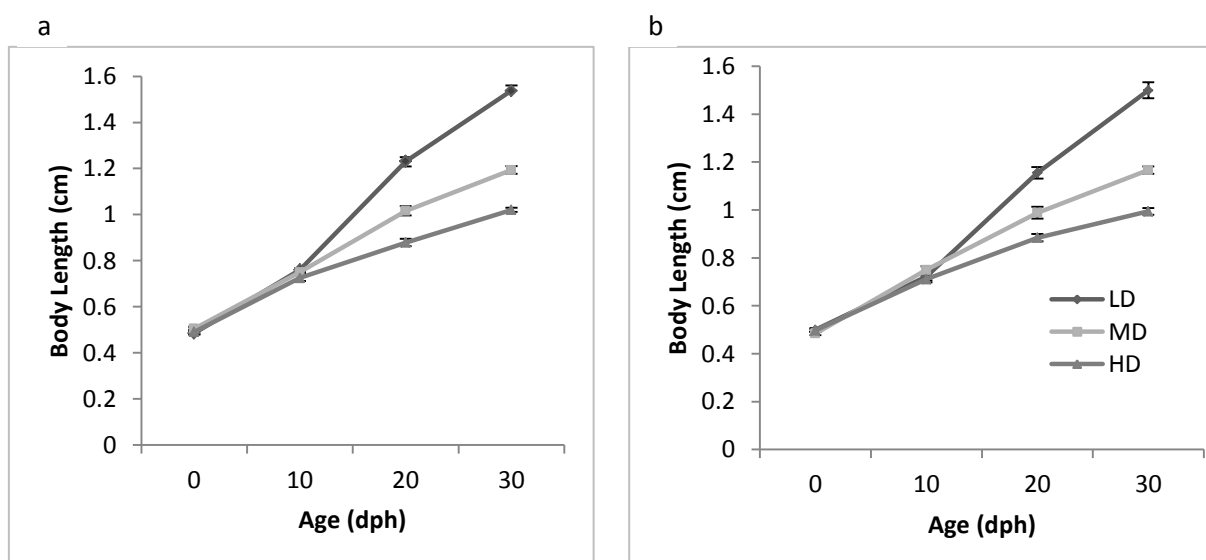


Figure 1. Mean body lengths (cm \pm s.e) over time of solvent control (a) and EE₂ exposed (b) fish maintained at densities of 5 (Low Density, LD), 15 (Medium Density, MD) and 30 (High Density, HD) fish per aquarium.

Consistent with the range finding study (for details of results see SI), EE₂ caused significant reductions in body lengths compared to solvent controls at 20

dph ($t_{32.768} = 2.314$, $p = 0.027$) in treatments where food availability was highest i.e. the Low Density (LD) scenario, but this was not the case at 10 or 30 dph ($p > 0.05$) (Fig. 2b). Body lengths did not differ between solvent control and EE₂ treatments at any time point in treatments with more limited food availabilities i.e. Medium Density (MD) and High Density (HD) scenarios ($p > 0.05$) (Fig. 2). Final wet weights at 30 dph were progressively higher with increasing food availability (control: $S = -0.707$, $p < 0.001$; 100 ng EE₂ L⁻¹: $S = -0.740$, $p < 0.001$). There were no significant differences, however, in final wet weights between solvent control and EE₂ treatments for any food availability level ($p > 0.05$).

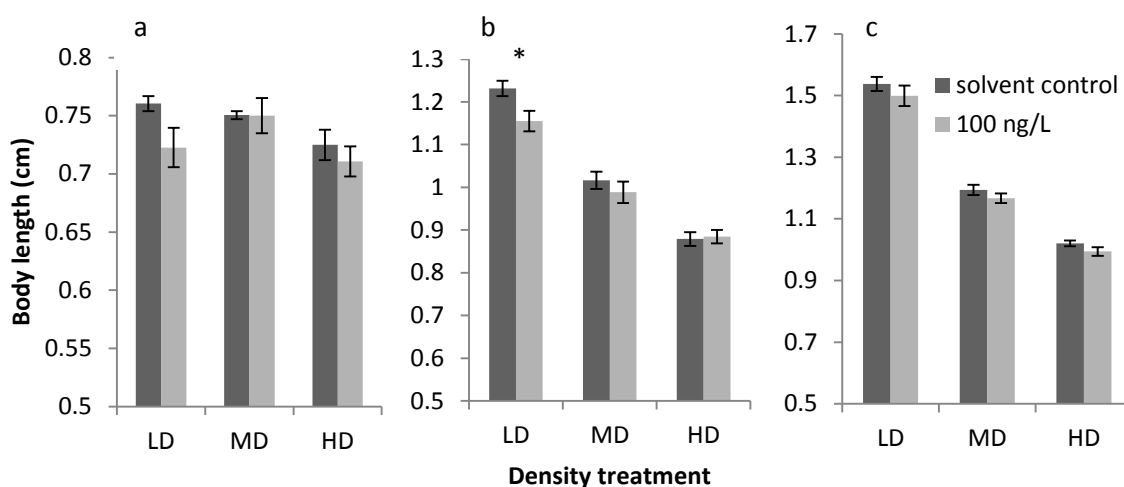


Figure 2. Mean absolute body lengths (cm) (\pm s.e) of stickleback maintained at densities of 5 (Low Density, LD), 15 (Medium Density, MD) and 30 (High Density, HD) fish per aquarium under solvent control or EE₂ exposure conditions (100 ng L⁻¹) at (a) 10, (b) 20 and (c) 30 dph. Statistically significant differences ($p < 0.05$) are represented by an asterisk.

3.1.3. Growth conversion efficiency

Within the EE₂ exposure treatment and the solvent control treatment growth conversion efficiency (cm day⁻¹ mg food⁻¹) was significantly increased with decreasing food availability according to Pearson's correlation (r_{10}) for each 10 day growth increment (Table 1).

Table 1. Pearson's correlation coefficients (r_{10}) showing increasing growth conversion efficiencies with decreasing food availability in solvent control and EE₂ treatments for each time increment.

Treatment \ Time increment	Solvent control		100 ng EE ₂ L ⁻¹	
	r ₁₀	p value	r ₁₀	p value
0–10 dph	0.962	< 0.001	0.913	< 0.001
10–20 dph	0.686	0.014	0.862	< 0.001
20–30 dph	0.850	< 0.001	0.870	< 0.001

Comparing the EE₂ exposure treatment and the solvent control treatment, growth conversion efficiency (cm day⁻¹ mg food⁻¹) was significantly reduced in the LD treatment between 0 and 10 dph (19%; $t_{5.491}$ df = 3.697, $p = 0.0133$) and 10 and 20 dph (22%; $t_{3.941}$ df = 3.849, $p = 0.0188$) (Fig. 3a and b) but not significantly reduced between 20 and 30 dph (4%; $t_{4.228}$ df = 0.376, $p = 0.725$) (Fig. 3c). In the MD and HD treatments, there were no statistically significant differences in growth conversion efficiency at any time points ($p > 0.05$). Thus, as food limitation increased with increasing fish stocking density and increasing body size over time, the effect of EE₂ on growth conversion efficiency was reduced.

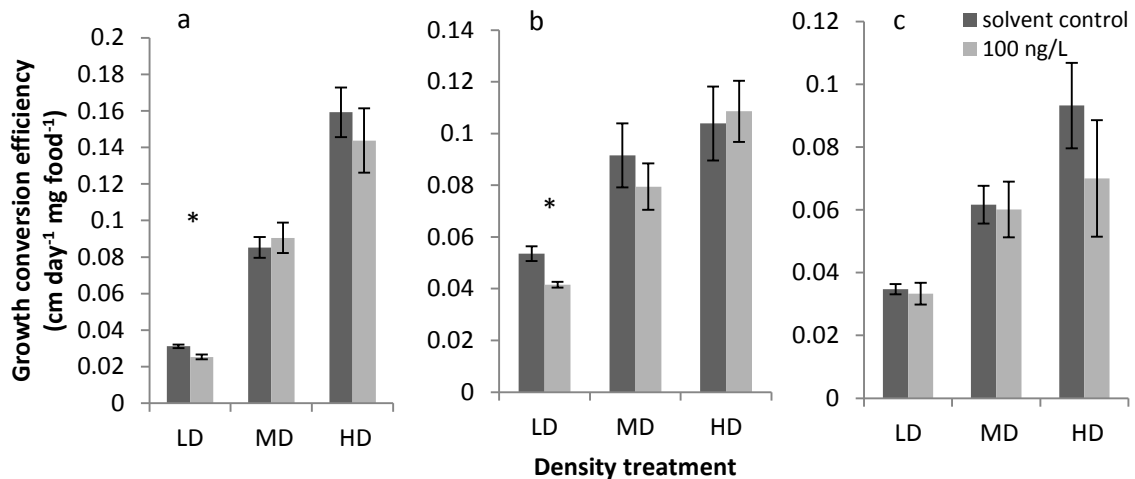


Figure 3. Mean growth conversion efficiency (cm day⁻¹ mg food⁻¹) (\pm s.e) of stickleback maintained at densities of 5 (Low Density, LD), 15 (Medium Density, MD) and 30 (High Density, HD) fish per aquarium under solvent control or EE₂ exposure conditions (100 ng L⁻¹) measured for periods between 0 and 10 (a), 10 and 20 (b) and 20 and 30 (c) dph. Statistically significant differences ($p < 0.05$) are represented by an asterisk.

3.2. Model analysis

3.2.1. Sensitivity analysis

The model was robust generally to changes in parameter values from all sub-models (< 10% change in output variable for 10% change in input variable), with the exception that body mass was sensitive to some growth parameters (SI, Table A2).

3.2.2. Model validation

Body lengths from the default model (i.e. Model 2) over each time period under the different food rations displayed a good fit to those measured in the empirical validation study (Study 2), where the mean (\pm s.d) R^2 value of all food ration data was 0.95 ± 0.044 (Fig.4). Similarly, predicted wet weights at 60 dph fitted closely with the mean wet weights reported in empirical Study 2 ($R^2 = 0.86$).

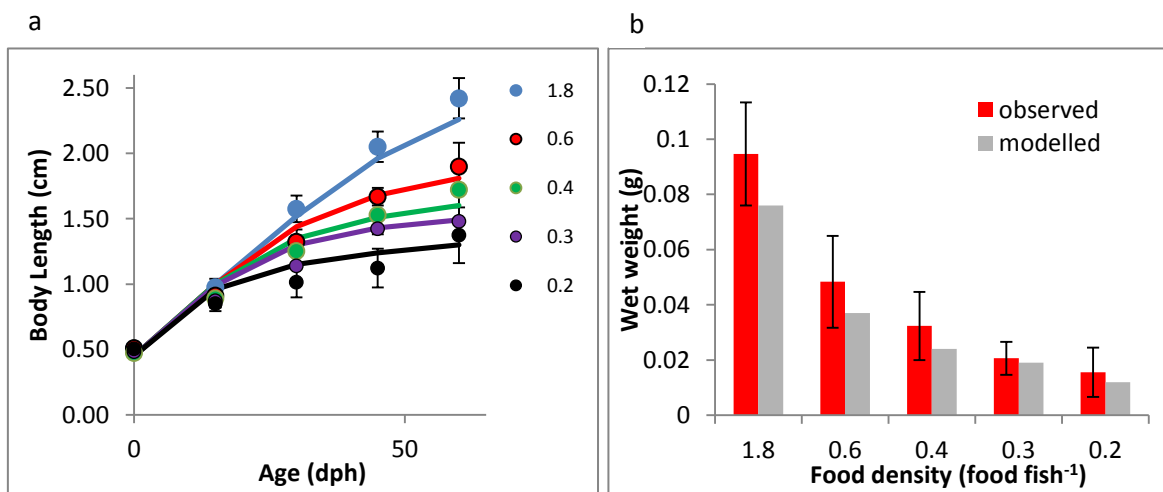


Figure 4. Body lengths of observed (circles) and modelled (lines) stickleback from 0 to 60 dph (a) and wet weights of modelled and observed stickleback at 60 dph (b) under varying food availabilities (fish densities). Figure 3a shows data for food densities ranging from 0.2 – 1.8 mg fish⁻¹ day⁻¹. Observed laboratory data are displayed as means (\pm s.d), whereas modelled outputs represent a single model run.

The 'Non-adaptive foraging model' (Model 1) mostly under-predicted final body lengths and masses of sticklebacks at 60 dph, and this was particularly evident for body mass (model fits not shown, outputs from Model 1 can be found in the

Sl.). The ‘Adaptive foraging model’ (Model 2) provided a better visual fit to the data, where model outputs more closely resembled the measured growth patterns (i.e. body lengths over time) and final body masses at 60 dph. Statistical analyses confirmed that the ‘adaptive foraging model’ (Model 2) better fit the empirical data from Study 2 (Table 2).

Table 2. Comparison of AIC and Akaike weights (AIC_w) for Model 1 (‘Non-adaptive foraging model’) and Model 2 (‘Adaptive foraging model’) when compared to empirical measurements of body length over time and body mass at 60 dph under varying food rations. A lower AIC value represents a better model fit, whereas the AIC_w represents the relative likelihood of the model.

	AIC		AIC _w	
	Model 1	Model 2	Model 1	Model 2
Body length over time	-107.7	-114.0	0.04	0.96
Body mass at 60 dph	-37.8	-39.5	0.3	0.7

3.3. *Simulation experiments*

The model was used to explore two key findings from the EE₂ exposure study (Study 1): (i) growth conversion efficiency increases with higher fish density and reduced food availability and (ii) the inhibitory effect of EE₂ on growth conversion efficiency reduces as food limitation increases (Fig. 3). We focus on the time point between 10 and 20 dph for all further analyses because this is where the effect of EE₂ on growth was detected in the exposure study (Study 1). The earlier time point between 0 and 10 dph, which additionally showed significant effects of EE₂ on growth conversion efficiency, included a larval phase in the model where fish were not exogenously feeding and were therefore not responsive to food ration during this time.

3.3.1. *Growth conversion efficiency increases with reduced food availability*

The purpose of investigating this pattern is to explore the relative roles of body size (i.e. metabolic scaling) vs adaptive behaviour in determining growth conversion efficiency under control conditions and to ensure that the model provides good fits to the observed control growth conversion efficiencies prior to

investigating the interactive effects of EE₂ and food limitation. Possible mechanisms underlying the increase in growth conversion efficiency at higher fish stocking densities (and lower food availabilities) in Study 1 were explored first using the ‘Non-adaptive foraging model’ (Model 1) which accounts for metabolic scaling i.e. fish with lower rations are smaller and thus metabolic rate is higher and *vice versa* (e.g. Sibly et al. 2013; Glazier, 2014; Hirst et al. 2014). We then used the ‘Adaptive foraging model’ (Model 2), which assesses for the role of movement adaption in determining growth conversion efficiency. Model outputs were compared with the observed patterns of growth conversion efficiency in Study 1. Outputs of growth conversion efficiencies from both versions of the models were compared to those observed in the solvent control treatment for all food availability levels.

Model simulations demonstrated that fish mass (i.e. metabolic scaling) was important in determining the patterns of increasing growth efficiency with decreasing food availability. However, model outputs including adaptive foraging behaviour (Model 2) generally provided a better fit with the empirical data (Fig. 5).

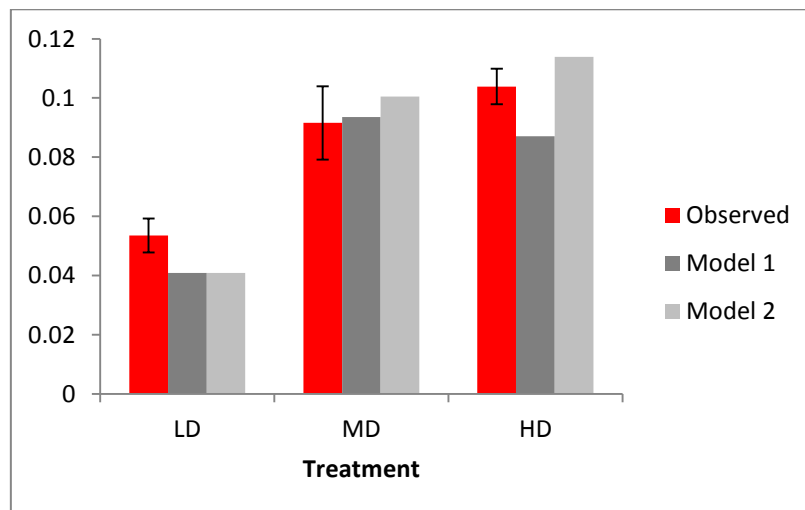


Figure 5. Growth conversion efficiency of observed and modelled stickleback maintained at densities of 5 (Low Density, LD), 15 (Medium Density, MD) and 30 (High Density, HD) fish per aquarium. Model 1 excludes adaptive foraging behaviour, whereas Model 2 includes it. Observed laboratory data are displayed as mean standard body lengths (cm ± s.d), whereas modelled outputs represent a single run.

3.3.2. *The inhibitory effect of EE₂ on growth conversion efficiency reduces as food limitation increases*

We used the energy budget model to explore possible physiological mechanisms (i.e. partitioning of energy budgets) which may explain why the effect of EE₂ on growth conversion efficiency was less apparent with reduced food ration between 10 and 20 dph.

We tested three hypotheses:

1. The reduction in growth conversion efficiency caused by EE₂ is constrained by body size (via metabolic scaling); reductions in fish growth rate are less likely to be detected/resolved with decreasing food availability and body size.
2. Adaptive foraging behaviour (i.e. where AMR is dependent only on food availability) compensates for further reductions in somatic growth rate caused by EE₂ exposure at low food availabilities.
3. Additional physiological adaption i.e. where movement costs are reduced further in EE₂ exposed treatments compared to controls, limits further reductions in growth rates caused by the chemical at low food availabilities.

To test these hypotheses, an EE₂ toxicant effect sub-model was added to the energy budget model where growth conversion efficiencies of fish aged 10-20 dph were reduced by 22%, to match those observed in the LD treatment of the empirical exposure study (Study 1). The 'Non-adaptive foraging behaviour' model (Model 1) was used to test hypothesis 1. Removing adaptive foraging behaviour from the model ensured that outputs only emerged from the prescribed physiological partitioning of energy budgets of individual fish, and not as a result of adaptive behaviours. The 'Adaptive foraging model' was used to test hypothesis 2. To test hypothesis 3, the adaptive foraging behaviour was adjusted further so that when the toxicant effect was present, AMR was reduced to 2/3 of the default AMR. Since there are no studies quantifying reductions in movement as a response to toxicant exposure, this value represents the AMR of fish which are conserving energy as a result of long-term periods of food stress (Wieser et al., 1992).

Model simulations were then compared to empirical results quantifying the reduction of growth conversion efficiency compared to solvent controls in the EE₂ exposure study (Study 1) for each food ration: LD -22%; MD -13%; HD +4%. Hypotheses 2 and 3 best explained the results observed in the empirical exposure study, where growth efficiencies were -22%, -11%, and -1% of control values, for LD, MD and HD treatments, respectively (Fig. 6).

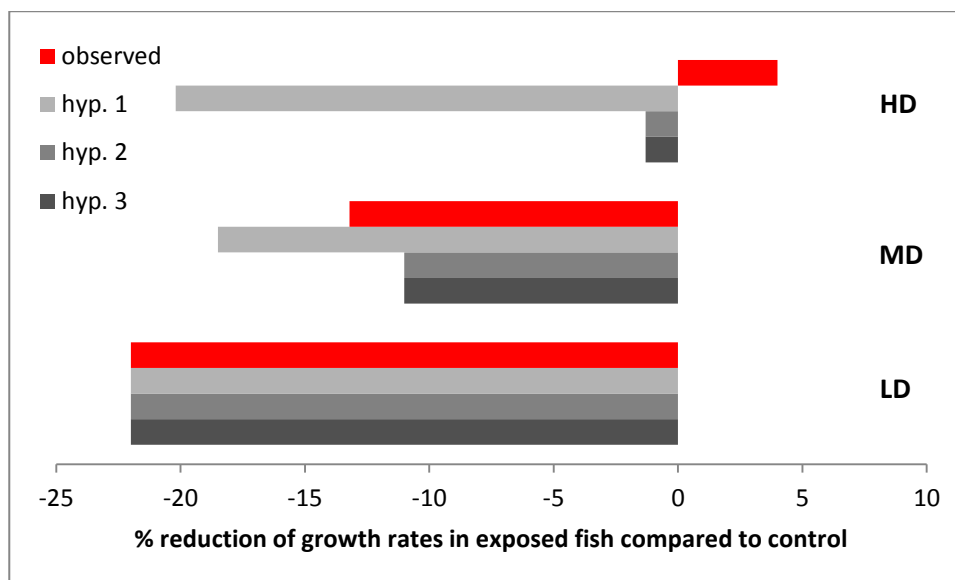


Figure 6. Predictions (according to hypotheses 1–3) of percentage reduction in growth conversion efficiency ($\text{cm day}^{-1} \text{ mg food}^{-1}$) of EE₂ exposed fish relative to controls for food rations imposed in the empirical exposure study (Study 1) and modelled outputs for each hypothesis. HD = High Density ($30 \text{ fish aquaria}^{-1}$); MD = Medium Density ($15 \text{ fish aquaria}^{-1}$); LD = Low Density ($5 \text{ fish aquaria}^{-1}$).

4. Discussion

We combined the use of empirical exposure studies and a mechanistic model to explore the interactive effects of EE₂ and food limitation on the growth of early life stage stickleback. The empirical study demonstrated that the inhibitory effects of EE₂ on somatic growth (body length and growth conversion efficiency) observed in high food availability treatments were not discernible in low food availability treatments. We used an energy budget model to explore the potential for physiological and behavioural mechanisms to compensate for the inhibitory effects of chemical exposure and food limitation on growth.

EE₂ is an oestrogen receptor agonist and the direct toxicological effects of exposure on fish are well established in the literature, including reductions in

somatic growth rates in early life stage fish (Lange et al., 2000; Papoulias et al., 2000; Shved et al., 2008; Van den Belt et al., 2003; Zha et al., 2008). There are several possible underlying mechanisms which may explain this effect. These include the fact that EE₂ has been shown to interfere with growth hormone (GH) and insulin-like growth factor (IGF) in tilapia (Shved et al., 2008), which are known to mediate somatic growth in fish (Reinecke et al., 2005). Equally chemical exposure can lead to reduced growth rates as energy is reallocated to cope with the effects of the chemical (or other) stressors (Calow, 1991; Sibly and Calow, 1989). As an example, exposure to environmentally relevant concentrations of EE₂ incurs a metabolic cost through the production of the yolk protein precursor vitellogenin (Copeland et al., 1986; Tyler and Sumpter, 2006). This has been displayed in early life stage fathead minnow, where exposures to 10 ng EE₂ L⁻¹ induced vitellogenin synthesis with a window of enhanced sensitivity between 10 and 15 dph (Van Aerle et al., 2002), which coincides with the time point in the present study at which the EE₂ effect on body length was significant. Other physiological costs associated with exposure to high levels of EE₂ during early life stages in fish include hepatic and renal toxicity (Zha et al., 2008; Zillioux et al., 2001). Fish are especially susceptible to toxic effects on growth in early life stages, particularly during sensitive ontogenetic stages such as sexual differentiation and organ development (Mohammed, 2013). During early life stages, somatic growth rates are at their highest and this is essential for maximising survivorship in the wild (e.g. under predation and competition pressure (Lorenzen, 1996)); thus chemicals which affect early life stage growth may have ecological consequences for fish populations.

The interactive effects of EE₂ and food limitation have not previously been explored; however, significant effects of food limitation on toxicity of other chemicals have been reported in fish. Hopkins et al. (2002), for example, demonstrated increased sensitivity to coal-combustion wastes of starving lake chubsuckers (*Erimyzon sucetta*). Interactive effects of food limitation and exposure to PCBs were additionally reported by Jørgensen et al. (1999) in Arctic charr. Conversely, carp were less resistant to copper when food was abundant than when food was limited, which was linked to increased hepatic metallothionein in starved fish protecting against copper exposure (Hashemi et al., 2008). These results in carp are similar to the findings in our study in that

fish which are already stressed by food limitation demonstrated an increased level of resilience to additional chemical stressors compared to fish maintained on high food rations. In many cases, multiple stressors interact to cause cumulative effects on organisms; however, we have demonstrated that these effects may not always be additive. Fish have inherent abilities to be able to adapt their behaviour under periods of food stress because they are continuously subjected to fluctuations of food availabilities in the wild (Milinski, 1979; Dill, 1983; Beyer; Beyers et al., 1999). It is logical, therefore, that these inherent adaptive abilities may help organisms to cope when they are subject to additional stressors, and our results support this.

It is well established in the literature that movement costs are adapted in the presence of food limitation (Bagamian et al., 2004; Méndez and Wieser, 1993; Wieser, 1991; Wieser et al., 1992). Movement may also be adapted under toxicant exposure; for example, Handy et al. (1999) demonstrated that rainbow trout (*Oncorhynchus mykiss*) reduced locomotion in response to Cu exposure, and suggest that this reduction is a metabolic 'sparing effect' to enable detoxification of Cu without impairing growth rates. In the absence of chemical exposure, individuals adapt their physical activity when food is limited in order to maximise growth rates and our modelled simulations (including adaptive foraging behaviour) supported this. When adaptive foraging behaviour was removed, the model under predicted outputs of length and mass. The model supported the hypothesis that the adaption of movement already exhibited as a result of food limitation compensated for the additional growth effects caused by EE₂ exposure. There are other potential physiological adaptations which may explain the observed results. For example, assimilation efficiency could increase when individuals are subjected to physiological stress (Rønnestad et al., 2013). Thus, we cannot say if reduced movement was entirely responsible for the reduced chemical effects observed when food was limited in our study; adaptive movement is an example of one of numerous possible adaptive mechanisms.

Integrated approaches, such as the one presented here, have the potential to provide insights into multiple stressor effects that are difficult to capture in the lab alone. It should be highlighted, however, that although energy budget models are useful for testing informed data-driven hypotheses, they cannot give

definitive answers unless the outputs are confirmed in the lab. In this sense, hypothesis testing simulation experiments may be useful for refining future lab studies. In addition, the empirical results showed that the effect of EE₂ on growth was not significant between 20 and 30 dph in the LD treatment. This may have been because as growth rates began to slow down naturally it gave the EE₂ treated fish an opportunity to catch up. However, it is also possible that the constant food ration maintained throughout the study resulted in food becoming limiting in the LD treatment as the fish got larger over the final time increment (fish received 5% of their final body weight as food at 30 dph). It is therefore difficult to conclude if this reduced EE₂ effect over time is an indication of the physiological susceptibility of individuals at different life stages / ages or a result of experimental error.

The magnitude of stress that can be tolerated by an organism is a function of the individual's capacity to adapt to the effects of the stressor(s) (Beyers et al., 1999). The resilience of organisms to chemical exposure is therefore determined by the potential for adaptation of physiological and/or behavioural processes. However, these adaptations may have unintended consequences and lead to tradeoffs; for example, reducing movement costs may affect the chances of encountering prey, which may in turn affect growth and survival (Hughes, 1998; Milinski, 1993; Railsback et al., 1999). Thus, animals in the wild may have less scope for physiological adaptation than animals in laboratory controlled conditions. Equally, the level of effect observed in laboratory studies where animals are maintained under optimal conditions may not accurately represent the level of effect observed in the environment, where additional stressors will contribute to the level of effect and to adaptive responses (Forbes and Calow, 1997; Calow and Forbes, 1998). Although there are environmental risk assessment frameworks for assessing the effects of exposure to multiple chemicals on organisms (Kortenkamp, 2007; 2008), there is currently no explicit consideration for the combined effects of chemical exposure and natural stressors. The energy budget model presented here provides a tool to assess the effects of toxicant exposure, whilst accounting for the potential cumulative effects of natural stressors, such as food availability, and taking into account the finite physiological energy budgets of individuals. We have demonstrated in this

study that physiological adaptations can compensate somewhat for the effects of multiple stressors in early life stage fish.

5. Conclusions

An integrated approach is presented by which the underlying mechanisms of multiple stressor effects can be explored using informed hypotheses. Using an *in vivo* study, the interactive effects of food limitation and chemical exposure on early life stage stickleback were assessed. An energy budget model was confronted with multiple hypotheses from which we concluded that stickleback may adapt their physical activity to cope with the effects of these two stressors. The combined laboratory study – energy budget model approach has shown potential for providing more detailed mechanistic assessments of multiple stressor effects.

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Chapter 3. Supplementary Information

*Interactive effects of food limitation and environmental oestrogen exposure in the three-spined stickleback (*Gasterosteus aculeatus*).*

Part 1: Full model description (ODD)

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Part 4: References

Part 1: Full model description (ODD)

Overview

Purpose

The model was developed to provide realistic predictions of energy allocation in the three-spined stickleback under varying conditions and stressors. The model will be used to explore the differential allocation of energy to different life-history processes in the presence of food limitation and under periods of chemical stress.

Entities, state variables and scales

The entities in the model are a single spatial unit and one stickleback with its own energy budget. The model environment mimics a 2 L fish tank comprising of a single patch. The individual fish goes through three life stages: egg, larva, and juvenile. The stickleback is characterised by the state variables age, body weight (g, dry weight), body length (cm, total length) and energy reserves (kJ (juveniles only), converted into g to account for total body weight, see *process overview and scheduling*). Spatial units are characterised by water volume (L), food density (g) and food quality (kJ g⁻¹). The overall environment is characterised by temperature.

Process overview and scheduling

Each juvenile in the model possesses its own energy budget; the energy budgets of eggs and larvae are implicit as it is assumed that there is sufficient energy in the yolk sac to undergo maintenance, and maximum growth for larvae. Each of the following processes (highlighted in bold) will occur each time step in sequential order. The modelled time step represents one day and simulations cease once the individual is aged 60 days post hatch.

Update landscape: Food density and energy content of the patch is replenished at the start of each day.

Ingestion and energy intake: Juveniles assimilate a fixed proportion of energy dependent on the maximum ingestion rate of the individual, which is dependent on body length, and the energy content of the food.

Maintenance: Juveniles pay maintenance costs from assimilated energy or energy reserves according to their active metabolic rate, which is influenced by food availability. Individuals die if the costs of maintenance are greater than the sum of assimilated energy and energy reserves. Mass and temperature have scaling effects on metabolic rate.

Growth: Larvae grow at a constant rate. Juveniles undergo somatic growth if assimilated energy is available after the costs of maintenance have been paid. This sub-model determines total length (cm) and structural mass (g).

Energy reserves: Once the costs of maintenance and somatic growth have been paid, juveniles store energy in the form of lipids. The total energy content of the reserves (kJ) is converted into mass (g), which is added onto the structural mass of the individual. When food is limited reserves are used to cover maintenance costs until they are depleted. Fluctuations in energy reserves result in fluctuations in total body mass.

Change life-stage: Eggs develop into larvae and larvae into exogenously feeding juveniles once each life stage has fully developed at the end of a temperature dependent incubation period. It is assumed that when larvae develop into juveniles, the energy reserves in the yolk sac have been fully depleted.

Design concepts

Basic principles: Individual energy budgets follow fundamental principles of physiological ecology (Sibly and Calow, 1986) and scale with body mass and temperature according to allometric laws as reviewed in Sibly et al. (2013). Food availability and temperature influence the allocation of available energy to life-history processes.

Adaption: Movement decisions are adaptive in the model, whereby the amount of energy allocated to movement is dependent on the hunger level of the individual and food density. When food is limited, movement costs initially increase as the individual searches for food, before decreasing to conserve energy (Wieser, 1991; Wieser et al., 1992). Movement rules implicitly follow some aspects of optimal foraging theory; if maintenance costs exceed the

energy assimilated from the ingested food, movement is subsequently adapted to maximise energy gains (Charnov, 1976).

Emergence: Growth rates and survival are emergent of the food density and energy content of the food in the environment and the cost of foraging.

Sensing: The individual senses food density.

Stochasticity: There is no stochasticity in the model.

Observation: Growth patterns (length and mass), ingestion rates and energy reserves are monitored daily.

Table A1. Default parameter values of stickleback energy budget model with sources.

Symbol & Definition	Value	Unit	Reference
E Activation energy	0.457	eV	Killen et al. (2010)
A_e Assimilation efficiency	0.95	---	Cui (1987)
E_x Energy content of food	24	kJ g^{-1}	Paffenhöfer (1967); Paul and Michael (1994)
B_o Taxon-specific normalization constant	7.8×10^6	$\text{kJ g}^{-1} \text{day}^{-1}$	calculated from Meakins (1975)
IG_{max} Maximum ingestion rate	0.006947	$\text{g cm}^{-2} \text{day}^{-1}$	Volsett and Bailey (2013)
h Half saturation coefficient	3.7×10^{-6}	g l^{-1}	Volsett and Bailey (2013)
A_{m0} Active metabolism multiplication factor	2	---	Meakins and Walkey (1975)
α Active metabolism slope	1	---	
E_c Energy content of tissue	3.5	kJ g^{-1}	Walkey and Meakins (1970) converted from wet to dry weight (*0.23) according to Chellappa (1989).
	Energy content lipid	39.4	kJ g^{-1} Jobling (1994); Schmidt-Nielsen (1997)
E_s Energy cost of synthesis tissue	1.7	kJ g^{-1}	calculated from Sibly and Calow (1986, pp. 54-5)

	Energy cost of synthesis lipid	14.7		kJ g^{-1}	Jobling (1994); Schmidt-Nielsen (1997)
L_m	Maximum asymptotic length	6.7		cm	Froese and Pauly (2016)
K	Growth constant	0.0193		cm day^{-1}	pers.obvs
L_b	Length at hatch	0.45		cm	pers.obvs
M_b	Mass at hatch	0.00156		g	Wootton (1973)
w_a	weight constant	0.0068		---	Froese and Pauly (2016)
w_b	weight exponent	3.28		---	Froese and Pauly (2016)
T_{ref}	Reference background temp	288.15		kelvin	

Details

Sub-models:

Ingestion and energy intake:

Individual ingestion rates are temperature dependent and follow a type II functional response, whereby food density (g l^{-1}) is modelled against ingestion rate ($\text{g cm}^{-2} \text{day}^{-1}$) as:

$$\text{Equation 1:} \quad \text{Ingestion rate} = IG_{max} e^{\frac{-E}{k} \left(\frac{1}{T} - \frac{1}{T_{ref}} \right)} \frac{X}{(h+X)} L^2$$

Where IG_{max} is the maximum ingestion rate ($\text{g cm}^{-2} \text{day}^{-1}$), X is food density (g l^{-1}), h is the half-saturation coefficient (g l^{-1}) and L is body length (cm). The maximum ingestion rate (IG_{max}) and half saturation coefficient (h) parameters were obtained by fitting the model to stickleback feeding data taken from Volsett and Bailey (2011). The original data recorded attack rate as a function of food density (artemia l^{-1}). Since the model requires ingestion rates rather than attack rates, failed attacks were taken into account using data from Heller and Millinski (1979) whereby the number of daphnia successfully ingested per attack was quantified. Artemia density was converted into artemia mass (g l^{-1}) as reported by Paffenhöfer (1967) and Paul and Michael (1994) and ingestion rates are corrected for body length.

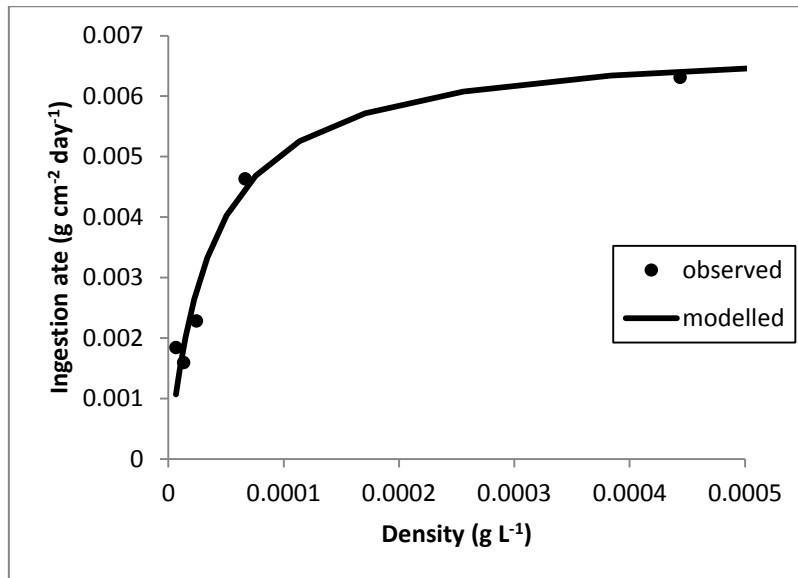


Figure A1. Modelled (black line) and measured (dots) ingestion rates for stickleback at varying food densities (*artemia nauplii*). With parameter values $IG_{\max} = 0.006947$ and $h = 0.0000367$

The model represents a tank environment where fish are fed *artemia nauplii* with an energy content of 24 kJ g^{-1} (Paffenhöfer, 1967; Paul and Michael, 1994). An assimilation efficiency of 0.95 was taken from Cui (1987). Since there are no reported assimilation efficiencies for the stickleback, the assimilation used here refers to the common minnow, *Phoxinus phoxinus*.

Maintenance:

Maintenance costs for juveniles comprise the active metabolic rate (AMR) below which the organism cannot survive:

Equation 2:
$$\text{Maintenance} = B_0 M^{\frac{3}{4}} e^{\left(-\frac{E}{kT}\right)} A_m$$

where B_0 is a taxon-specific normalization constant, $M^{3/4}$ is the scaling with body mass, $e^{\left(-\frac{E}{kT}\right)}$ is the exponential Arrhenius function, E is the activation energy and k is Boltzmann's constant ($8.62 \times 10^{-5} \text{ eV K}^{-1}$) (Peters, 1983; Gillooly et al., 2001; Brown and Sibly, 2012) and A_m is the active metabolism multiplication factor. The value of B_0 was estimated by solving eq. 2, where M is 1g, E is an activation energy of 0.457 and T is 288.15 K, to obtain a value of B_0 : $7,827,124 \text{ kJ g}^{-1} \text{ day}^{-1}$.

We assume that energy reserves in the yolk sac are sufficient to cover maintenance costs for eggs and larvae. Juveniles undergo active metabolism which incorporates basal metabolism and the energy costs of swimming. Meakins (1975) recorded the active metabolic rate of spontaneously active stickleback at 15°C as 0.153 kJ g⁻¹ day⁻¹, compared with a basal metabolism of 0.08 kJ g⁻¹ day⁻¹. This is consistent with the common assumption that the cost of active metabolism in fish is approximately twice the resting metabolic rate (Fry, 1947; Tytler and Calow, 1985). To account for active metabolism, we therefore apply a default multiplication factor (A_m) of 2 to the costs of basal metabolism.

Under periods of food limitation, however, fish adapt their active metabolism. Wieser (1991) suggested that in response to shortages of food, fish increase locomotory activity in search of food up to a critical point, after which energy is conserved and metabolic rate is decreased. This strategy has been reported in early life stages of some fish species (Méndez and Wieser, 1993, Wieser et al., 1992). The metabolic responses to food deprivation will vary depending on the life-history strategy of the fish, but generally predatory fish will increase activity in search of food (Wang et al., 2006). Hungry stickleback have been observed to increase swimming activity (Beukema, 1968) and this was also observed, yet not quantified, in a study undertaken by the authors. To account for this adaption in the model, the active metabolic rate multiplication factor (A_m) is adapted if the total energy gain from the patch is less than the total energy demand of the individual. The A_m parameter increases proportionally with increasing levels of starvation according to the linear equation,

Equation 3:
$$A_m = \alpha S + A_{m0}$$

where S is starvation relative to the daily requirements of the individual, A_{m0} is the active metabolic rate multiplication factor when starvation = 0, and α is the slope. If the energy gained from the food does not exceed the metabolic costs of moving to find the food, the individual will reduce movement costs to $A_m^{2/3}$ (Wieser et al., 1992). Additionally, if lipid reserves decline below a critical threshold of 50% of their potential maximum lipid reserves, the individual enters a starvation strategy where movement costs are decreased to $A_m^{2/3}$.

Growth:

If assimilated energy remains after the costs of maintenance have been paid, juveniles allocate energy to somatic growth. The energy costs of synthesising new tissue are calculated from body mass (g), which is allometric with body length (cm). The maximum growth rate (cm day⁻¹) of an individual under optimal conditions follows the von Bertalanffy (1957) growth function (VBGF):

Equation 4:
$$\text{Growth (cm day}^{-1}\text{)} = K (L_m - L)^3$$

where L_m is the asymptotic length (cm), K is the von Bertalanffy growth constant (cm day⁻¹), L is total length (cm). The model parameter K was parameterized by fitting the VBGF equation to length at age stickleback data obtained from a study undertaken by the authors at 15°C where growth was recorded in the first 60 days of life:

$$L_m \left(1 - \left(1 - \left(\frac{L_b}{L_m} \right) e^{-\frac{Kt}{3}} \right) \right)$$

where L_b is length at hatch (cm). An asymptotic length of 6.7 cm was obtained from Froese and Pauly (2016).

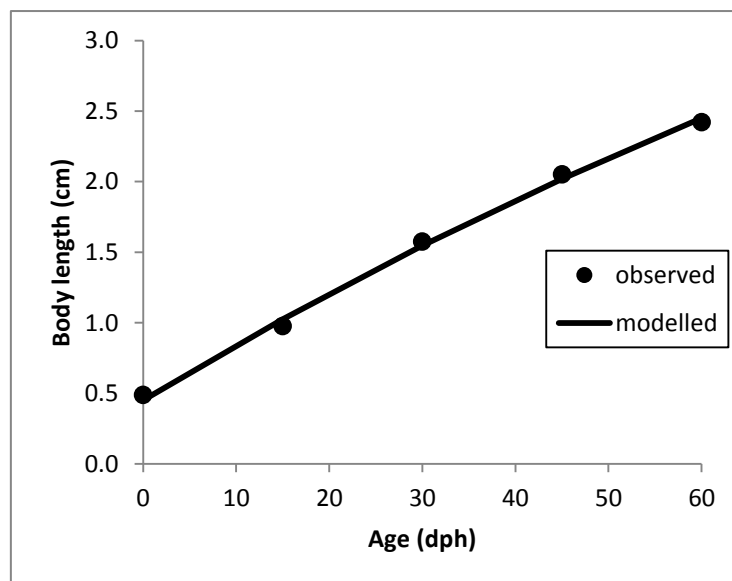


Figure A2. VBGF fit to age (dph) and length (cm) data collected from sticklebacks raised in laboratory conditions at 15°C in the first 60 days of life, where L_m is 6.7 and K is 0.0193.

Body length is converted into mass using the allometric length: mass equation,

Equation 5:
$$\text{Mass (g)} = m_a L^{m_b}$$

where M_a denotes the mass constant, L denotes body length (cm) , and m_b denotes the mass exponent. The equation was parameterised from 29,975 unsexed sticklebacks by Froese and Pauly (2014) using type 1 linear regression of log mass vs log length. The energy costs of synthesising new tissue are calculated as the daily addition of somatic body mass, taking into account the costs of synthesising new tissue ($E_c + E_s$). In juveniles, the allometric equation here is multiplied by $1/1.3$ to ensure that only the costs of structural growth are accounted for (see details below). If less energy is available than required for maximum growth rates, a lower rate is calculated for the energy available.

The average condition factor (CF, an index of the extent to which the total weight of a fish is high for its length, calculated as mass / length³ x 10⁶) of stickleback in the wild is 1.3 (Chellappa et al., 1995) and it is therefore assumed that body mass calculated from the allometric equation (Table, Eq. 6) refers to fish with a CF of 1.3. Juvenile mass fluctuates accordingly depending on individual energy reserves, whereby individuals with maximum energy reserves represent the highest possible mass, and individuals with no energy reserves refers only to the structural mass of the individual. The condition factor for the structural mass of the fish, or the somatic condition factor, is 1 (Chellappa et al., 1995). This sub-model accounts for the structural mass of the individual only, and body mass is therefore calculated as $(M_a L^{M_b}) \frac{1}{1.3}$. The mass of energy reserves are added onto the structural mass in 'energy reserves'. Since CF in larvae is assumed to be constant and independent of food availability, the mass calculated from the allometric equation refers to total mass for larvae.

Eggs do not grow and larvae grow at a constant rate of 0.033 cm day⁻¹ implicitly using energy reserves from the yolk sac.

Energy reserves and starvation:

Any remaining assimilated energy after the costs of maintenance and growth have been accounted for is stored as energy reserves in juveniles.

Energy is mainly stored as lipids (Chellappa et al., 1989) costing 14.7 kJ g⁻¹ for synthesis and storage and yielding 38 kJ g⁻¹ (Jobling, 1994; Schmidt-Nielsen, 1997). For simplicity, we assume that all energy is stored as lipid because glycogen accounts only for a very small proportion of total energy reserves

(Chellappa, 1989; Chellappa, 1995). According to Chellappa (1995), the maximum condition factor (see *growth*) reported in wild fish was 1.4 compared to a somatic condition factor of 1. Energy reserves in the model are thus stored up to a maximum threshold proportional to 40% of an individual's structural mass. The mass of energy reserves is added onto the structural mass of the individual to give a total mass.

In eggs and larvae, it is assumed that there are sufficient energy reserves in the yolk sac to cover maintenance costs, and growth costs in larvae. Energy reserves are utilised in juveniles in instances where the energy assimilated does not account for maintenance costs. Once all of the energy reserves are used up, the individual dies (Sibly et al., 2013).

Part 2: Appendices documenting additional details of model analysis

Appendix 1: Model sensitivity analysis

Table A2. Sensitivity analysis reported as the ratio of percentage change in output variables to 10% increases in input parameter values. Sensitive output values (> 0.1) are represented in bold.

<i>Parameter</i>	<i>Body Length</i>	<i>Body Mass</i>
Activation energy (E)	0	0
Assimilation efficiency (A_e)	0	0
Energy content of food (E_x)	0	0
Taxon-specific normalization constant (B_o)	0	0
Maximum ingestion rate (IG_{max})	0	0
Half saturation coefficient (h)	0	0
Active metabolism multiplication factor (A_{m0})	0	0
Active metabolism slope (α)	0	0
Energy content of tissue (E_c)	0	0
Energy cost of synthesis (E_s)	0	0
Maximum asymptotic length (L_m)	-0.08	-0.3
Growth constant (K)	-0.06	-0.22

Length at hatch (L_b)	0	0
Mass at hatch (M_b)	0	0
weight constant (w_a)	0	-0.11
weight exponent (w_b)	0	-0.35

Appendix 2: Validation of model 2 (no adaptive foraging behaviours).

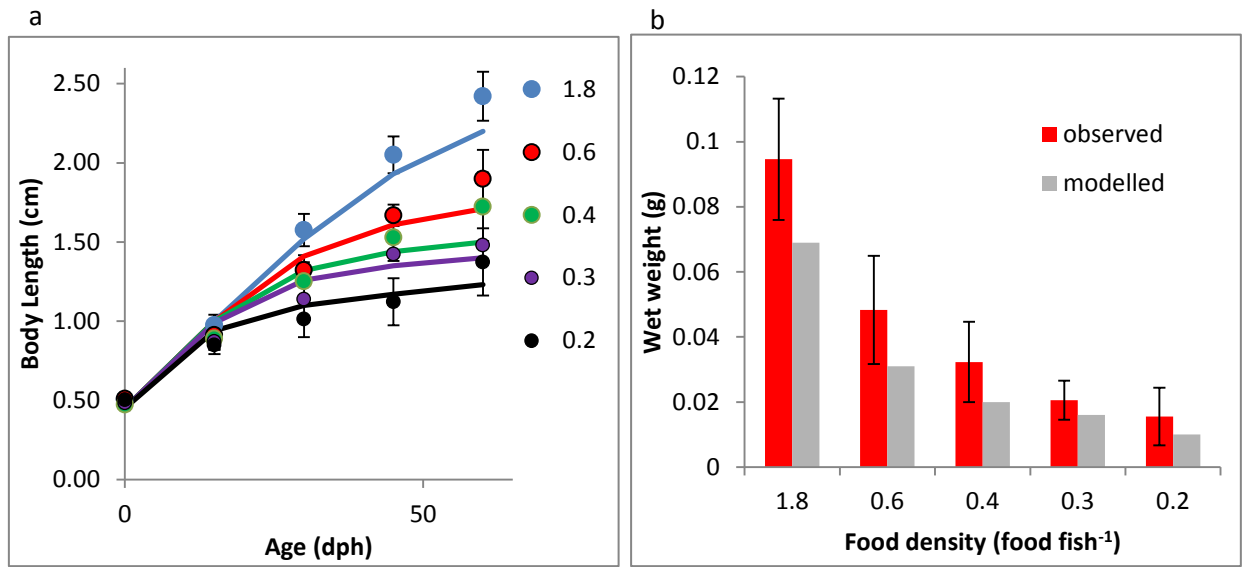


Figure A3. Body lengths over time (a) and final wet weights (b) of observed and modelled stickleback under varying food densities. Circles on graph a represent observed data and lines represent modelled data. Legend refers to food density per individual (mg). Observed data are displayed as mean lengths (\pm s.d), whereas modelled outputs represent a single run.

Appendix 3: Food densities used for model validation

Table A3. Food density per fish (g) inputs for model validation for each fish density treatment and each time point taken from the empirical validation study and accounting for fish mortalities.

Age (dph) Density (fish tank ¹)	0 – 15	15 -30	30 - 45	45 - 60
3	0.00183	0.00183	0.00183	0.00183
10	0.00063	0.00063	0.00063	0.00063
15	0.00041	0.00041	0.00041	0.00041
20	0.00033	0.00033	0.00033	0.00033
30	0.000195	0.00020	0.00022	0.00024

Appendix 4: Food densities used for assessing effects of food availability on growth efficiency (section 3.3.1).

Table A4. Food density per fish (g) inputs for model simulations of each fish density treatment and each time point taken from the empirical exposure study and accounting for fish mortalities.

Age (dph) Density (fish tank ¹)	0 – 10	10 -20	20 - 30
5	0.00088	0.00088	0.00088
15	0.000289	0.000289	0.000289
30	0.000147	0.000149	0.000153

Part 3: Appendices documenting additional details of empirical studies

Appendix 5: Additional details of model validation study.

Methods

Details of the food limitation study which was used to validate the model can be found in the main document (section 2.1.6). Here, we describe a simultaneous study which was undertaken alongside the food limitation study to assess if space limited the growth rates of stickleback. This study was undertaken to

ensure that any effects on growth rates observed in the food limitation study were not a result of limited space.

Animal and husbandry conditions were identical to those described section 2.1.6. Embryos were transferred into 2L tanks at densities of 3, 10, 15, 20 and 30 fish per tank. Four tanks per density were set up in a random block design. Food rations were calculated so that individual fish had access to 12% body weight of feed per day, resulting in space being the sole limitation for these colonies. Body lengths of fish were quantified every 15 days throughout the experiment using the methods described in the main document (section 2.1.3). Aquaria were siphoned daily to maintain high levels of water quality.

Results

There was no correlation between growth and density where food was not limited ($S = -0.02$, $p = 0.670$). Thus, space limitation had no effect on growth rates.

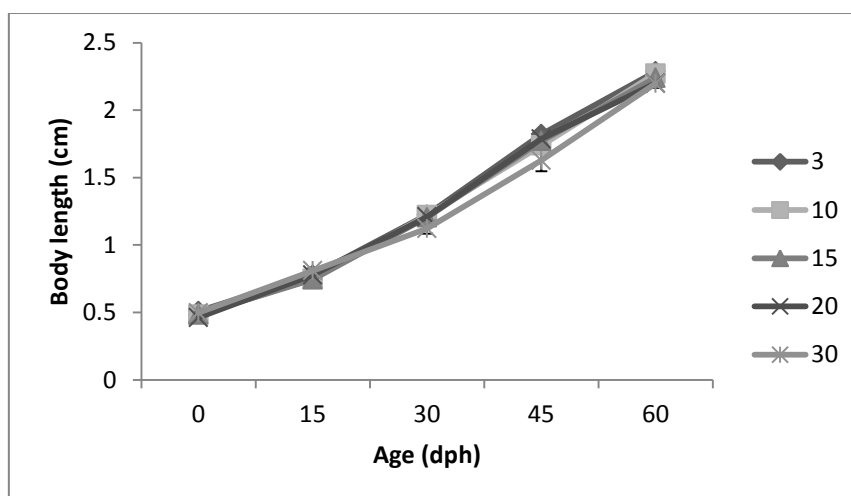


Figure A4. Mean total body length (\pm s.e) of stickleback stocked at 3, 10, 15, 20 or 30 fish per aquarium and fed 12% of body weight per day. Legend refers to fish density per 2L aquarium.

Appendix 6: Preliminary EE₂ exposure study

Stickleback exposed to 100 ng EE₂ L⁻¹ from hatch until 21 dph had significantly reduced final body lengths compared to solvent controls (0.001% ethanol (EtOH)) (Fig. A3). Stickleback were stocked at densities of 15 fish per 2 L aquaria and fed to satiation twice daily.

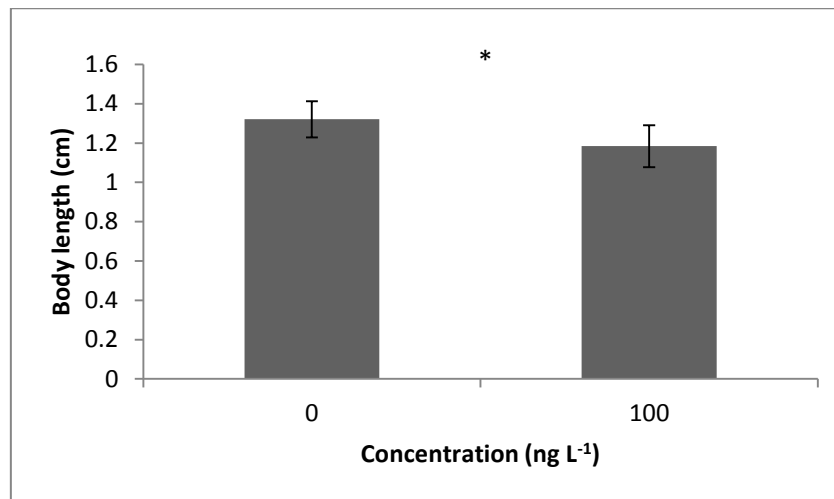


Figure A5. Mean total body lengths (\pm s.e) of stickleback aged 21 dph exposed to 0 (solvent control (0.001% EtOH)) or 100 ng EE2 L⁻¹ from 0 to 30 dph. Statistically significant differences ($p < 0.05$) are represented by an asterisk.

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Chapter 4. Applying a Mechanistic Model to
Predict Effects of Endocrine Disrupting
Chemicals on Fish Populations.

Abstract

The use of man-made chemicals is increasing as demands for improved healthcare and food security continue to grow. The potential environmental impacts of these chemical exposures on wildlife are of growing concern. Freshwater ecosystems are particularly vulnerable to these effects and wildlife populations, including fish, can be exposed to concentrations known to cause adverse effects on organisms. In addition, temperate fish populations often undergo sustained periods of food limitation in the wild which they have to cope with in addition to exposure to other stressors, including chemical exposures. The interactive effects of chemical exposures and food limitation on fish populations are difficult to establish and are not explicitly considered in current risk assessments.

Chemical effect mechanisms may be highly specific (e.g. the disruption of endocrine (hormone) signalling) or more general, (e.g. impacting physiology through the reallocation of energy reserves to metabolise or recover from the effects of chemicals). Mechanistic modelling approaches are being employed to predict how the effects of chemicals on the metabolism and energy budgets of individuals scale-up to the population level. Here an energy budget-individual-based model was developed and validated for the three-spined stickleback. Using two case study EDCs (EE₂ and trenbolone) with well-established mechanistic pathways, the model was applied to predict population level effects of EDC exposures. Additionally, the cumulative effects of chemical exposure and food limitation were assessed on stickleback populations. Findings suggest that the underlying mechanism of the chemical and the level of density dependent competition for food are important in determining the extent by which individual-level effects translate to effects on the population. The work illustrates that mechanistic modelling approaches can effectively integrate specific chemical effects, general effects on physiological energy budgets and density-dependent mechanisms with significant value for chemical risk assessments.

1. Introduction

Synthetic chemicals enter freshwater ecosystems directly from domestic and industrial effluents (Petrovic et al. 2002) and/or from diffuse agricultural runoff (Schulz 2004, Wauchope 1978). Of particular environmental concern are

endocrine disrupting chemicals (EDCs) which are considered of equal regulatory priority as Substances of Very High Concern (i.e. carcinogens and mutagens) (EC No 1272/2008). EDCs induce their effects through altering the synthesis, metabolism or function of natural endogenous hormones that in turn can lead to physiological stress to individuals and subsequently alter the allocation of energy to life-cycle processes (Calow, 1991; Beyers, 1999). Exposure to EDCs can cause direct effects on the allocation of energy to growth and/or reproduction e.g. synthetic oestrogens inducing inappropriate or excess vitellogenin synthesis, (Copeland et al. 1986; Tyler and Sumpter 1996; Wheeler et al. 2005; Ankley et al., 2010). Additional metabolic costs may also be incurred for example for the blood clearance of vitellogenin (Thorpe et al., 2007), or in the repair of damaged tissues (e.g hepatic and renal damage (Zha et al., 2008)).

In addition to chemical exposures, temperate fish populations are frequently subjected to environmental stressors, 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 are likely to have cumulative effects on fish populations (Holmstrup et al., 2010).

The development of mechanistic effect models, including population models, for chemical risk assessments has increased dramatically in the last decade (e.g. as a result of the CREAM (Chemical Risk Effects Assessment Models) initiative, see Grimm et al., 2009). The application of these models has influenced regulation, where results from population models are now considered when assessing adverse effects of EDCs on wildlife populations (EC No 2017/2100 and EC No 2018/6054). However, most of the models developed for risk assessments represent invertebrate taxa, and those which are developed for fish are limited in their ability to link mechanistic effects of chemical exposures to the adverse effects on populations. Existing population models often extrapolate directly from the adverse effects of chemicals on individual vital rates (survival, growth and reproduction) to quantify effects at the population level (e.g. Hazlerigg et al., 2014; Mintram et al., 2018), rather than representing the processes which link both levels of organisation. Individual-

based models (IBMs) offer the capability to simulate chemical effects on lifecycle traits directly and consider also compensatory processes, including density dependent compensation in vital rates. Energy budget models offer the additional benefits of quantifying direct and indirect (e.g. homeostatic) costs of chemical exposure on the partitioning of energy within organisms. Integrated approaches where energy budget models are combined with IBMs can be used to better link organism-level responses to population level effects. This approach has been used previously to predict chemical effects on invertebrate populations (Martin et al., 2013; Johnston et al., 2015) but has yet to be extended to other vulnerable taxa, including fish. Combining these modelling approaches also allows integration of potential cumulative effects of multiple stressors over time and space, including chemical and climatic stressors and food shortages, which is vital in providing realistic ecological assessments.

In order to mechanistically model the effects of EDC exposures, effect pathways which link key events from initial exposure and molecular initiating events (MIEs) to whole organism effects need to be established in order to determine the associated direct and indirect (including energetic) effects. An adverse outcome pathway (AOP) is a conceptual construct that portrays existing knowledge concerning the linkage between a direct MIE (e.g. oestrogen receptor agonism) and an adverse outcome at a population-relevant level (e.g. reduced reproductive success) (Ankley et al. 2010). The information inferred from AOPs can be used to explicitly characterise indirect, energy-mediated effects from specific chemical pathways (Goodchild et al., 2018; Murphy et al., 2018). There has been considerable interest in linking AOPs to energy budget 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). However, since AOPs are not chemical specific; rather, they link effect pathways for a specific MIE; additional information is needed to infer the full energetic effects of a specific chemical exposure. AOPs need to be used in conjunction with empirical data quantifying the physiological energy budgets of test organisms.

Here, a spatially explicit energy budget based-IBM for the three-spined stickleback (*Gasterosteus aculeatus*) was established using a published 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 follows the methods of Sibly et al. (2013) rather than traditional dynamic energy budget (DEB) theory (Kooijman, 2010). The primary reason being that DEB infers a 'kappa rule' which assumes that throughout the life-cycle, maintenance and somatic growth receive a fixed proportion of energy and any remaining energy is allocated to reproduction. Empirical evidence, however, supports that sticklebacks prioritise reproduction over growth since females continue to produce eggs whilst somatic growth ceases and body weight decreases under limiting food conditions (Wootton and Evans, 1976; Wootton, 1977; Wootton et al., 1978). DEB theory is therefore not deemed realistic for this species. Adopting the methodology of Jager and Zimmer (2012) and Johnston et al. (2014), it is assumed that chemicals impose stress on specific physiological parameters, which have predictable effects on life-cycle processes (e.g. reproduction) following energy allocation principles. Using two EDCs with two different effect mechanisms (17 α -ethinylestradiol (EE₂) – an oestrogen receptor agonist and 17 β -trenbolone – an androgen receptor agonist), the impacts of environmentally-relevant exposures of these EDCs on fish populations was assessed. Individual-level effects on fecundity for both EDCs 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 combination of energetic pathways by which EE₂ and trenbolone cause effects at the organism-level and assess how these effects extrapolate to the population level. The energetic pathways modelled here are justified in section 2.5 and all assumptions are explicitly stated. The interactive effects of food availability and chemical exposure on stickleback populations were subsequently assessed. EE₂ and trenbolone exposures were simulated in environments with low and high food availability in order to explore the role of environmental conditions in determining population susceptibility to these EDCs.

2. Methods

2.1. Test compounds

17 α -ethinylestradiol (EE₂) and 17 β -trenbolone were chosen as the two case study toxicants.

EE₂ is a synthetic oestrogen commonly used in the contraceptive pill and its mode of action (MoA) is oestrogen receptor agonism. Predicted concentrations of EE₂ in typical surface waters are estimated at 0.3 ng L⁻¹ but may reach up to 9 ng L⁻¹ in wastewater treatment plant effluents (Hannah et al., 2009); effects in fish have been widely reported within 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. The MoA of trenbolone is androgen receptor agonism. Trenbolone metabolites have been reported in downstream sites at concentrations up to 50 ng L⁻¹ (Durhan et al. 2005); however inputs of chemical mixtures into surface waters may result in higher total concentrations of androgenic chemicals. Exposure to trenbolone is known to cause weight gain in male and female fish, masculinisation of females (Seki et al. 2006) and reduced reproductive output in females (Ankley et al. 2003).

2.2. Test species

The energy budget-IBM population model 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 EE₂ and trenbolone were undertaken using the fathead minnow (*Pimephales promelas*); a model temperate freshwater fish species used widely for regulatory ecotoxicology studies in North America, Canada and Europe (OECD, 1992; OECD 2012; Ankley and Villeneuve, 2006). Sticklebacks and fathead minnows have short lifespans (1 – 3 years) and similar life-history strategies. Both share polygamous mating systems where multiple females lay eggs in nests which are fertilised externally and guarded by males which display notable secondary sex

characteristics and courtship behaviours. The species have similar growth rates, maximum body sizes and female fecundities. Molecular susceptibilities to EE₂ and trenbolone are also likely similar based on the level of conservation of their primary targets (oestrogen and androgen receptors, respectively) (Brown et al., 2014).

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 Evaluation” (TRACE) document (Augusiak et al., 2014; Grimm et al., 2014; Schmolke et al., 2010) and can be found in Chapter 5. The model was implemented in the free programming software NetLogo 6.0.1 (Wilensky, 1999).

The purpose of the energy budget-IBM is to simulate realistic population dynamics for the three-spined stickleback, including responses to spatial and temporal variation in environmental conditions, to investigate population level effects of EDC exposures. Individual energy budgets describe the allocation of assimilated energy from ingested food to maintenance, growth, reproduction and energy storage (Sibly et al., 2013) (Fig. 1). EDCs induce their physiological effects by altering the allocation of energy to these life-cycle processes (see section 2.5). Energy budget algorithms follow fundamental principles of physiological ecology (Sibly and Calow, 1986) and energy demands scale with body mass and temperature according to established allometric and thermodynamic laws (Sibly et al., 2013). The model was parameterized from field and laboratory data for the stickleback where possible; otherwise data from other fish species were used. Environmental conditions (water temperature and food density) were modelled based on field data from the UK (Turner et al., 2013; Wootton, 1994).

The energy-budget IBM represents a spatially explicit 20 m² static water body where spatial variation in food availability results in landscape heterogeneity. Individuals move across the waterbody according to the ideal-free distribution i.e. fish spread themselves evenly based on the energetic profitability of habitat patches (Milinski, 1979, 1984). Food availability and temperature drive spatial

and temporal variation in the allocation of available energy to key life-history processes and thus regulate population dynamics.

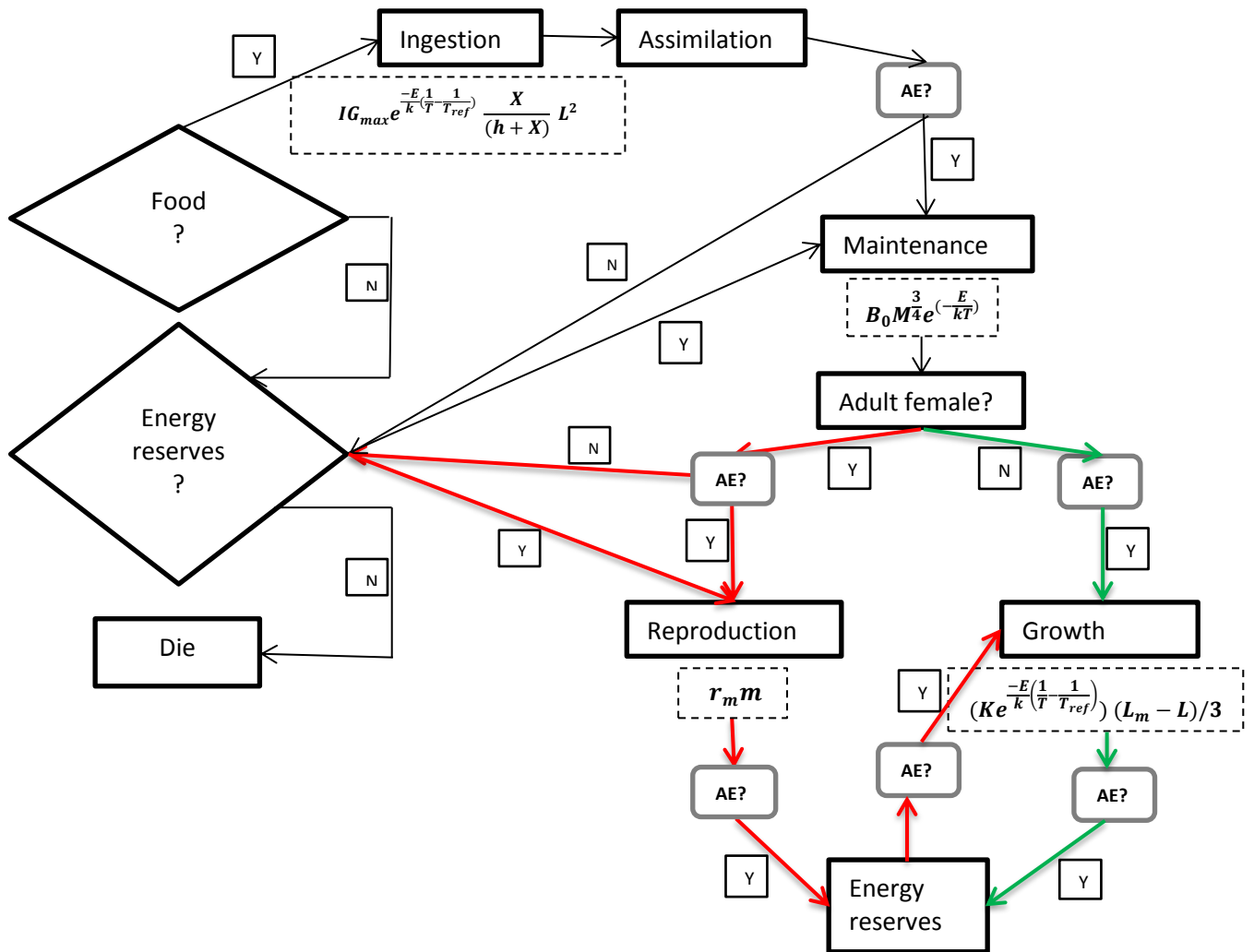


Figure 1. Conceptualisation of the physiological processes undertaken in juvenile and adult stickleback on a daily basis which all demand allocations of resources (energy). Diamond boxes represent energy sources; square black boxes represent life-history processes; grey boxes calculate if there is sufficient assimilated energy (AE?) to undertake the next process; and dashed boxes are relevant algorithms. 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. 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 subsidized from reserves.

The energy budget model (excluding chemical exposure) was verified at the organism level by comparing modelled outputs of somatic growth rates (body lengths over time and wet weights at 60 dph) to laboratory controlled empirical data for stickleback maintained under different levels of food availability (recorded from our own study, details for this study can be found in Chapter 5, 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 contrasting environmental conditions. 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. Temperatures and food densities were estimated as accurately as possible from the available meta-data obtained from the studies on those wild populations (Chapter 5, section 7). All 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 in Chapter 5.

We subsequently used the energy budget-IBM to assess the population level effects of exposure to EE₂ or trenbolone. We assessed how the effects of these chemical exposures on populations differ between systems with high and low food availabilities.

2.4. Effects of EE₂ and trenbolone exposure on stickleback populations

Effects of EE₂ and trenbolone exposure on fathead minnow fecundity were taken directly from the literature. Armstrong et al. (2015) quantified egg production during 21 days of exposure to EE₂ 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 exposure to trenbolone, Ankley et al. (2003) reported significant reductions in cumulative egg production of 60% at 50 ng L⁻¹ and almost complete inhibition of egg production at 0.5, 5 and 50 µg L⁻¹. In order to maintain environmentally relevant concentrations, we

simulated exposures of the reported effects at 1.5 ng L⁻¹ for EE₂ and 50 ng L⁻¹ for trenbolone. These are, however, at the higher end of reported environmental concentrations for these chemicals (see section 2.1).

2.4.1. Establishing energetic pathways linking effects of EE₂ and trenbolone on fecundity

EE₂ is an oestrogen receptor agonist which directly limits energy input to egg production by inhibiting endocrine (FSH/LH) and paracrine signalling and subsequently limiting ovarian development and ovulation in female fish (AOP-wiki; AOP 29 <https://aopwiki.org/aops/29>). Exposures have also been reported to inhibit female breeding behaviours (Coe et al., 2010) via neurological pathways (AOP-wiki; AOP 29 <https://aopwiki.org/aops/29>) which directly impact egg production/release and spawning success. Low dose exposures to EE₂ can also incur substantial metabolic costs primarily via the increased production of vitellogenin (VTG) (AOP-wiki; Armstrong et al., 2015). Additional metabolic costs include increased production of vitelline envelope protein (Finne et al., 2011) and general sub-cellular costs associated with maintaining homeostasis (Goodchild et al., 2018) and repairing tissue damage (e.g. hepatic and renal damage as a result of increased VTG production (Zha et al., 2008)).

As an androgen receptor agonist and a progesterone receptor agonist, trenbolone reduces VTG production in the hepatocytes by reducing LH/FSH synthesis, consequently reducing E₂ synthesis (AOP-wiki; AOP 23 <https://aopwiki.org/aops/23>). Reducing VTG synthesis directly limits energy input to female fecundity without incurring a significant metabolic cost. The androgenic effects of trenbolone exposure result in the induction of male secondary sexual characteristics (SSCs), including in females (Ankley et al., 2003), which incur an additional maintenance cost. This is quantified as the occurrence of nuptial tubercles in fathead minnow (Ankley et al., 2003), but in stickleback notable SSCs include nuptial colouration and eye colouration in males (Barber et al., 2000). A further metabolic cost of masculinisation in stickleback is the induction of spiggin (a glue protein synthesised by males to aid nest building) (Katsiadaki et al., 2002) Trenbolone exposure can also incur significant metabolic costs via its action as an anabolic steroid, promoting somatic growth by increasing muscle mass (Buttery et al., 1984). As with EE₂,

there will also be sub-cellular metabolic costs associated with maintaining homeostasis (Goodchild et al., 2018).

2.4.2. Incorporating energetic costs of chemical exposures into the energy budget-IBM

Direct effects on female fecundity caused by endocrine disruption are implemented in the model by altering the maximum rate of energy allocation to the reproduction parameter (rm) in the reproduction sub-model. Metabolic costs associated with chemical exposure are implemented by altering the taxon-specific normalization constant (B_0) parameter in the maintenance algorithm. Increased somatic growth costs are implemented by altering the mass constant in the length: mass allometric equation (m_a). This method supposes that the % reduction in egg production reported in the experimental studies is proportional to the % reduction in the physiological parameter.

For EE₂ exposures, we assume that the observed reductions in fecundity are equally a result of increased maintenance costs (B_0 parameter) and direct reductions in reproduction (rm parameter); whereas for trenbolone we assume that the effect is equally a result of direct reductions in reproduction (rm parameter), increased costs of synthesising muscle mass (m_a parameter), and increased maintenance costs (B_0 parameter). Thus, we reduced and increased the rm and B_0 parameter, respectively, by 19.5% during exposure to EE₂ (following a total observed reduction in fecundity of 37% (Armstrong et al., 2015)), and reduced the rm parameter and increased the B_0 and m_a parameter by 20% (following a total observed reduction in fecundity of 60% (Ankley et al., 2003)) during exposure to trenbolone. We assume that each mechanism is of equal importance in determining the whole organism effect because there is insufficient data to accurately quantify the proportions. The only way to infer accurate proportions of each mechanism would be to use data which quantified the chemical exposure at different food rations. The exact mechanisms could then be inferred via calibration of the model; however, this extent of data is not available for these chemicals.

2.4.3. Simulation experiments

We simulated EE₂ and trenbolone exposures at 1.5 and 50 ng L⁻¹, respectively, for the duration of the breeding season (May - July) for 10 years. This scenario was repeated in an environment with high and low food availability, where food density was increased or decreased by 50% from the default food density values (based on field data from the UK, see Chapter 5). Population level effects are quantified by comparing mean population abundance and biomass of exposed and 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). Mean cumulative number of eggs produced over the 10 year exposure period is compared with control populations.

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 became independent of replicate number ($\pm 5\%$). Thus, population level effects are considered relevant if the mean abundance over 10 years exceeds a 5% deviation from the mean control abundance.

3. Results

3.1. Model verification

The energy-budget model accurately predicted the empirical observations of somatic growth rates maintained under varying food rations well (body lengths: R² = 0.94; wet weights: R² = 0.71) (Fig 2).

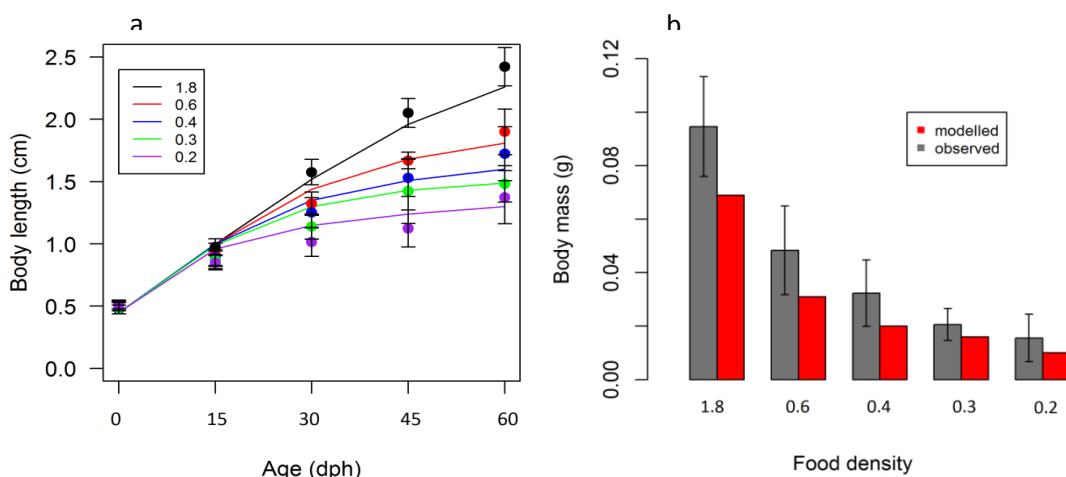


Figure 2. Body lengths over time (a) and final wet weights (b) of observed and modelled stickleback under different food allocations. Circles on graph a represent observed data, lines represent modelled data and legend refers to food amount per individual (mg). Observed data are displayed as mean lengths (\pm sd), whereas modelled outputs represent a single run.

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 (Fig. 3) 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).

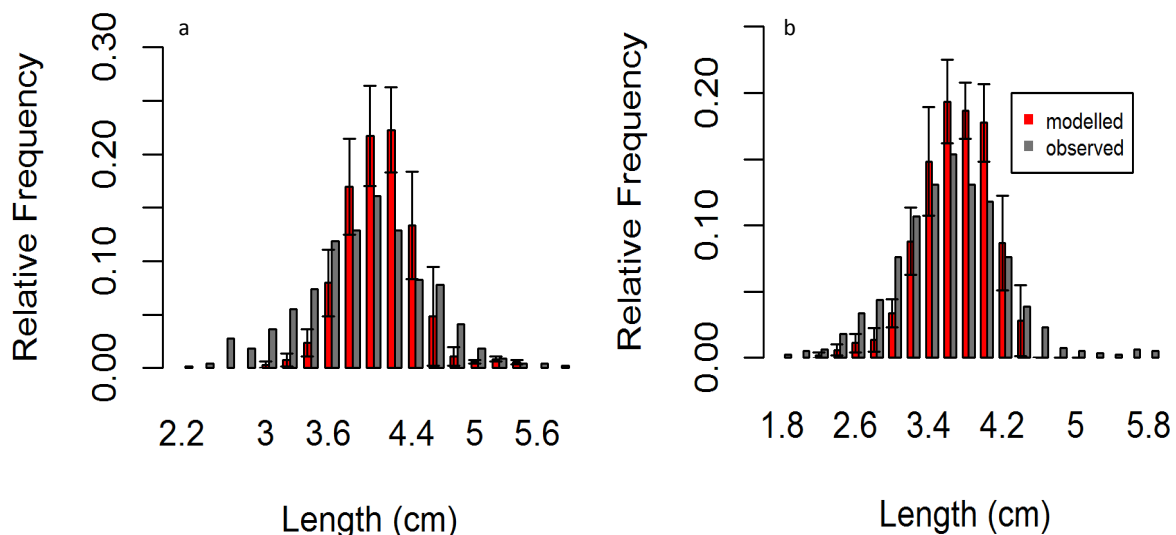


Figure 3. Comparisons of modelled and observed data for size distributions in spring (Feb/March (a)) and autumn (October (b)). Modelled size distributions represent mean values from 15 replicate runs (\pm sd) over 5 years. Observed data were obtained from a stickleback population in the River Rheidol (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 also showed good predictions for mean body lengths of stickleback from Lake Frongoch (R^2

= 0.66) (Fig. 4b). It is notable that there is very little variation around modelled mean body lengths from Lake Frongoch because food competition was eliminated in these modelled scenarios in order to accurately match food densities in the field (see Chapter 5, Section 7).

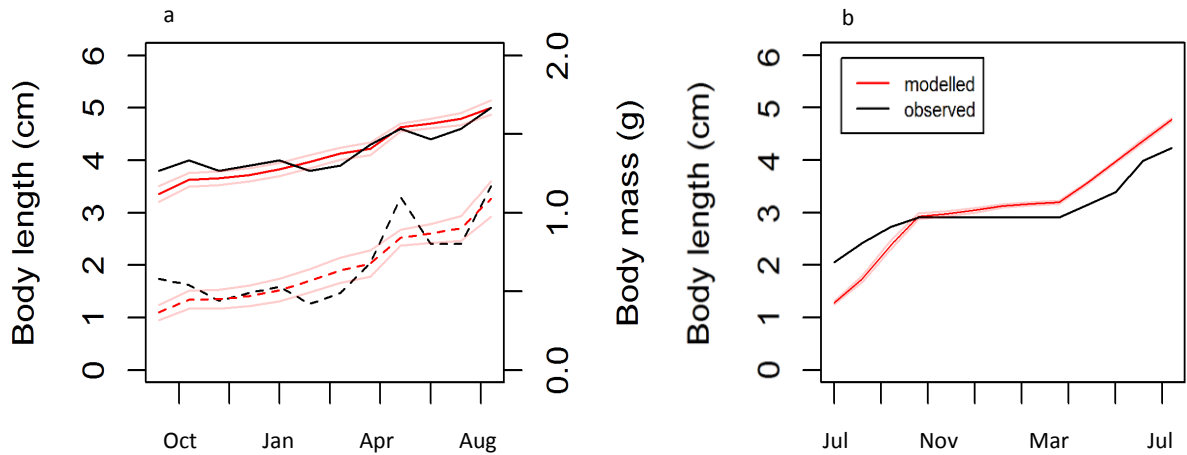


Figure 4. Comparisons of modelled and observed data for mean body length and mass over a year from September to August (a) and mean body length over a year from July to July (b). Modelled data represents mean values from 15 replicate runs (\pm sd) over 5 years and observed data were obtained from a stickleback population in the River Rheidol (Wales, UK) after Wootton et al. (1978) (a) and Lake Frongoch (Wales, UK) after Allen and Wootton (1982) (b).

3.2. Chemical exposure simulation

Trenbolone exposure reduced cumulative egg production in environments with high (21% reduction) and low (25% reduction) food availabilities and this translated into significant reductions in population abundance (9% and 12% reduction, respectively). EE₂ exposure reduced cumulative egg production in the low food availability environment (16% reduction), but not in the high food availability environment, which translated into significant reductions in population abundance (9% reduction) for the former. In the high food availability environment, neither EE₂ nor trenbolone affected population biomass; however in the low food availability environment, both trenbolone and EE₂ exposures caused an increase in population biomass (13% and 6% increase respectively) (Table 1). Modelled changes in population abundance and biomass over the 10 year exposure period are shown in Figure 5.

Table 1. Effects of EE₂ and trenbolone exposure on mean cumulative egg production, population abundance and population biomass over 10 years in low and high food availability environments. Data is expressed as percentage deviation from control simulations and represents mean values of 15 simulations. Significant deviations (based on a statistical effect threshold of 5%) are highlighted in bold.

	Cumulative egg production		Population abundance (fish m ⁻²)		Population biomass (g m ⁻²)	
	Low food	High food	Low food	High food	Low food	High food
Trenbolone	- 25	- 21	- 12	- 9	+ 13	+ 1
EE ₂	- 16	- 3	- 9	- 3	+ 6	- 1

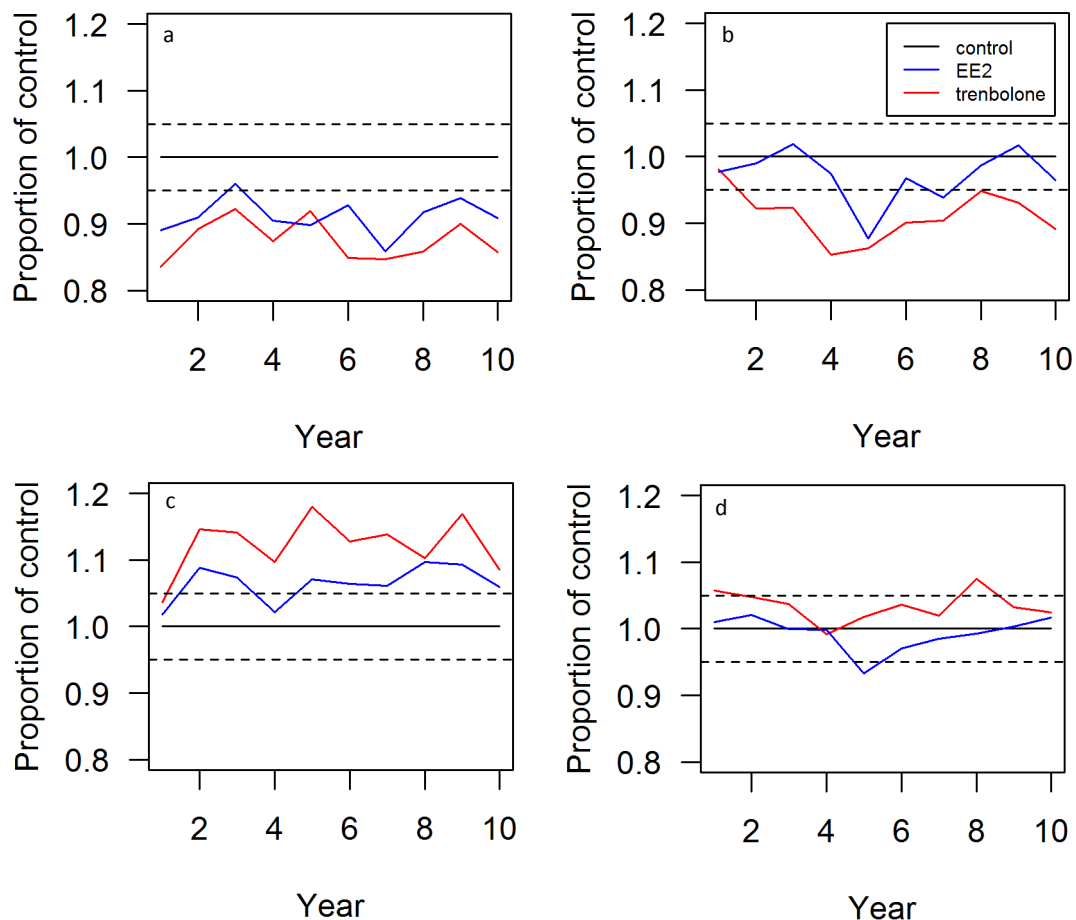


Figure 5. Effects of EE₂ and trenbolone exposure on mean population abundance (a,b) and population biomass (c,d) over 10 years in low (a,c) and high (b,d) food availability environments. Data are expressed as proportion

deviation from control simulations and represent mean values of 15 simulations.

Dashed lines represent the statistical effect threshold of 5%.

4. Discussion

An existing IBM for the three-spined stickleback was enhanced by incorporating individual energy budgets. Its application in predicting chemical effects on populations was demonstrated through the implementation of effects on individuals via mechanistic pathways established in AOPs and wider peer reviewed literature for each chemical. The model validated well against both individual life-cycle processes (somatic growth) and population dynamics (population abundance and size structure) of wild stickleback populations. This indicates that the model captured the individual physiology of sticklebacks accurately and resulted in representative modelling of stickleback population dynamics.

Our modelled results suggest that the underlying mechanism of the chemical (direct EDC effects and secondary metabolic effects) and the environmental conditions of the ecosystem (i.e. food availability) can co-determine the extent by which individual-level effects of chemical exposure translate into effects on fish populations. When food availability was high, EE₂ for the exposure regime adopted had no effect on cumulative egg production, population abundance or population biomass. This is because there was sufficient energy for all reproduction costs (related to fecundity) on top of the increased maintenance costs (related to excess VTG synthesis and metabolism). The direct endocrine disrupting effects of EE₂ on individual fecundity were inconsequential at the population level due to density dependent compensation in growth and mortality. In contrast, the effects of EE₂ on stickleback populations were clearly apparent when food availability was low, because there was insufficient energy available allow for maximum reproduction rates and the increased maintenance costs. Unlike EE₂, trenbolone exposure impacted on stickleback populations when food availability was high because increased metabolic costs (via increased somatic growth and maintenance) did not allow maximum reproduction rates to be met; thus, direct effects on fecundity were significant at the population level. This effect mechanism was exacerbated in the system with low food availability. Population biomass, on the other hand, was not affected

by EE₂ exposures and increased as a consequence of trenbolone exposures. This emerged from the buffering capacity of density dependent competition, whereby reductions in population abundance caused by the chemical resulted in reduced food competition and consequently larger individuals.

A mechanistic modelling approach offers a way to assess how indirect effects of EDCs on an individual's metabolism may contribute to their direct (MoA) effects on populations. This approach aids also in understanding the resilience of populations to chemical stressors under different environmental conditions. In particular, wild fish species in temperate climes are frequently subjected to periods of food limitation; 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 (Chapter 3; Hashemi et al., 2008)) chemical exposure effects in individual fish. Determining these interactive effects at the population level experimentally in the field is unfeasible and consequently food-web models (e.g. AQUATOX, Park et al., 2008) are often used as a substitute. Energy budget-IBMs, however, provide highly detailed assessments for specific species and result in more tractable multiple stressor effect scenarios for target model species. They also require less information for model parameterisation than full ecological food-web models. Use of complex IBMs however requires a certain level of expertise and any use of these models in a regulatory context will require training of risk assessors to do so. TRACE documentation can aid with this process.

The simulations presented show that effects on population abundance (number of individuals) were always greater than on population biomass (total mass of the population). Recognising and acknowledging distinctly different responses in population number versus population biomass is crucial for population protection versus preservation of ecosystem functioning. In the latter case, food web modelling (e.g. AQUATOX, park et al., 2008) is largely concerned with preservation of energy flow and population biomass (e.g. as described by the biomass spectrum (Kerr and Dickie, 2001)). It is arguably more challenging to conserve wildlife populations by maintaining their abundance and genetic diversity. Although effects on population abundance may be reduced

significantly (statistically speaking) compared to control populations, it is difficult to establish the ecological relevance of these effects, as they will be different for different species in different situations (e.g. food availabilities). In addition, due to a lack of demographic data for most fish species, it is difficult to establish if a reported effect falls within the natural variation for that population (Hamilton et al., 2016). However, even 'small' reductions in population abundance as a result of single chemical exposures can be greatly exacerbated by other chemicals (Kortenkamp, 2007), and physical (e.g. temperature (Gordon, 2003)) and biological (e.g. predation and disease (Rehberger et al., 2017)) stressors. Populations remain the cornerstone of nature conservation based on diversity-stability hypothesis (McCann, 2000; Ives and Carpenter, 2007) and statistically significant reductions in the abundance of keystone populations should therefore be given due consideration in conservative environmental risk assessments (EFSA, 2010)

As the use of man-made chemicals increases with the demands of the growing human population, risk assessors require more integrated and accurate tools to predict the effects of multiple stressors on wildlife populations and ecosystems. Here we integrate AOPs describing the translation of molecular effects to individual level effects (bottom-up approach) with population modelling describing population dynamics regulated by inter-individual-environment interactions e.g. competition for resources (top-down approach) (Kramer et al. 2010). However, AOPs are a simplification of the complicated mechanistic effects of chemicals on exposed individuals and, like any modelled system, are limited by knowledge gaps (Leist et al., 2017). In order to link AOPs with energy budgets, assumptions need to be made about the links between energy-regulation processes and the mechanistic pathway of the chemical, since this information is not currently included within AOPs (Goodchild et al., 2018). We demonstrated how AOPs can be used in conjunction with additional mechanistic data from the literature to help solidify these energetic links and fill data gaps in order to define the most plausible mechanisms underlying direct and indirect effects of EDCs. Ensuring clarity and transparency about the assumptions made when linking mechanistic data with energy budget models will aid in building confidence in this approach. A limitation, however, remains that it is virtually impossible to accurately infer the proportions of the direct EDC effect vs

secondary metabolic costs. Nonetheless, since mechanistic models are generally designed to aid in management decisions using an investigative approach, rather than providing exact predictions, multiple scenarios can be modelled when uncertainties relating to the chemical mechanism arise.

Mechanistic population modelling approaches have been used to inform on a number of environmental challenges (Martin et al., 2013; Johnston et al., 2015). Simulation models such as these are necessary to investigate scenarios which cannot be practically undertaken in the field or the laboratory. Current risk assessments often consider only one stressor at a time because of the difficulties associated with defining, conducting and interpreting realistic, representative multiple stressor studies. The results presented suggest that chemical exposure assessments need to take into consideration the productivity of the ecosystem and food availability when predicting effects at the population level. This is consistent with the general theory that exposing a population to a chemical that impairs population growth (e.g. by reducing individual survival or reproductive rates) when the population is at or approaching carrying capacity can reduce the intensity of intraspecific resource competition, which in turn reduces the impact of the chemical on population abundance (Forbes et al., 2001). This theory has been reported in multiple empirical and modelling studies (e.g. Grant, 1998; Liess 2002; Moe et al., 2002; Hayashi et al., 2009) and explains why the population in the present study was more resilient to chemical exposure under high food availability compared to low food availability.

Chemical exposures, and their interactions with other stressors, are threatening the health of freshwater ecosystems and there is a demand for the development of more holistic tools to assess the risks associated with chemical exposures. Here, a fully evaluated model complex is presented with which the impacts of chemical exposures can be assessed in the context of a fluctuating (and anthropogenically modified) environment for populations of temperate small sized freshwater fish with a short lifespan, relatively low fecundity and a high level of parental investment (i.e. high juvenile survival rate). The model can be adapted to represent the environmental conditions of different geographical regions, including thermal and hydrological regimes, and is thus more adaptable than standard IBMs, whilst simultaneously maintaining equitable realism.

5. Conclusions

There is a demand for more generic and accurate tools to assess multiple stressor scenarios within chemical risk assessments. Using a combined stickleback energy budget-IBM, the underlying mechanisms by which chemical effects, including the major direct effect pathways for AOPs and indirect effects via disruptions of basic homeostasis and energy budgets, may determine the extent by which individual-level effects translate up to population level effects. Our results suggest that food limited populations may also be more susceptible to chemical exposure effects. Integrating mechanistic data, inferred from AOPs and chemical-specific data, into individual-based 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.

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Chapter 5. A TRansparent and
Comprehensive model Evaludation
(TRACE) document for a sticleback
energy budget-IBM.

This is a TRACE document (“TRAnsparent and Comprehensive model Evaluation”) which provides supporting evidence that our model presented in:

< Chapter 4. Applying a mechanistic model to predict effects of endocrine disrupting chemicals on fish populations. >

was thoughtfully designed, correctly implemented, thoroughly tested, well understood, and appropriately used for its intended purpose.

The rationale of this document follows:

Schmolke A, Thorbek P, DeAngelis DL, Grimm V. 2010. Ecological modelling supporting environmental decision making: a strategy for the future. *Trends in Ecology and Evolution* 25: 479-486.

and uses the updated standard terminology and document structure in:

Grimm V, Augusiak J, Focks A, Frank B, Gabsi F, Johnston ASA, Kułakowska K, Liu C, Martin BT, Meli M, Radchuk V, Schmolke A, Thorbek P, Railsback SF. 2014. Towards better modelling and decision support: documenting model development, testing, and analysis using TRACE. *Ecological Modelling*

and

Augusiak J, Van den Brink PJ, Grimm V. 2014. Merging validation and evaluation of ecological models to ‘evaluation’: a review of terminology and a practical approach. *Ecological Modelling*.

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Problem formulation

This TRACE element provides supporting information on: The decision-making context in which the model will be used; the types of model clients or stakeholders addressed; a precise specification of the question(s) that should be answered with the model, including a specification of necessary model outputs; and a statement of the domain of applicability of the model, including the extent of acceptable extrapolations.

Summary: Population dynamics of temperate freshwater fish are driven by, among other things, seasonal changes in resource availability and temperature. Chemical exposure can cause physiological stress to organisms and consequently alter population structure. To investigate realistic population level effects of chemical exposure on the three-spined stickleback, we have developed an IBM in which individuals possess their own energy budgets so that food availability, temperature, and chemical exposure alter the allocation of energy to life history processes. The stickleback is a relevant study species for assessment of European waters and is a model species in many studies of ecology, physiology, and ecotoxicology. Model outputs provided a good match to empirical patterns of individual life-history processes and population structures. This document provides confidence that the model is fit for use in chemical risk assessments.

The model was designed to provide predictions of chemical effects on populations in the context of realistic environmental conditions and stressors. The model should act as a potential higher tier risk assessment (EFSA, 2013; EC No 2017/2100; EC No 2018/6054) using results from lower tier toxicity tests to predict the extent to which effects on individuals extrapolate up to the population level. Additionally, the model can be used as a predictive tool to highlight chemicals worthy of further investigation and to explore the characteristics which affect the susceptibility of populations to specific chemical exposures. Thus, the main stakeholders will include risk assessors and chemical regulators, as well as the academic community.

The stickleback was selected because it is one of the only European fish species recommended as a standard OECD test species (OECD, 2012; OECD, 2017). It has a global distribution and is particularly abundant throughout Europe, the US and Canada. Thus, the stickleback is a relevant species for assessing the risks of chemicals to European waters and its use as a model species in ecotoxicology means that literature data on toxicant effects are readily available. Importantly, the stickleback is one of the most well studied fish species in terms of ecological and demographic data, making it an ideal candidate for the development and validation of an IBM. The stickleback is representative of a temperate freshwater fish species with annual spawning, a short lifespan, low female fecundity, and male investment in breeding behaviours and paternal care. We envisage that the model will also use data from lower tier toxicity tests on species with a similar life-history strategy, such as the fathead minnow, to give a more general indication of chemical effects on fish populations.

The basis of the model is that the physiological processes undertaken by individuals, which are affected by food availability and temperature, determine population dynamics. Chemical exposures have physiological effects on individuals which impact growth, reproduction, and survival. Thus, the model provides a mechanistic approach to quantifying population level effects of chemical exposure, whilst accounting for fluctuations in environmental conditions. The model was developed using available data from the literature and from personal observations and follows general theory of fish ecology and

physiology. Model outputs provided good matches to empirical data of individual life-history processes and to population dynamics.

An IBM with no energy budget component can be found in Mintram et al. (2018). This model is more appropriate if the underlying mechanisms of the chemical effect are not well understood as it allows direct extrapolation from lower tier toxicity tests to populations.

1 Model description

This TRACE element provides supporting information on: The model. Provide a detailed written model description. For individual/agent-based and other simulation models, the ODD protocol is recommended as standard format. For complex submodels it should include concise explanations of the underlying rationale. Model users should learn what the model is, how it works, and what guided its design.

Summary:

In this section we provide a complete model description, following ODD (Overview, Design concepts and Details) guidelines (Grimm et al., 2006, 2010). Here, we describe all of the assumptions, equations, parameter values and data sources which were used in the development of the IBM.

Purpose

The purpose of the model is to simulate realistic population dynamics of the three-spined stickleback, including responses to spatial and temporal variation in environmental conditions, to investigate population level effects of chemical exposures.

Entities, state variables and scales

The entities in the model are the spatial units (comprising the landscape) and individual fish with their own energy budget. The model environment represents a 2 dimensional 20 m² pond comprising of 500 20 L patches measuring 20cm (length) * 20 cm (width) * 50cm (depth). Individuals are distinguished into one of four life stages: eggs, larvae, juveniles or adults. All sticklebacks are characterised by the state variables age, body weight (g), body length (cm).

Juveniles and adults are further characterised by energy reserves (kj), sex (male or female) and location. Energy reserves are implicit for eggs and larvae as their life-cycle processes are not dependent on food availability. Adult males possess the state variable breeding status: Boolean; if they are able to establish territories then they are nesting and exhibit nesting behaviour, territory size (m^2) and courtship success probability. Adult females have an inter-spawning interval (days between spawnings) which determines the frequency of spawning events, and a batch size (eggs per spawning event). Spatial units are characterised by water volume (L), food density (g) and food quality (kj/ g). The overall environment is characterised by temperature and season.

Process overview and scheduling

Each individual in the model possesses its own energy budget. Each of the following processes (highlighted in bold) will occur over each time step in sequential order. Entities are processed in a random sequence and individual fish update their state variables each day.

Update time and landscape: Temperature and season are updated. Food density and energy content of each patch is replenished at the start of each day.

Survival: An individual's daily mortality rate is determined by four main factors: developmental mortality (eggs only), density dependent egg cannibalism (eggs only), starvation (juveniles and adults), and background mortality (including predation; all life-stages).

Movement: Foraging individuals (juveniles and adults) move across patches according to the ideal free distribution (Milinski, 1979, 1984) in order to maximise ingestion rates. Sexually mature adult males move to find nesting sites in the breeding season, and adult females move to find a breeding partner.

Ingestion and energy intake: Juveniles and adults assimilate a fixed proportion of energy dependent on the maximum ingestion rate of the individual (determined by food availability), the energy content of the food, and the number of individuals on the patch. The food density of the patch depletes accordingly each day. Eggs are ingested throughout the breeding season by all eligible fish in the population, including the parent male.

Maintenance: Juveniles and adults pay maintenance costs from assimilated energy or energy reserves according to their active metabolic rate, which is

increased for breeding males to account for the costs of breeding behaviours. Individuals die if the costs of maintenance are greater than the sum of assimilated energy and energy reserves. Mass and temperature have scaling effects on metabolic rate.

Reproduction: Fish reproduce during the breeding season. Females allocate energy to reproduction prior to growth if there is sufficient assimilated energy remaining after maintenance costs have been paid. Males establish territories and implicitly undertake courtship behaviours. All male reproductive parameters are set prior to maintenance so that the costs of breeding can be paid for in 'maintenance'. Male courtship behaviours and territory quality are criteria that females use to choose where they deposit their eggs.

Growth: Larvae grow at a constant rate. Juveniles and adults undergo somatic growth if assimilated energy is available after the costs of maintenance, and reproduction for adult females, have been paid. If there is insufficient energy to achieve maximum growth, growth rates are adjusted accordingly. This sub-model determines total length (cm) and structural mass (g).

Energy reserves: Juveniles and adult males allocate energy to reserves once the costs of maintenance and somatic growth have been paid. Energy is stored in the form of lipids; the total energy content of the reserves (kJ) is converted into mass (g), which is added onto the structural mass of the individual. When food is limited reserves are used to cover maintenance and reproduction costs until reserves are depleted. Fluctuations in energy reserves result in fluctuations in total body mass.

Change life-stage: Eggs develop into larvae and larvae into exogenously feeding juveniles once they are fully developed at the end of a temperature dependent incubation period. Juveniles develop into adults during the breeding season once they reach the length at which sexual maturity is onset.

Design concepts

Basic principles: Individual energy budgets follow fundamental principles of physiological ecology (Sibly and Calow, 1986) and scale with body mass and temperature according to known allometric laws (Sibly et al., 2013). Food availability and temperature drive seasonal discrepancies in the allocation of

available energy to key life-history processes and thus regulate population dynamics.

Emergence: Landscape heterogeneity emerges as a result of individuals depleting food reserves over a single day. Population dynamics (age and size structure, spatial distribution and abundance of fish) emerge as food density and quality results in differential energy allocation among individuals at different life stages.

Adaptation: Several behaviours in the model are implicitly adaptive. Reproductive behaviour is the primary adaptive trait driving reproductive success as male traits (nest building, courtship, territory size, and nest quality) determine female choice. Juvenile and adult movement is implicitly adaptive as individuals disperse themselves based on patch profitability to maximise energy gain. Adult males search for optimal breeding grounds to establish territories which reduce mortality risk and increase the chance that a female will spawn in his nest.

Sensing: Stickleback sense the presence of conspecifics in the local (territorial males and spawning females) and global (all adults and juveniles) environment. Adults sense the presence of eggs and/or larvae in the nest and adjust their territorial behaviour accordingly. Juveniles and adults sense patch conditions (food density/ quality) and adjust their movement (move to find a higher quality patch) and behaviour (e.g. establish territory, spawn) accordingly.

Interaction: Direct interaction occurs during breeding where a paired male and female carry out both communicative signalling (courtship) and physical contact (fertilisation). These interactions are modelled implicitly. Each male will continue interacting with its fertilised eggs and larvae until the larvae leave the nest. Indirect interaction occurs via competition for food and territory sites and mates during reproduction.

Stochasticity: Habitat types are distributed randomly within the system. At initialisation, age, position, and sex are the results of stochastic processes.

Observation: Total abundance, number of individuals at each life stage, and population biomass (g) will be recorded daily.

Input data: Water temperature and food density data is read from an external file. Water temperature recordings were taken throughout the year at a lake in

Slapton Ley, Devon, at a depth of 0.5 m (Turner et al., 2013). Food density trends follow trends in water temperature (described later in this section).

Initialisation: The model begins on January 1st with only juveniles present within the system. An initial density of 100 juveniles was chosen as it is within the range of stickleback found in the wild (see section 2, *calibration*) but low enough to maximise model speed. Age is drawn from a uniform distribution (i.e. set randomly) between 142 and 232 days post hatch (dph) based on a three month breeding season between May and July. Length (cm) is set according to age (Eq. 5b) and weight (g) is allometric to body length (Eq. 6). The position of individuals within the pond is allocated based on a preference for complex vegetated habitat, as displayed in wild populations (Candolin and Selin, 2012). Juveniles are randomly allocated to a vegetated patch; as densities have been observed at up to 60 fish m⁻² in the wild (Whoriskey and FitzGerald, 1985), there is assumed to be no exclusion of juveniles from vegetated patches outside of the breeding season. Energy reserves are set to 50% of their maximum to represent declined reserves in winter.

The pond patches are setup with 10% vegetation cover following a wild pond which was mapped by Whoriskey and Fitzgerald (1987). All of these vegetated patches are considered potential territories for males during the breeding season. The remaining patches are set as open water, all of which are set as potential breeding-grounds; however this can be changed by the user. All vegetative patches have an energy content set randomly between 17 and 25 kJ g⁻¹ (Wootton, 1994). Food density and temperature is read from the external file.

Sub-models

Table 1. Equations used in each sub-model of the IBM with parameter descriptions and units. Parameter values and sources can be found in table 2.

Sub-model	Equation	Parameter descriptions
Survival	<p>Eq 1. Natural mortality</p> $M_m = M_u M^b$	<p>M_w: Natural mortality probability at mass M</p> <p>M_u: Natural mortality probability</p>

		<p>at unit mass (1 g)</p> <p>M: mass (g)</p> <p>b: Allometric scaling factor</p>
	<p>Eq 2. Egg cannibalism</p> $EC = EC_a D + EC_b$	<p>EC: Egg cannibalism probability</p> <p>EC_a : EC constant</p> <p>D : Global adult and juvenile (fish length >= 1.5 cm) density (fish m⁻²)</p> <p>EC_b : EC intercept</p>
Ingestion	<p>Eq 3. Ingestion rate</p> $IG = IG_{max} e^{\frac{-E}{k}(\frac{1}{T} - \frac{1}{T_{ref}})} \frac{X}{(h + X)} L^2$	<p>IG: Ingestion rate (g cm⁻² day⁻¹)</p> <p>IG_{max}: maximum ingestion rate (g cm⁻² day⁻¹)</p> <p>X: Food density (g l⁻¹)</p> <p>h: half-saturation coefficient (g l⁻¹)</p> <p>L: Body length (cm)</p> <p>E: Activation energy (eV)</p> <p>k: Boltzmann's constant (eV K⁻¹)</p> <p>T: Temperature (K)</p>
Maintenance	<p>Eq 4. Metabolic rate</p> $MR = B_0 M^{\frac{3}{4}} e^{\left(-\frac{E}{kT}\right)}$	<p>MR : Metabolic rate</p> <p>B₀: taxon-specific normalisation constant</p> <p>M: Mass (g)</p> <p>E: Activation</p>

		energy (eV) k: Boltzmann's constant (eV K ⁻¹) T: Temperature (K)
Growth	Eq 5. Growth rate $GR = (Ke^{\frac{-E}{k}(\frac{1}{T} - \frac{1}{T_{ref}})}) (L_m - L)/3$	GR: Growth rate (cm day ⁻¹) K: Growth constant (cm day ⁻¹) L _m : Asymptotic length (cm) L: Length (cm) E: Activation energy (eV) k: Boltzmann's constant (eV K ⁻¹) T: Temperature (K)
	Eq 6. Length: mass conversion $M = m_a L^{m_b}$	M: Mass (g) m _a : mass constant m _b : mass exponent
Reproduction	Eq 7. Territory size If D > 20 fish m ⁻² TS = 0.063 If D < 1.3 fish m ⁻² TS = 0.54 If 20 > adult male density > 1.3 fish m ⁻² $TS = T_a D^{T_b}$	TS : Territory size (m ²) T _a : TS constant D: Male density (fish m ⁻²) T _b : TS exponent
	Eq 8. Courtship success $CS = C_a Ln(TS) + C_b$	CS : Courtship success probability C _a : CS constant TS : Territory size

		(m ²) C _b : CS intercept
	Eq 9. Reproduction rate $R = r_m m$	R: Maximum reproduction rate (kj day ⁻¹) r _m : maximum reproduction rate per unit mass (kj g ⁻¹ day ⁻¹) M: Mass (g)

Update environment and landscape

Date and season are updated every time step. Temperature (K) and food density (g) are read from an external file and are updated every 5 days. Temperature data was taken from a lake in Devon, UK (Turner et al., 2013) at a depth of 0.5 m which matches the depth of the modelled system. This data was interpreted from a graph which reported monthly mean temperatures. Food density data follows the patterns of temperature, such that food increases with increasing temperature, but the minimum and maximum food densities were chosen based on the available literature (see section 2). The energy content of each vegetated patch changes daily and is set randomly between 17 and 25 kj g⁻¹ (Wootton 1994) to maintain a heterogeneous environment. Open water patches have a food density of 0.

Survival

Egg mortality occurs in the form of developmental mortality (all natural mortality caused by mutations, incorrect egg development and infections), egg cannibalism, and predation. Laboratory data on developmental mortality from the literature and from a study undertaken by the authors in the absence of predation showed a mean hatching success of 92%. There is no evidence that hatching success is affected by temperature (within ranges which eggs would be subject to in the wild) or incubation time, as displayed in the parameterisation data (Hagen, 1967; Candolin et al., 2008; personal observations). To accommodate this, we set a constant daily mortality rate (N_e)

of 0.014 for the first 6 days of incubation (the minimum incubation time in the model). This results in a constant overall rate of developmental egg mortality independent of development time.

Stickleback eggs are subject to high levels of cannibalism which can vary in intensity between populations (Foster 1988, Ostlund-Nilsson et al., 2006). In the wild, eggs in the nest are predated by raiding males and females (hetero-cannibalism) and by the guarding male parent (filial cannibalism) (Pitcher, 1986). Whoriskey and FitzGerald (1985) quantified density dependent cannibalism in the stickleback in a semi-wild study and this data was used in the model (Table 1, Eq. 2). The study did not explicitly account for resource availability; however, a study by Candolin (2000) demonstrated that low rations did not significantly increase cannibalism in the stickleback. Similarly, Klug et al., (2006) found that male condition in the sand goby, a species with a similar breeding strategy to the stickleback, did not affect the rate of egg cannibalism. There is therefore little empirical evidence for the energy-based hypothesis for egg cannibalism presented by Rohwer (1978), whereby cannibalism is presented as a strategy to increase body condition when food supplies are low. We therefore do not explicitly relate egg cannibalism to food density in the model; rather, we relate cannibalism to overall fish density as recorded by Whoriskey and FitzGerald (1985). Cannibalistic fish ingest eggs once the food density of the patch is depleted. Egg predation by other species is assumed to be minimal because of the paternal guarding investment undertaken by the male stickleback. It is therefore assumed in the model that non-cannibalistic egg predation only occurs when the guarding male dies.

Background mortality (including predation) affects larvae, juveniles and adults. Since there are no data quantifying mortality rates for larval stickleback in the wild, this parameter was calibrated (see *calibration* for details). Daily mortality rates (M) are considered to stay constant for the duration of the larval stage to account for low motility potential and consequent high rates of predation. For juveniles and adults, background mortality is set according to the natural mortality at unit mass equation (Table 1, Eq. 1) where an increase in body mass results in a reduced daily mortality rate (Lorenzen, 1996). This equation incorporates all forms of mortality including predation, background mortality and starvation, and follows the general theory that as fish get older, and larger,

mortality decreases (Wootton, 2002). Since starvation is already included in the model, the M_u parameter, which was reported as 0.00781 by Lorenzen et al (1996) for temperate pond fish (when converted from annual to daily mortality), was reduced and calibrated (see section 2, *calibration* for details).

Juveniles and adults are subject to starvation if the sum of assimilated energy and energy reserves does not cover maintenance costs (see *maintenance*).

Movement

Eggs and larvae do not move. Foraging stickleback (juveniles and adults outside of a breeding cycle) follow the ideal free distribution as described by Milinski (1979) and Milinski (1984) where individuals distribute themselves between food patches in the ratio of the patch profitability (Pitcher 1986). Once sexually mature, adult males move to find nesting sites and adult females move to find receptive breeding partners (see *Reproduction*).

During the breeding season, movement is adapted so that non-cannibalistic fish continue to follow the ideal-free distribution as described above, but cannibalistic fish are excluded from adult male territories as they pose a threat to offspring in the nest. Thus, if there are no unowned patches (i.e. patches which are not within a males territory) which follow the ideal free criteria, cannibalistic fish move to any owned patch with a food density > 0 . If there are no unowned patches with a food density > 0 , cannibalistic fish move to any unowned patch.

Ingestion and energy intake

Individual ingestion rates follow a type II functional response and are dependent on temperature and food density (Table 1, Eq. 3). The model was parameterised from data which quantified attack rate as a function of food density (Volset and Bailey, 2011) and ingestion rate as a function of attack rate (Heller and Millinski, 1979). If the density of food on the patch is insufficient to meet the maximum ingestion rates of all the individuals on that patch, each individual acquires an equal amount of food proportional to their body mass.

Ingestion rates of cannibalistic individuals in the breeding season are additionally dependent on the number of eggs in the system. Cannibalistic fish are defined in the model as individuals with a body length ≥ 1.5 cm, since mouth gape size prevents smaller fish from cannibalising eggs. There are no

studies quantifying mouth gape size in stickleback, so a body length ≥ 1.5 cm was considered to be a sensible estimate, based on observations in the laboratory (personal observation), to represent individuals capable of cannibalism. After ingesting all of the available food on the patch, cannibalistic fish ingest eggs until they have reached their maximum ingestion rate or until there are no more eggs available. Individuals do not explicitly move to acquire eggs; rather, the density of eggs available for ingestion is spread evenly throughout the population of cannibalistic fish and the additional energy is added to each individual energy budget. Since the proportion of filial cannibalism and nest raiding by other individuals is unknown (Pitcher, 1986) and varies between populations (Foster 1988, Ostlund-Nilsson et al., 2006), this seems the most effective way to model egg ingestion.

Sticklebacks are omnivorous but the diet is generally dominated by two prey categories: zooplankton and the larvae and pupae of chironomids (Wootton, 1974). The energy content of food (E_x) was taken as $17 - 25$ kJ g^{-1} dry weight (Wootton, 1974) and an assimilation efficiency (A_e) of 0.95 was taken from Cui (1987). Since there are no reported assimilation efficiencies for the stickleback, the assimilation used here refers to the common minnow, *Phoxinus phoxinus*. Eggs have an energy content equal to E_c . The total energy assimilated in a day is thus equal to: ingestion rate * $((E_x + E_c) * A_e)$.

Maintenance

In the model we assume that eggs and larvae have sufficient energy in the yolk sac to cover maintenance costs and thus maintenance is not explicitly modelled for these life stages. Maintenance costs in juveniles and adults follow the active metabolic rate (AMR, basal metabolism + the energy costs of swimming), below which the organism cannot survive (Table 1, Eq. 4). Meakins (1975) recorded the routine rate of spontaneously active stickleback to be approximately twice that of their basal metabolic rate (BMR), which is consistent with general theory (Fry, 1947; Tytler and Calow, 1985). It is assumed that the daily costs of swimming in a static water body remain relatively constant from day to day because the fish do not migrate and do not need to swim against flow rates. To account for active metabolism, we therefore apply a multiplication factor of 2 to the costs of BMR (see section 2).

Over the course of the breeding season, the costs of maintenance in adult males are significantly increased as individuals undergo a complex series of movement-based behaviours (Chellappa et al., 1989). These behaviours consist of establishing a territory, nest building, courtship displays, and the guarding and fanning of young. The period between territory establishment and larvae fleeing the nest is defined as a breeding cycle and typically lasts 14 days. Meakins and Walkey (1975) (recalculated by (Smith, 1991)) estimated the costs of active metabolism during a breeding cycle to be 4.3 times greater than the costs of BMR. This multiplication factor is applied to the costs of BMR.

Energy reserves are utilised in juveniles and adults in instances where the energy assimilated does not cover maintenance costs. Once all of the energy reserves are used up, the individual dies (Sibly et al., 2013).

Growth

Eggs do not grow. Fish length (total length, cm) is used as the primary element of growth in the model. Larvae grow at a constant rate of 0.033 cm/ day at 15 °C (pers. obs). Larvae always grow at their maximum rate because they rely on their yolk sac for energy. Juveniles and adults increase in body length according to the von Bertalanffy growth function (Table 1, Eq. 5) which results in decreasing growth rates as fish get larger. The model was parameterised from laboratory fish ($n = 12$) reared at 15°C for the first 60 days of life (personal observations) and using data from Froese and Pauly (2016). Larvae growth rates are dependent on temperature, whereas juvenile and adult growth rates are dependent on temperature and food availability. Thus, as fish density increases and resources become limiting, growth rates of juveniles and adults decrease. Body length is converted to mass according to the allometric equation (Table 1, Eq. 6), parameterised from 29,975 sticklebacks (Froese and Pauly, 2016). The energy costs of synthesising new tissue are calculated as the daily addition of somatic body mass, taking into account the costs of synthesising new tissue ($E_c + E_s$). In juveniles and adults, the allometric equation here is multiplied by 1/1.3 to ensure that only the costs of somatic growth are accounted for (see details below). If less energy is available than required for maximum growth rates, a lower rate is calculated for the energy available.

The average condition factor (CF, an index of the extent to which the total weight of a fish is high for its length, calculated as $\text{mass} / \text{length}^3 \times 10^6$) of stickleback in the wild is 1.3 (Chellappa et al., 1995) and it is therefore assumed that body mass calculated from the allometric equation (Table 1, Eq. 6) refers to fish with a CF of 1.3. Juvenile and adult mass fluctuates accordingly depending on individual energy reserves, whereby individuals with maximum energy reserves represent the highest possible mass, and individuals with no energy reserves refers only to the structural mass of the individual. The condition factor for the structural mass of the fish, or the somatic condition factor, is 1 (Chellappa, et al., 1995). This sub-model accounts for the structural mass of the individual only, and body mass is therefore calculated as $(M_a L^{M_b}) \frac{1}{1.3}$. The mass of energy reserves are added onto the structural mass in '*energy reserves*'. Since CF in larvae is assumed to be constant and independent of food availability, the mass calculated from the allometric equation refers to total mass for larvae.

Reproduction

This process is executed by adult stickleback in the breeding season (1st May to 30th June). Adults are sexually mature once they have reached a total length of 4.5 cm (Froese and Pauly, 2016; Paepke, 1984).

Males

Each day, males which have reached sexual maturity acquire nests on available vegetated patches and establish territories around these patches according to their territory size (Table. 1, Eq. 6). If there are no vegetated patches available, males will establish territories around open water patches. Thus, a male may establish a nest on a single vegetated patch, but the territory surrounding this nest may be a mixture of vegetated and open water patches. This is consistent with evidence from wild stickleback demonstrating that individuals show a preference for concealed nest sites over open water nest sites (Black, 1971; Moodie, 1972; Kynard, 1978; Hagen, 1967; Sargent and Gebler, 1980; Krakk et al., 2000). Territories are established on a first come first serve basis so that competition for territories is random and independent of size. Although there is some evidence that larger males display a competitive advantage over smaller males when choosing territory sites (Rowland, 1989; Dufresne et al., 1990;

Kraak et al., 2000), we did not implement size-dependent territory acquisition into this sub-model because it significantly slowed down the IBM without causing any changes in overall outputs. Since male sticklebacks ferociously guard their territories, it is presumed that once a male has established a nest, he cannot be excluded from that nest. A male which acquires a territory is defined as a nesting male. When searching for territories, non-nesting males search for un-owned patches within a radius that is set to be slightly larger than his allocated territory size. This ensures that patches are allocated to an individual's territory in a near continuous manner surrounding the turtle, as is likely to occur in the wild. The chances that a male will successfully court a female is set by his courtship success probability (Table 1, Eq. 7), which is dependent on territory size.

The breeding season lasts 3 months (90 days) during which a single male can complete as many breeding cycles as possible until he is outcompeted or dies (Kynard, 1978; Wootton, 1984). Each breeding cycle lasts 14 days and consists of a nest building phase (day 0); a courtship phase (days 1 - 4) which determines if a female will spawn; and a parental phase (nest guarding and fanning of embryos and fry: days 2 - 14) (Wootton, 1984; Kynard, 1978; van de Assam, 1967). A male can acquire a maximum of 5 clutches in days 1 - 4 of each breeding cycle; after which point he will reject any more females into his territory (van de Assam; 1967; Wootton, 1984). If a male fails to acquire a clutch within this period, the cycle is set back to 1 (courtship phase). This is to avoid males guarding a territory for a full breeding cycle without being able to acquire any egg clutches after day 4. The owner will abandon his territory at day 14 of his cycle and begin searching for another space as previously described (Kynard et al., 1978). A male can acquire a final clutch in an already established territory on the last day of the season (day 90) resulting in these males completing their final cycle into August (up to day 104). In wild populations, the time spent in parental care of fry is often variable and can range from abandonment prior to the development of free-swimming fry (van de Assam, 1967; Kynard, 1978), to continuing the parental phase until the fry leave the nest independently (Kynard, 1978). In the model, this is dependent upon which day of the breeding cycle the clutch was acquired. It is assumed that if a male is

removed from his territory through mortality, all of the eggs in his nest will die as they are unable to survive without aeration or guarding from predators.

Males do not fast during the breeding season, but they rarely leave their territory during a breeding cycle in order to maximise the survival of their young (Wootton 1984). In the model, males undergoing a breeding cycle can only forage on the patch that they occupy (i.e. the nest patch), and can forage as usual outside of a cycle.

Females

Once the breeding season commences, temperature and photoperiod cues result in adult females which have reached a given length to become sexually mature and begin spawning (McPhail, 1977; Baggerman, 1958). During each inter-spawning interval (ISI), energy is accumulated for expenditure on egg production and at the end of the ISI an egg batch is deposited if there is a receptive male available. A female's ISI is a function of body mass whereby larger females have a shorter ISI (Wootton, 1974). Females with a weight ≤ 0.49 have an ISI of 9, females with a weight ≥ 0.94 have an ISI of 3 and females between these weights have an increasing ISI with weight between 3 and 9 days (Brown-Peterson and Heins, 2009; Wootton et al., 1995). These ISI's were defined based on an empirical study undertaken by Wootton (1973). The maximum rate of energy allocation to egg production per day increases linearly with mass (Table 1, Eq. 9). The number of eggs produced from the accumulated energy is calculated as the total energy accumulated within an ISI / $M_h (E_c + E_s)$. Females produce eggs up to a maximum number per egg batch, which is calculated as the maximum rate of energy allocation to egg production per day * ISI. This prevents females continuously producing eggs if there are no receptive males. An average fertilisation rate of 0.935 is used for all females, as the literature indicates no changes in fertilisation rate with age or size (Frommen et al., 2008; Barber and Arnott, 2000).

Females first search for an available nest in a vegetated patch (Kraak et al., 1999); if none are available she will search for an open water nest. In the wild, although only one female can visit a nest at a given time, more than one female can visit each day (van den Assem, 1967; Wootton, 1984). In the model, two females can visit a nest at one given time to account for the one day time step implemented. An available territory is therefore defined as containing one male

guarding no more than 4 clutches in days 1 – 4 of his breeding cycle, with no more than 1 female already present. The probability that she spawns in the nest of the owner of that territory is determined by the courtship success probability of the male owner.

When energy accumulated from ingested foods does not meet the requirements of reproduction between successive spawnings, the cost of egg production is subsidised from available energy reserves (Wootton 1977, Wootton 1994). In the lab, females will continue to produce eggs even when the food supply is not sufficient to maintain their body weight (Wootton 1977, Wootton and Evans 1976), suggesting that reproduction will continue to be prioritised until energy reserves run out. This is also supported in wild populations, where most individuals lose mass and decrease in somatic condition (Crivelli and Britton 1987, Wootton et al., 1978) and energy reserves are depleted throughout the breeding season (Wootton, et al., 1978).

Energy reserves

Energy is mainly stored as lipids (Chellappa, et al., 1989) costing 54 kJ g^{-1} for synthesis and storage and yielding 39.3 kJ g^{-1} (Jobling 1994, Schmidt-Nielsen 1997), giving a cost of synthesis of 14.7 kJ g^{-1} . According to Chellappa (1995), the maximum condition factor (see *growth*) reported in wild fish was 1.4 compared to a somatic condition factor of 1. Energy reserves in the model are thus stored up to a maximum threshold proportional to 40% of an individual's structural mass. For simplicity, we assume that all energy is stored as lipid because glycogen accounts only for a very small proportion of total energy reserves (Chellappa et al., 1989; Chellappa, 1995). The mass of energy reserves is added onto the structural mass of the individual to give a total mass.

Energy reserves in eggs and larvae are implicit as we assume that there is sufficient energy in the yolk sac to cover maintenance, and maximum growth costs in larvae.

Change life-stage

Eggs develop into endogenously feeding larvae following a temperature-dependent incubation period of 11 days at 15°C (pers obs). Larvae develop into exogenously feeding juveniles following a temperature-dependent incubation period of 4 days at 18.5 degrees (Swarup, 1958). Juveniles develop

into sexually mature adults once they reach 4.5 cm (Froese and Pauly, 2016; Paepke, 1984).

Table 2. Default parameter values of stickleback PEB model with sources. All fish related weights refer to wet weights, whereas food density parameters refer to dry weights.

Symbol & Definition	Value	Unit	Reference
E Activation energy	0.457	eV	Killen et al. (2010)
A_e Assimilation efficiency	0.95		Cui (1987)
E_x Energy content of food	Unif (17, 25)	kJ g^{-1}	Wootton (1994)
B_o Taxon-specific normalization constant	7.8×10^6	$\text{kJ g}^{-1} \text{ day}^{-1}$	calculated from Meakins (1975)
IG_{max} Maximum ingestion rate	0.006947	$\text{g cm}^{-2} \text{ day}^{-1}$	Volsett and Bailey (2013)
h Half saturation coefficient	0.0000367	g l^{-1}	
E_c Energy content of tissue	7	kJ g^{-1}	Peters (1983)
Energy content lipid	39	kJ g^{-1}	Jobling (1994); Schmidt-Nielsen (1997)
E_s Energy cost of synthesising tissue	3.6	kJ g^{-1}	calculated from Sibly and Calow (1986, pp. 54-5)
Energy cost of synthesis lipid	14.7	kJ g^{-1}	Jobling (1994); Schmidt-Nielsen (1997)
L_m Maximum asymptotic length	6.7	cm	Froese and Pauly (2016)
K Growth constant	0.0193	cm day^{-1}	pers.obvs
L_h Length at hatch	0.45	cm	pers.obvs

M_h	Mass at hatch	0.00156	g	Wootton (1973)
L_p	Length at sexual maturity	4.5	cm	Paepke (1984)
M_a	mass constant	0.0068	---	Froese and Pauly (2016)
M_b	mass exponent	3.28	---	Froese and Pauly (2016)
M_u	Natural mortality probability at unit mass	0.0051		Lorenzen (1996) and calibrated
b	Allometric scaling factor	-0.427		Lorenzen (1996) calibrated
M_l	Larval background mortality rate	0.26		
EC_a	Egg cannibalism constant	0.0049		Whoriskey and FitzGerald (1985)
EC_b	Egg cannibalism intercept	-0.0133		
N_e	Egg natural mortality rate	0.014		Hagen (1967); Candolin et al. (2008); pers. obs
rm	Maximum rate of energy allocation to reproduction	0.74	$\text{kJ g}^{-1} \text{day}^{-1}$	Hagen (1967)
f	Fertilisation rate	0.935		Frommen et al. (2008); Barber and Arnott (2000)
ISl_a	inter-spawning interval constant	-13.215		Wootton (1974)
ISl_b	inter-spawning interval intercept	15.444		Wootton (1974)
T_a	Territory size constant	0.653		van de Assam (1967)
T_b	Territory size exponent	-0.797		

C_a	Courtship constant	0.0577		van de Assam
C_b	Courtship intercept	0.895		(1967)
T_{ref}	Reference background temp	288.15	kelvin	

2 Data evaluation

This TRACE element provides supporting information on: The quality and sources of numerical and qualitative data used to parameterize the model, both directly and inversely via calibration, and of the observed patterns that were used to design the overall model structure. This critical evaluation will allow model users to assess the scope and the uncertainty of the data and knowledge on which the model is based.

Summary:

The model follows general principles and theory of fish ecology and physiology. The model was parameterized from data in the literature wherever possible. The parameters not included here did not require any processing and were simply taken as presented from their sources in table 2. The model was calibrated using available knowledge and data using parameters which could not be accurately obtained from the literature.

Parameterisation details

Food densities

Seasonal fluctuations in food densities followed the trends of temperature fluctuations, which were derived from empirical data (Turner et al., 2013). However, minimum and maximum food densities were estimated from available data in the literature. Wootton (1994) concluded from the available literature that a typical daily consumption rate of wild stickleback is between 2 and 10% of body wet weight in the wild, depending on temperature. In the winter when food rations are at their lowest, individuals maintain an average body weight of 0.5 g (calculated using an IBM in Mintram et al., 2018) and abundances are at their

lowest (Hurst 2007, Toney and Coble 1979, Wootton 2007) resulting in little competition for food patches. Thus, the lowest food density was calculated as 0.01 g per patch. During the breeding season, temperatures and food densities are at their highest and the average adult weighs 1 g. This gives an average food density of 0.1 g per fish. However, to account for the high level of food competition during the summer from juveniles, this was increased to 0.15 g per patch.

Parameterisation of mortality sub-model

Larval, juvenile and adult background mortality is set according to the natural mortality at unit weight equation where an increase in body weight results in a reduced daily mortality rate according to the equation:

Equation 1.
$$M_m = M_u W^b$$

(Lorenzen, 1996) where M_W is the natural mortality probability at mass M , M_u is the natural mortality probability at unit mass, and b is the allometric scaling factor. The model was parameterised by Lorenzen (1996) using the complete Thail estimator, a robust non-parametric regression model, from 103 data points derived from the published literature and specific to fish in temperate regions. This equation incorporates all forms of mortality including predation, background mortality and starvation. Since starvation is already included in the model, the M_u parameter was reduced for the IBM and calibrated (see *calibration* in section 2)

Developmental mortality (N_e) caused by infections, mutations, and incorrect egg development was determined from three laboratory controlled studies (n = 12, Table 3). A constant hatching success of 92 % gives a daily mortality probability of 0.014 for a 6 day incubation period.

Table 3. Hatching success measurements in the absence of predation

Source	hatching success %
Hagen, 1967	82
Hagen, 1967	95
Hagen, 1967	75
Hagen, 1967	93

Hagen, 1967	94
Hagen, 1967	96
Hagen, 1967	92
Hagen, 1967	98
Hagen, 1967	96
Hagen, 1967	82
Hagen, 1967	98
Hagen, 1967	92
Hagen, 1967	90
Hagen, 1967	97
Hagen, 1967	82
Hagen, 1967	100
Hagen, 1967	93
Hagen, 1967	97
Hagen, 1967	95
Hagen, 1967	100
Hagen, 1967	89
Hagen, 1967	92
Candolin et al. 2008	82
pers.obvs.	94.5

A semi-wild density dependent cannibalism study undertaken by Whoriskey and FitzGerald (1985) provided data to quantify egg mortality caused by cannibalism from conspecifics. The model was fit to the linear equation,

Equation 2.
$$EC = EC_a D + EC_b$$

Where EC is the daily egg cannibalism probability, EC_a denotes the egg cannibalism constant, D represents adult and juvenile density with body length ≥ 1.5 cm (fish m^2), and EC_b denotes the intercept. The original data reported total egg mortality over an average of 7 days, which was converted into daily mortality for the model. The model was fit to 4 data points (means) using least squares. Cannibalism increases with the density of juveniles and adults. A minimum daily rate of egg cannibalism of 0.0073 was set to account for filial

cannibalism at low densities; this was the lowest recorded in the study by Whoriskey and FitzGerald (1985).

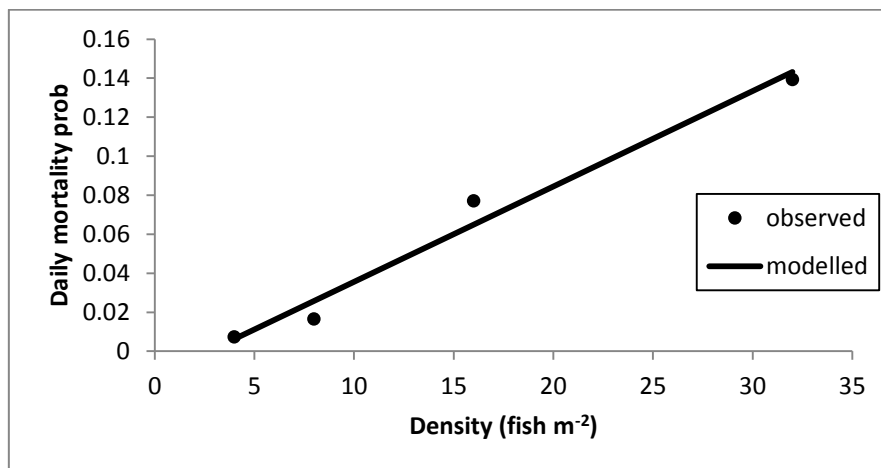


Figure 1. Data from a density dependent cannibalism study fitted to a linear model, with parameter values $EC_a = 0.0049$ and $EC_b = -0.0133$.

Parameterisation of ingestion sub-model

Individual ingestion rates follow a type II functional response (Holling, 1959) where food density affects ingestion rate up to an asymptote. Ingestion rates are also proportional to the surface area ($M^{2/3}$) of the individual (Kooijman and Metz, 1984) and to temperature, according to:

Equation 3.
$$IR: IG_{max} e^{\frac{-E}{k}(\frac{1}{T} - \frac{1}{T_{ref}})} \frac{X}{(h+X)} L^2$$

where IR is the ingestion rate ($g \text{ day}^{-1}$), IG_{max} is the maximum ingestion rate ($g \text{ cm}^{-2} \text{ day}^{-1}$), X is food density ($g \text{ dry weight l}^{-1}$), h is the half-saturation coefficient ($g \text{ dry weight l}^{-1}$), L is body length (cm), and $\frac{-E}{k}(\frac{1}{T} - \frac{1}{T_{ref}})$ is the Arrhenius function which relates temperature to a reference temperature. The maximum ingestion rate (IG_{max}) and half saturation coefficient (h) parameters were obtained by fitting the model to feeding data taken from Volsett and Bailey (2011) using least squares. The original data recorded attack rate as a function of food density (artemia l^{-1}). Since the model requires ingestion rates rather than attack rates, failed attacks were taken into account using data from Heller and Millinski (1979) whereby the number of daphnia successfully ingested per attack was quantified. Artemia density was converted into artemia mass ($g \text{ dry weight l}^{-1}$) as reported by Vanhaecke et al. (1983) and ingestion rates were corrected for body length.

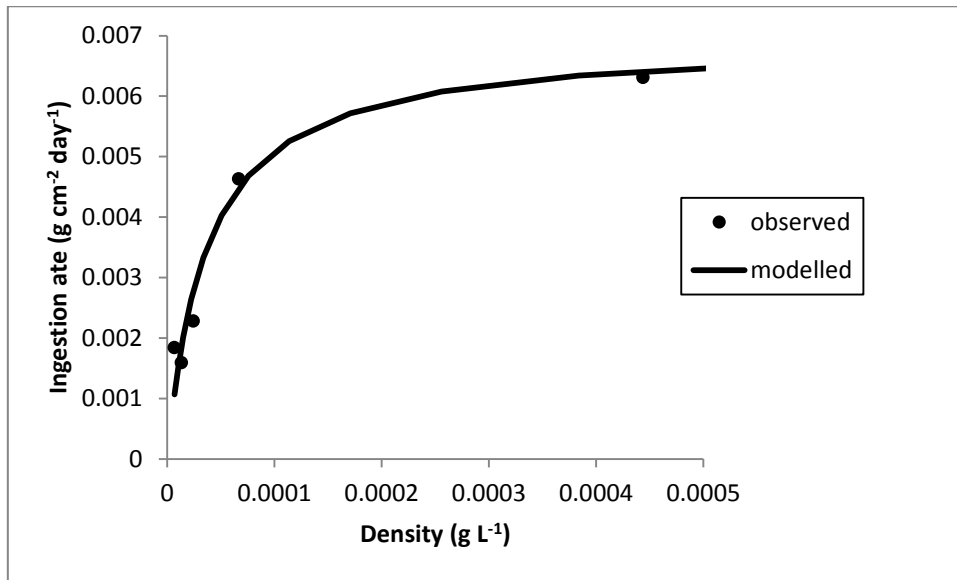


Figure 2. Modelled (black line) and measured (dots) ingestion rates for stickleback at varying food densities (*artemia nauplii*), with parameter values $IG_{max} = 0.006947$ and $h = 0.0000367$

Parameterisation of maintenance sub-model

Basal metabolic rate is modelled according to the equation:

Equation 4.
$$MR = B_0 M^{\frac{3}{4}} e^{\left(\frac{-E}{kT}\right)}$$

where MR is the metabolic rate (kJ day^{-1}), B_0 is a taxon-specific normalization constant ($\text{kJ g}^{-1} \text{day}^{-1}$), $M^{3/4}$ is the scaling with body mass, $e^{-E/kT}$ is the exponential Arrhenius function where E is the activation energy (eV), k is Boltzmann's constant ($8.62 \times 10^{-5} \text{ eV K}^{-1}$) and T is temperature (K) (Peters, 1983, Gillooly et al., 2001, Brown and Sibly, 2012). Meakins (1975) estimated basal metabolic rate as 0.08 kJ/g/day . The value of B_0 was then estimated by solving eq. 2, where M is 1g , E is an activation energy of 0.457 and T is 288.15 K , to obtain a value of B_0 : $7.8 \times 10^6 \text{ kJ g}^{-1} \text{day}$.

Parameterisation of growth sub-model

The maximum growth rate of an individual under optimal conditions follows the von Bertalanffy (1957) growth function (VBGF):

Equation 5.
$$GR = \left(K e^{\frac{-E}{k} \left(\frac{1}{T} - \frac{1}{T_{ref}} \right)} \right) (L_m - L) / 3$$

where GR is growth rate (cm day^{-1}), L_m is the asymptotic length (cm), K is the von Bertalanffy growth constant (cm day^{-1}), L is total length (cm) and $\frac{-E}{k} \left(\frac{1}{T} - \frac{1}{T_{ref}} \right)$

$\frac{1}{T_{ref}}$) is the Arrhenius function which relates temperature to a reference temperature. The model parameter K was parameterized using least squares by fitting the VBGF equation to length at age data obtained from a study undertaken by the authors at 15 °C where growth was recorded in the first 60 days of life, where L_b is length at hatch (cm) and t is age (dph):

Equation 5b.
$$L_m(1 - (1 - \left(\frac{L_b}{L_m}\right))e^{-\frac{Kt}{3}})$$

(Sibly et al., 2013) An asymptotic length of 6.7 cm was obtained from Froese and Pauly (2016).

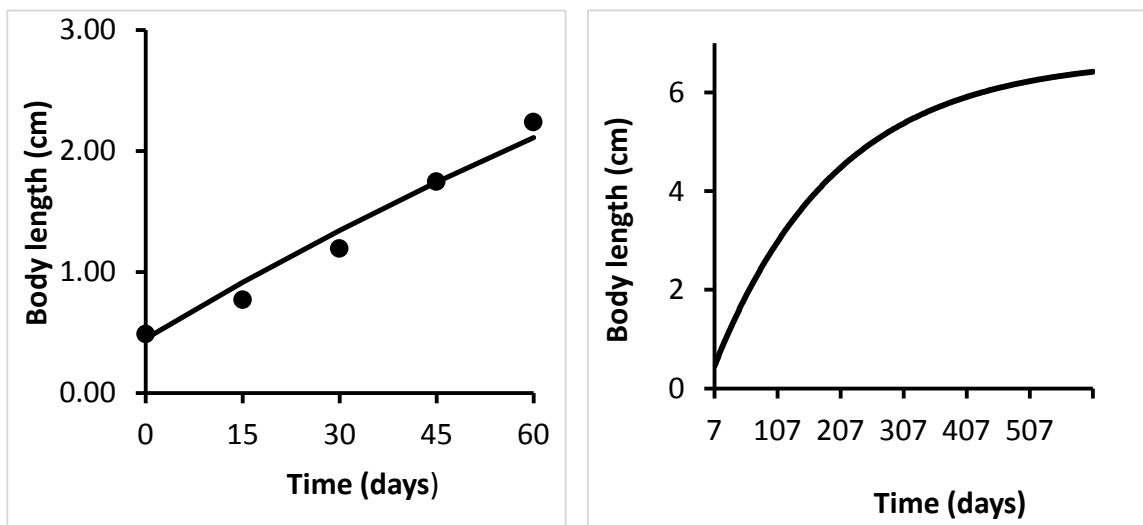


Figure 3. VBGF fit to age (dph) and length (cm) data collected from sticklebacks raised in laboratory conditions at 15°C in the first 60 days of life (a), and modelled length at age predictions over the whole life cycle (b), where L_m is 6.7 and K is 0.0193.

Parameterisation of length: mass

Weights and lengths of 29,975 unsexed sticklebacks were parameterised by Froese and Pauly (2016), using type 1 linear regression of Log W vs Log L, to the allometric equation,

Equation 6.
$$\text{Mass} = m_a L^{m_b}$$

where m_a denotes the weight constant and m_b denotes the weight exponent.

Parameterisation of reproduction sub-model:

Territory size (m^2) is density dependent and is set according to the power function equation:

Equation 7.
$$TS = T_a D^{T_b}$$

Where TS is the territory size (m^2), T_a denotes the territory size constant, T_b denotes the territory size exponent and D represents fish density (fish m^2). The model was fit to 5 data points from a laboratory controlled experiment (van den Assem, 1967) using non-linear least squares (Fig. 4). Minimum and maximum territory sizes in the model are set based on the ranges observed in this study (0.063 – 0.54 m^2).

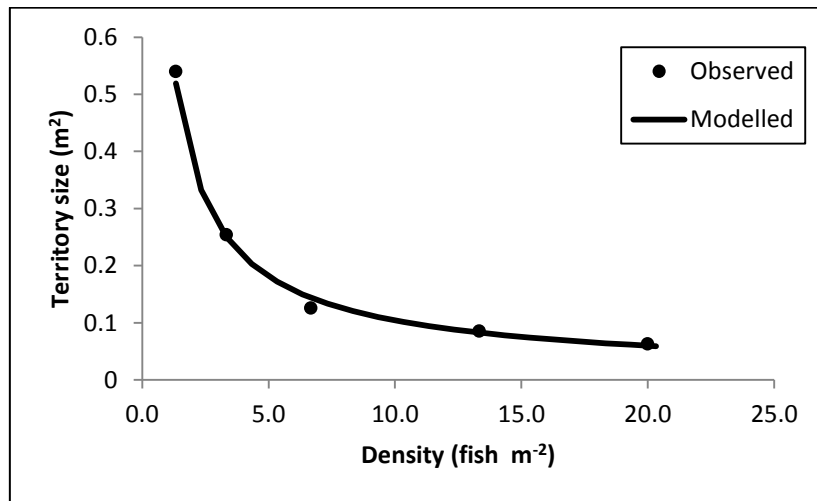


Figure 4. Observed and modelled data for male territory size as a function of global fish density, with parameter values $T_a = 0.653$ and $T_b = -0.797$.

A male’s courtship success probability is then calculated as a function of his territory size according to the logarithmic equation:

Equation 8.
$$CS = C_a \ln(TS) + C_b$$

where CS is the courtship success probability, C_a denotes the courtship constant, TS denotes territory size (m^2) and C_b denotes the courtship intercept. The model was fit to 6 datapoints from a laboratory controlled experiment (van den Assem, 1967) using least squares (Fig. 5).

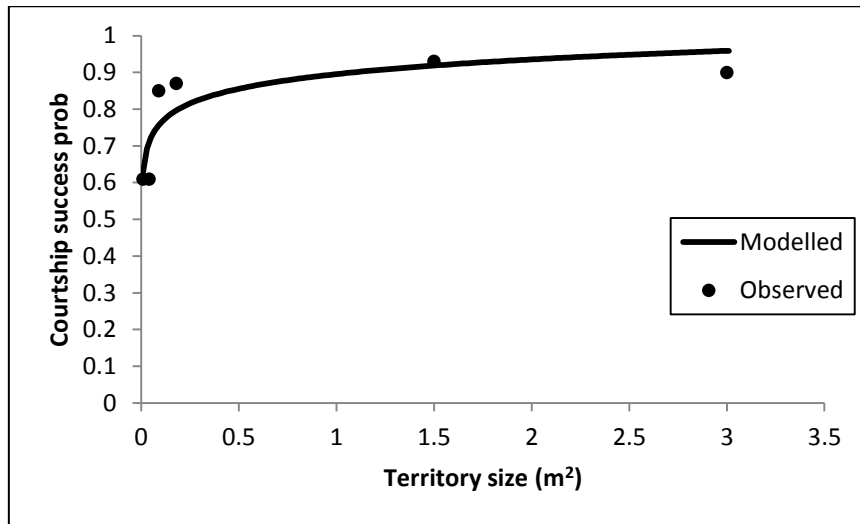


Figure 5. Courtship success modelled as a function of territory size using the logarithmic equation, with parameter values $C_a = 0.0577$ and $C_b = 0.895$.

Parameterisation of reproduction rate:

Reproduction rate increases with increasing body length and is set according to the equation:

Equation 9.
$$R = r_m m$$

where R is the maximum rate of energy allocation to reproduction (kJ day^{-1}), r_m is the maximum rate of energy allocation to reproduction per unit of adult mass ($\text{kJ g wet weight day}^{-1}$), and m is body mass (g). Hagen (1967) recorded a maximum egg number per spawning of 229 for a fish measuring 5.4 cm. It is important that reproduction was scaled with mass, rather than length, because mass fluctuates throughout the breeding season depending on the mass of energy reserves and the equation above accounts for this; therefore the allometric length: mass equation was used to convert 5.4 cm to 1.72 g. A maximum egg number/ day of 76 was yielded when an inter-spawning interval of 3 days was used (Wootton, 1973). The energy costs of producing one egg is equal to: $Mb(E_c + E_s)$, where the energy content of tissue (E_c) is 7 kJ g^{-1} (Peters, 1983) and the energy cost of synthesis (E_s) is 3.6 kJ g^{-1} (Sibly and Calow, 1986). The cost of producing one egg is therefore 0.17 kJ egg^{-1} . This gives a maximum rate of energy allocation (r_m) of $0.74 \text{ kJ g}^{-1} \text{ day}^{-1}$.

Calibration

The model was calibrated using parameters in the model which could not be accurately obtained from the literature. Larval mortality rates (N_e) for wild fish

are highly variable and there are no values for mortality of endogenously feeding stickleback larvae. McGurk (1986) collated studies which reported daily mortality rates between 0.04 and 0.69 for marine larvae. Comparatively, for freshwater fish, Houde (1994) estimated an average survivorship from hatch until metamorphosis into exogenously feeding fish as 5%. Since mortality of stickleback larvae is likely to be lower than average because of paternal guarding in the nest, survivorship was considered to be higher than the average reported by Houde (1994). An N_e parameter between 0.33 and 0.44 would result in a total survivorship of between 10% and 20% at the temperatures used in the model and this was considered a sensible range based on the assumptions and data available. The model was additionally calibrated using the M_u parameter which determines background mortality rates. The original parameter calculated total background mortality for temperate pond fish, including starvation. This parameter was therefore reduced since starvation is already included in the model. The model was calibrated using these two parameters so that abundances outside of the breeding season remained within the range known to occur in the wild (2 – 28 fish m^{-2} (Krokhin, 1970; Reimchen, 1990; Reimchen, 1994; Whoriskey and FitzGerald, 1985; Wootton and Smith, 2000), but densities can reach up to 63 fish m^{-2} in August/September (Penczak and O'Hara, 1983, Whoriskey and FitzGerald, 1985)).

3 Conceptual model evaluation

This TRACE element provides supporting information on: The simplifying assumptions underlying a model's design, both with regard to empirical knowledge and general, basic principles. This critical evaluation allows model users to understand that model design was not ad hoc but based on carefully scrutinized considerations.

Summary: The conceptual model by which the IBM was designed is displayed in figure 6. The model follows general principles of fish ecology and physiology and was developed using data from empirical studies in the published literature wherever possible. Additional details on the simplifying assumptions of the model can be found in sections 1 and 2. The rationale for including food and temperature in the model is additionally described in this section as well as the design of the spatial environment.

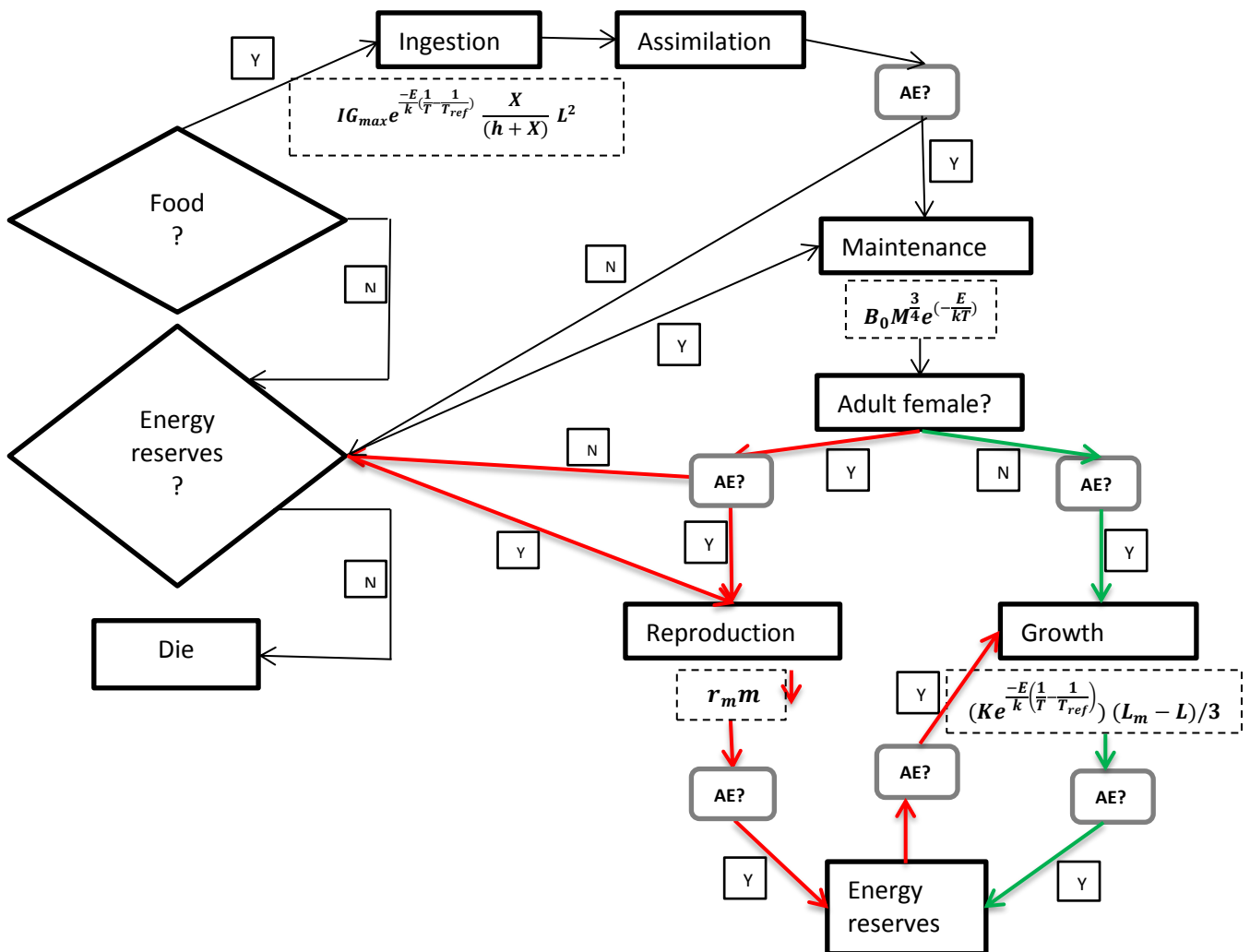


Figure 6. Conceptualisation of the physiological processes undertaken in juvenile and adult stickleback on a daily basis which all demand allocations of resources (energy). Diamond boxes represent energy sources; square black boxes represent life-history processes; grey boxes calculate if there is sufficient assimilated energy (AE?) to undertake the next process; and dashed boxes are relevant algorithms. 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. 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 subsidized from reserves.

Food and temperature

The stickleback is a temperate fish species, meaning that life-history processes, and consequently population dynamics, are heavily influenced by seasonal changes in temperature and food availability. The decision to incorporate food and temperature into the model was taken during the model analysis phase of an existing IBM (Mintram et al., 2018). This model has no energy budget component, so in order to match validation data in both autumn and spring, seasonal growth was enforced using an adapted version of the von Bertalanffy equation (Somers, 1988; Hoenig and Choudary-Hanumara, 1982). Since the stickleback has a global distribution, this method of incorporating seasonal growth limits the model's application for use in regions with higher or lower temperatures. This was reflected in the model validation, where model outputs matched those of a UK population well, but could not match growth rates of a larger population from Germany without adapting default parameter values. Moreover, without the explicit incorporation of temperature and food availability, the variation around mean population dynamics (population abundance and size distributions) could not be fully captured because environmental conditions cannot be altered year to year. In the energy budget-IBM, environmental conditions can be altered (i) to represent different populations and (ii) to include annual variability.

Incorporating temperature and food availability into the model allows for a more mechanistic approach of incorporating seasonal growth and mortality. It also allows for the assessment of other anthropogenic stressors, such as climate change, or natural stressors, such as resource limitation, on stickleback populations.

Movement and food distribution

The IBM is spatially explicit and is divided into habitat patches which contain varying levels of food energy. Individual fitness in the model is therefore largely

determined by movement decisions between these patches. Juveniles and adults maximize energy gains by moving to the most profitable patch, which is dependent on the total amount of energy, and the number of exogenously feeding individuals on the patch. This is based on ecological theory and is referred to as the ideal free distribution (Milinski, 1979; 1984). If there is not enough food on a patch to accommodate the maximum ingestion rates of all the individuals on the patch, the food density is divided proportionally between the individuals based on their size.

The spatial system of the model is imposed based on available data from the literature. Food is contained within vegetated patches and all vegetated patches contain the same density of food, which changes temporally according to the input file, but the energy content of food on each patch is set randomly each day from a given range (Wootton, 1994). This creates a spatially heterogeneous environment for individuals to move around. Individual fitness, and consequently aspects of population dynamics, emerges from the spatial explicitness implemented in the model and the movement rules established for individuals.

4 Implementation verification

This TRACE element provides supporting information on: (1) whether the computer code implementing the model has been thoroughly tested for programming errors, (2) whether the implemented model performs as indicated by the model description, and (3) how the software has been designed and documented to provide necessary usability tools (interfaces, automation of experiments, etc.) and to facilitate future installation, modification, and maintenance.

Summary:

In order to ensure that the code performs as specified in the ODD, a number of tests were undertaken. These tests included syntax checking of the code, visual testing through the NetLogo interface, the use of print statements and spot tests with agent and patch monitors to check against calculations in Excel, stress tests with extreme parameter values, and tracking individual entities throughout a simulation.

The model was implemented in NetLogo 6.0.1; a free software platform for implementing IBMs. Due to the complexity of the model, model testing was split into three phases. Firstly, the original IBM without an energy-budget component described in Chapter 2 was thoroughly and rigorously tested by the authors and by an additional code reviewer. The details of the tests undertaken of this model can be found within this chapter. Secondly, a spatially implicit model containing one individual was tested independently to assess the energy budget components of the model. Testing consisted of comparing model outputs against calculations in an Excel spreadsheet for each algorithm under a range of environmental conditions. The third phase of testing was a thorough testing of the final combined energy-budget IBM by an independent code reviewer and myself (the model developer). Testing for this model involved extracting sub-models and testing them independently by comparing outputs against calculations in an Excel spreadsheet. The NetLogo interface was used as a tool to monitor changes in model outputs under differing environmental conditions. Spot checks of individual entities and patches were used to check the correct implementation of algorithms and sub-model sequencing.

5 Model output verification

This TRACE element provides supporting information on: (1) how well model output matches observations and (2) how much calibration and effects of environmental drivers were involved in obtaining good fits of model output and data.

Summary: This section firstly describes which parameters were directly estimated through calibration and the criteria used to calibrate the model. Model outputs of individual life-history processes were then compared to empirical observations in order to verify the energy-budget components of the model. Here, we assessed how modelled rates of growth compared to observed data when individuals were subject to varying levels of food stress.

Calibration

The criteria for model calibration were for model outputs of population abundance to remain within the ranges reported in the literature. Population abundance data was collated from the literature for stickleback over a global

scale. Outside of the breeding season, stickleback densities ranged from 2 – 28 fish m⁻² (Krokhin, 1970; Reimchen, 1990; Reimchen, 1994; Whoriskey and FitzGerald, 1985; Wootton and Smith, 2000), but densities can reach up to 63 fish m⁻² following the end of the breeding season into September (Penczak and O'Hara 1983, Whoriskey and FitzGerald, 1985). The model was considered to be a good fit to the data when the mean abundances fell within the ranges reported in the literature between September and May (i.e. outside of the breeding season) (Fig. 6). In August, the mean modelled density was 30 fish m⁻², with a maximum density of 36 fish m⁻², which is within the range reported by Penczak and O'Hara (1983) and Whoriskey and FitzGerald (1985) for populations where breeding has recently ended.

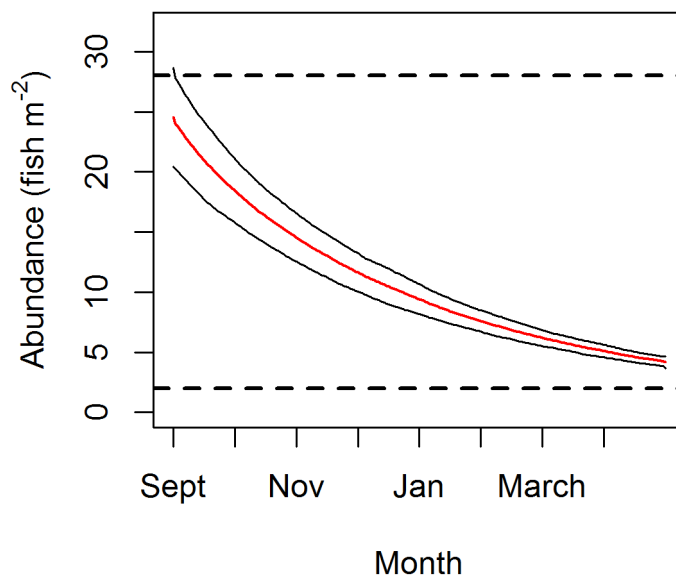


Figure 7. Modelled mean (\pm s.d) population abundances (fish m⁻²) between 1st September and 1st May. Modelled outputs represent 15 replicate runs over 20 years. Dashed lines represent the min and max abundances reported in the literature outside of the breeding season.

Individual life-cycle processes

In order to assess how well the energy budget component of the model matches empirical data, we compared model outputs of growth to observed data from individuals maintained under varying levels of food stress. To do this, we adapted the model so that it possessed only a single individual with its own energy budget on a single patch. This version of the model is deterministic; all

simulations in this section therefore represent data from one individual during a single run.

Model predictions of growth rates were compared to empirical data derived from the laboratory study described in Chapter 3 (section 2.1.6). In this study, somatic growth rates (body lengths over time and final wet weights) of stickleback maintained under varying levels of food availability were quantified from 0 - 60 dph. Food densities matched those used in the lab study (see Chapter 3, SI) and an energy content for *Artemia nauplii* of 24 kJ g⁻¹ was taken from Paffenhöfer (1967) and Paul and Michael (1994). Growth rates from this study at the highest food ration were used to parameterise the growth sub-model. Thus, we are using this empirical data to verify that modelled individuals respond to food shortages appropriately.

Modelled body lengths over each time step and final wet weights under different food rations generally matched the patterns of those measured in the lab well (body lengths $R^2 = 0.94$, wet weights $R^2 = 0.71$, Fig. 8). However, the model has a tendency to under predict body mass and this is a result of the constant active metabolic rate (AMR) used in the model. Data from the literature supports that movement is adaptive under periods of food stress in early life stages of several fish species in the lab (Méndez and Wieser 1993, Wieser et al., 1992) including stickleback (Beukema 1968). Preliminary testing of the model supported that AMR is adapted so that juveniles increase movement in an attempt to search for food up to a critical point, after which energy is conserved and metabolic rate is decreased (Wieser 1991). This version of the model provided a better match to this empirical data; however, we decided not to implement this adaption into the population model because it is unclear how it would translate into the field in the presence of other stressors, such as predation and abiotic conditions. Since this model is intended for risk/hazard assessments, keeping AMR as a constant without any adaption provides a worst case scenario which is preferable to including uncertain, potentially buffering, adaptations.

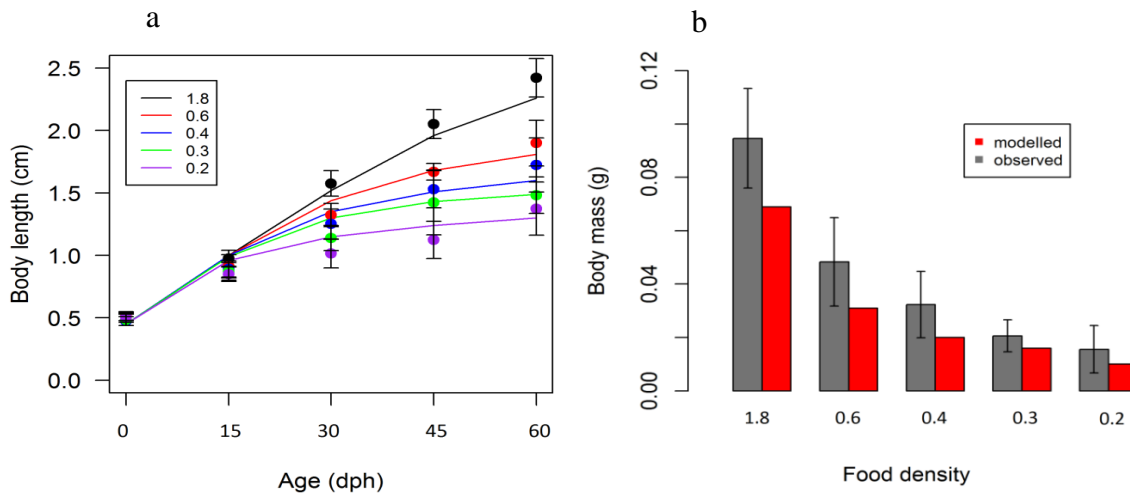


Figure 8. Body lengths over time (a) and final wet weights (b) of observed and modelled stickleback under varying food densities. Circles on graph a represent observed data and lines represent modelled data. Legend refers to food density per individual (mg). Observed data are displayed as mean lengths (\pm s.d), whereas modelled outputs represent a single run.

6 Model analysis

This TRACE element provides supporting information on: (1) how sensitive model output is to changes in model parameters (sensitivity analysis), and (2) how well the emergence of model output has been understood.

Summary:

Preliminary model analysis consisted of determining representative replicate run numbers. A full local sensitivity analysis was then undertaken, where parameter values were altered by $\pm 10\%$.

Preliminary analysis showed that 15 replicate model runs were necessary to get robust means and standard deviation. Replicate number was considered to be robust once the difference in the average and the standard deviation of the population abundance became independent of replicate number ($\pm 5\%$) when ticks = 3600.

A local sensitivity analysis was performed, where parameters from each sub-model formulation were altered by $\pm 10\%$. The effects of the changed parameters were assessed by comparing the mean population abundance and population biomass at a single time point over 5 years following a 10 year spin up period.

Table 4. Sensitivity of population abundance and biomass to changes in parameter values for each sub-model.

Symbol & Definition		Percentage change in abundance (\pm s.d)		Percentage change in biomass (\pm s.d)	
		-	+	-	+
E	Activation energy	-1.8	3.0	-1.9	-2.6
A_e	Assimilation efficiency	2.7	3.7	1.2	0.3
E_x	Energy content of food	1.0	-0.6	0	0.2
B_o	Taxon-specific normalization constant	11.3	3.7	0.4	0.2
IG_{max}	Maximum ingestion rate	0.7	3.9	-2.4	0.6
h	Half saturation coefficient	-1.8	-1.7	-0.3	0.4
E_c	Energy content of tissue	0.7	-0.2	-2.0	-1.7
	Energy content lipid	-1.6	1.1	-1.3	-0.65
E_s	Energy cost of synthesis	2.1	0.3	1.5	0.9
	Energy cost of synthesis lipid	3.7	0.8	-0.3	-0.8
L_m	Maximum asymptotic length	-0.5	2.6	0.4	1.8
K	Growth constant	-1.1	-2.5	1.0	0.2
L_h	Length at hatch	1.6	-0.4	1.3	0.5
M_h	Mass at hatch	5.7	5.3	0.2	-0.8
L_p	Length at sexual maturity	6.5	4.5	-0.5	-0.3
M_a	weight constant	1.0	4.6	1.6	2.2
M_b	weight exponent	-1.1	-3.8	2.5	0.2
M_l	Larval background mortality	1.6	6.7	1.9	3.3

	rate				
EC_a	Egg cannibalism constant	1.6	2.5	1.2	1.4
EC_b	Egg cannibalism intercept	-5.7	-3.0	-1.0	3.3
N_e	Egg natural mortality rate	-0.1	-1.1	-3.0	-2.0
rm	Maximum rate of energy allocation to reproduction	0.4	-1.3	-1.0	-0.5
f	Fertilisation rate	1.4	2.1	0	-0.2
ISI_a	inter-spawning interval constant	-3.7	-2.0	2.7	1.7
ISI_b	inter-spawning interval intercept	0.1	0.9	-0.4	1.2
T_a	Territory size constant	0.6	-5.4	0.4	0.3
T_b	Territory size exponent	2.6	3.2	0.3	1.0
C_a	Courtship constant	4.8	2.8	1.0	0.6
C_b	Courtship intercept	2.8	6.3	-1.4	0.5
-	Egg incubation time	1.1	2.9	-1.1	0.2
-	vegetation cover (at initialisation)	-10.1	3.5	-11.4	10.6
-	duration of breeding season	5.7	-2.2	5.6	-8.4

The model was most sensitive to alterations in the taxon-specific normalisation constant (B_0) of the maintenance sub-model and the number of vegetation patches within the environment. The sensitivity of the model to the former is largely dependent on the amount of food in the environment; the model is more sensitive to this parameter at lower food availabilities because it determines the amount of energy that can be allocated to subsequent life-cycle processes. The model's sensitivity to the latter highlights the importance of incorporating a spatially explicit environment within the IBM. In addition, the model was

sensitive to alterations in the duration of the breeding season and this is consistent with the IBM in Mintram et al. (2018) and is reflective of the low fecundity and relatively narrow breeding season of the stickleback. The model was generally robust to alterations in all other parameters. Interestingly, the IBM in Mintram et al. (2018) was most sensitive to alterations in sex ratio whereas the energy budget IBM presented here was not. The relative importance of this parameter has therefore decreased with the increasing complexity of the model. Overall, this sensitivity analysis highlights the important role of food availability in determining population dynamics.

7 Model output corroboration

This TRACE element provides supporting information on: How model predictions compare to independent data and patterns that were not used, and preferably not even known, while the model was developed, parameterized, and verified. By documenting model output corroboration, model users learn about evidence which, in addition to model output verification, indicates that the model is structurally realistic so that its predictions can be trusted to some degree.

Summary:

In this section, we compared modelled outputs to independent empirical data. We collated stickleback population dynamics data from two UK populations at different times of year so that the realism of the model can be assessed. Modelled environmental conditions were adapted to match the field conditions of the data as accurately as possible and temperature and food availability were kept constant between years. All model simulations represent outputs from default parameter values.

Details of studies used for model validation

Model validation was undertaken using population abundance data, size distribution data, and seasonal growth data for two populations in the UK. These populations were chosen for model validation primarily because the food availabilities within each location are contrasting, but also because sufficient information on environmental conditions could be obtained. The first population, located in the River Rheidol, Wales, represents a favourable habitat for the stickleback as food is abundant. 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 habitat for the stickleback as food is limited throughout the year and individuals are therefore comparatively smaller.

Population abundance data was recorded by Wootton et al (2005) and Wootton (2007) from the River Rheidol population. Field abundances were recorded every year in spring (Feb/March) and autumn (October) over 11 and 21 years, respectively. Mean modelled population abundances were recorded on the 1st March and 15th October each year to represent the mid values of the field data collection periods. Size distribution data was additionally reported from this population (Wootton, 2007). To compare modelled and observed size distributions, the proportion of individuals within each size class was calculated in October and February/March for five years. Modelled size distributions represent the mean frequency of individuals across the whole of each sample period. Wootton et al. (1978) reported body lengths and mass of female sticklebacks from the River Rheidol over a single year (Sept to Sept). In addition, Allen and Wootton (1982) reported body lengths of all fish (0+ cohort) from Lake Frongoch over a single year (July to July); body lengths from this study were converted from standard length to total length using a conversion factor of 1:1.21 (Greenback and Nelson, 1959) for comparison with modelled data. Mean modelled body lengths and mass of all fish (Allen and Wootton, 1982) or female fish (Wootton et al. 1978) were recorded on the 15th of each month for 5 years. All modelled data represents mean values of 15 runs following a 10 year spin up period.

The River Rheidol population is not considered to be resource limited (Wootton et al., 1978) and so food densities were maintained between 2 and 10% of body weight as described in section 2. Temperature additionally ranges between 5 and 19° C in the sampled area of the River Rheidol (Wootton et al., 1978) and so the environmental conditions described in section 2 were maintained for validation against this population. Monthly water temperatures for Lake Frongoch were obtained from Allen and Wootton (1984) and range from 3 to 19° C. Food densities for this population were estimated by Allen and Wootton (1982). The authors estimated food consumption per day based on recorded growth rates of fish; to implement this into the model, each individual was

allocated an exact amount of food per day so that food competition no longer occurred. This ensured that each individual in the model consumed the same density of food as estimated by Allen and Wootton (1982).

Model outputs vs empirical data

Model simulations of stickleback populations in the field showed good predictions of population abundances in the River Rheidol in spring over 11 years (modelled mean = 6.1 fish m²; observed mean = 4.7 fish m²) and autumn over 21 years (modelled mean = 17.2 fish m²; observed mean = 13.5 fish m²) (Wootton et al., 2005; Wootton, 2007). The model additionally showed good predictions for size distributions (Fig. S9) 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).

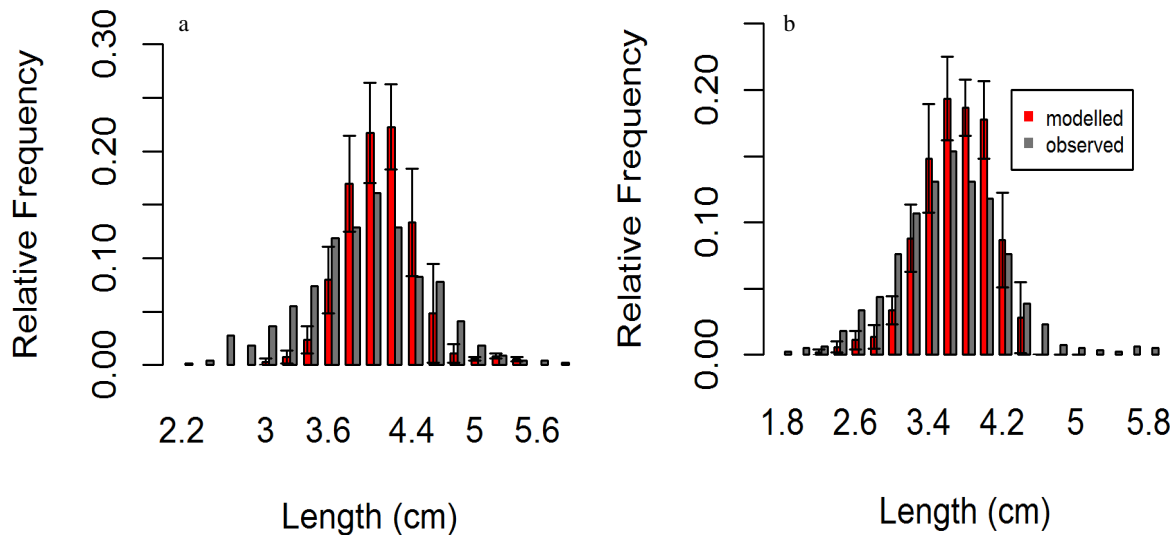


Figure 9. Comparisons of modelled and observed data for size distributions in spring (Feb/March (a)) and autumn (October (b)). Modelled size distributions represent mean values from 15 replicate runs (\pm sd) over 5 years. Observed data were obtained from a stickleback population in the River Rheidol (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. 10a) (Wootton, 1997). Under less favourable environmental conditions, the model also showed good predictions for mean body lengths of stickleback from Lake Frongoch ($R^2 = 0.66$) (Fig. 10b). It is notable that there is very little variation around modelled

mean body lengths from Lake Frongoch because food competition was eliminated in these modelled scenarios in order to accurately match food densities in the field.

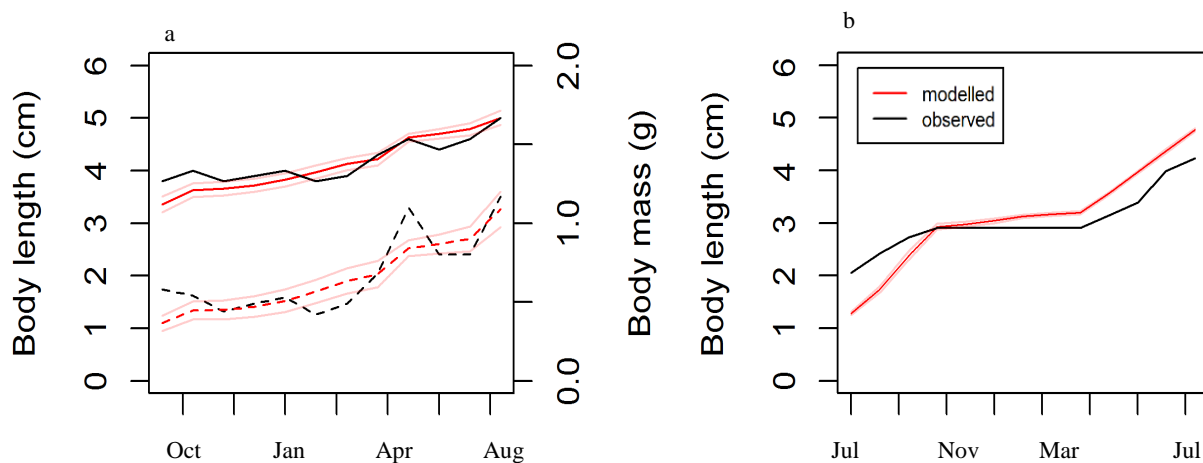


Figure 10. Comparisons of modelled and observed data for mean body length and mass over a year from September to August (a) and mean body length over a year from July to July (b). Modelled data represents mean values from 15 replicate runs (\pm sd) over 5 years and observed data were obtained from a stickleback population in the River Rheidol (Wales, UK) after Wootton et al. (1997) (a) and Lake Frongoch (Wales, UK) after Allen and Wootton (1982) (b).

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Chapter 6. Final Discussion

There are approximately 32,500 species of fishes on the planet (Nelson, 2006) of which more than 15,000 inhabit freshwater habitats. Despite covering only 0.3% of available global water (IUCN), freshwater ecosystems are hotspots for biodiversity (Reid et al., 2013) making their management and conservation a priority. Chemical exposures are threatening the health of freshwater ecosystems across the globe as the use of existing and novel chemicals continues to grow. The demand for more efficient agricultural practises (Tilman et al., 2002) alongside an increased consumption of pharmaceuticals by a growing and ageing population (Van Boeckel et al., 2014; Deo and Halden, 2013) is resulting in increasingly complex mixtures of man-made chemicals entering many freshwater environments. Endocrine disrupting chemicals (EDCs) are of particular environmental concern because of their ability to cause adverse effects on non-target organisms (including fish) and their populations at low and environmentally relevant concentrations (Fuhrman et al., 2015). Environmental risk assessments (ERAs) are conducted for the licensing of chemical products to ensure that new and existing chemicals do not cause adverse effects on wildlife populations (EC No 1107/2009).

In this thesis, novel modelling tools were developed to better inform ERAs to support more effective management decisions on the regulation of chemicals. The overall aim was to provide researchers and risk assessors with new information and tools to help identify the most harmful exposure scenarios for EDCs, and to explore the intrinsic factors (i.e. life-history strategy and population resilience) which affect population susceptibility. This body of work has contributed new knowledge to better understand the impacts of EDC exposures on fish populations. This chapter provides a critical analysis of the key findings obtained in this thesis, the implications of these findings for ERA and for fish conservation in general and recommended priorities for future research.

1. Key findings and take-home messages

Three key take-home messages can be concluded from the findings of this thesis: (i) population models can be used to realistically predict the effects of EDCs in wild fish; (ii) population models can be used to identify intrinsic (life-history strategy and density dependence) and extrinsic (abiotic conditions and

exposure scenario) factors which affect population susceptibility; (iii) mechanistic (e.g. energy budget) models can be used to explore mechanisms (e.g. adaptive behaviours) which may be causing observed effects in the lab.

Chemical exposures do not occur as isolated stressor scenarios for wild fish populations (Backhaus et al., 2013; Løkke et al., 2013; Beyer et al., 2014; van den Brink et al., 2016) and so it is important to establish how additional stressors may impact chemical effects. For this reason, chemical exposures and food limitation are the two constant themes throughout this thesis. These stressors are rarely explicitly considered together in laboratory studies and studies exploring their interactive effects at the population-level are almost non-existent. Although some modelling studies incorporate density dependence (which implicitly incorporates food competition) when analysing chemical effects (e.g. Hazlerigg et al., 2014), an explicit consideration for food availability is required to truly assess the interactive effects of chemical exposure and food limitation. It must be highlighted that the interactive effects of these two stressors on individual fish (presented in Chapter 3) were different to those reported for populations (using the model presented in Chapter 4). In Chapter 3, laboratory and modelling studies indicated that adaptive behaviours may compensate for some chemical effects in food limited fish. However, it was decided that these adaptive behaviours would not be implemented into the population model because of associated uncertainties. For example, the lab study quantified the effects of a single chemical (EE₂) on a very narrow life stage (0-30 days post hatch) under controlled conditions. It is unclear how these effects would extrapolate to the field, to other chemicals, and to other life stages. Most importantly, ERA is designed to be conservative so it is important to exclude uncertain, potentially buffering, behaviours to ensure a worst-case scenario.

Each of the models presented throughout this thesis is the product of a circular process of pattern orientated modelling (Grimm et al., 2005). The models were developed and tested, limitations and errors were identified, and this process was repeated until the model provided sufficient predictions of real-world scenarios. This pattern proved important for identifying the key drivers within each model. The most evident example of this is in Chapter 2, where the model could not provide good predictions of field data in autumn because seasonal

growth was not initially included. Extensive model analysis revealed the importance of seasonal growth on all life-history processes (i.e. growth, reproduction and survival). Similarly in Chapter 3, AIC analysis was used to identify the importance of adaptive foraging behaviour in food stressed individuals, and limitations identified in the IBM developed in Chapter 2 were used to support the incorporation of an energy budget model in Chapters 4 and 5. It is important, therefore, that models are thoroughly analysed using as much validation data as possible before they are used to address scientific questions. TRACE documentation can aid with this as it requires modellers to document all stages of model development, analysis and verification.

It is also important to consider the limitations of each of the models and techniques used throughout this thesis. Firstly, the IBM in Chapter 2 generally validated well against field data, but it did not capture the extent of the variation around the mean population dynamics (population abundance and size distribution data) displayed in the field data. Moreover, it proved somewhat limited (i.e. could not provide predictions for both populations without altering the default maximum growth parameter) when validating against two different stickleback populations (a UK and German population) because of the enforced method of incorporating seasonal growth. The IBM presented here is useful for predicting how a population may react to EDC exposure regimes under a given set of environmental conditions; however it may overlook the most vulnerable individuals in the population (i.e. the smallest) and does not account for favourable or unfavourable years (i.e. low or high population abundances). Indeed, this limitation was highlighted by a reviewer on submission of this manuscript and further justified the incorporation of an energy-budget model into this IBM so that environmental conditions could be explicitly captured. Since this model was developed for use within ERAs, single-endpoint effects were focused upon and these results therefore do not necessarily represent the full effects of the case study chemical. Although it is possible to model the effects of multiple endpoints using this IBM, there may be difficulties associated with interpreting the results i.e. identifying mechanisms behind observed effects.

The energy budget-IBM is the most complex model described in this thesis; it is inevitable, therefore, that this complexity will be traded off by some limitations and uncertainty. This model requires a higher level of mechanistic detail for a

chemical effect than the IBM described in Chapter 2. Uncertainties will therefore occur if there is insufficient data to determine the underlying mechanism(s) of a given chemical effect (e.g. direct vs indirect effects of an EDC). Moreover, it is particularly difficult, if not impossible, to accurately determine the proportions of the direct EDC effect vs secondary metabolic costs. Here, two of the most well described EDCs were used as case study toxicants so that uncertainties associated with knowledge gaps could be reduced. An additional challenge is presented when the individual-level chemical effects quantified in the literature are unavailable for the model species. In this case, there was significantly more data available for EDC exposures in the fathead minnow than for the stickleback; it was therefore necessary to extrapolate across these species. Since current ERA extrapolates results from a narrow range of laboratory conditioned model species to establish effects on all wild fish populations, the approach of extrapolating between two similar species provides less uncertainty than the current method used in ERA.

2. Implications of my thesis for understanding chemical exposures in an ecological context

Following significant efforts by researchers and industry to support the inclusion of mechanistic effect models into ERA (Grimm et al., 2009; Galic et al., 2010; Forbes et al., 2011), conclusions drawn from reliable models can now be used in the ERA of EDCs (EC No 2017/2100 and EC No 2018/6054). It is therefore a critical time to explore the potential for different modelling approaches to support (and hopefully improve) current ERA and support more targeted and effective protection for fish populations.

A range of different techniques were used in this thesis to explore the potential for holistic approaches to improve our understanding of chemical exposure effects and to identify key drivers affecting population susceptibility and resilience. The work undertaken here has significantly contributed to the fields of ecology and environmental risk assessment as follows.

Firstly, the work provides an environmentally realistic tool by which to extrapolate from individual- to population-level effects for the three-spined stickleback (Chapter 2). This model was designed to be accessible to users using the NetLogo interface and does not require excessive training for further

application. Additionally, all of the models presented are documented using standardised frameworks (i.e. ODD or TRACE) to increase transparency and to aid their future use by researchers and risk assessors. Current ERA uses dose-response experiments to quantify Predicted No Effect Concentrations (PNECs) and establish ecological risk based on a ratio between Predicted Environmental Concentrations (PECs) and PNECs. Using the IBM in Chapter 2, I demonstrate that ecological risk is dependent on a much greater number of factors associated with the exposure and not the concentration of the chemical alone; although concentration does affect the level of chemical effect on populations, population dynamics were also heavily influenced by the exposure regime of the chemical and density dependent interactions. The model can be used as a general tool to explore and identify the safest (if any) exposure regimes to ensure effective population protection.

The need to incorporate multiple stressors when determining chemical effects has recently been recognised by risk assessors (Backhaus et al., 2013; Løkke et al., 2013; Beyer et al., 2014; van den Brink et al., 2016). The challenge remains, however, that these assessments often require complex and expensive experimental designs, a high number of animals and the results obtained can be difficult to interpret. Combining traditional laboratory studies with modelling techniques may help in addressing these challenges (Løkke et al., 2013; Sokolova, 2013). The results presented in this thesis have added to existing knowledge surrounding the interactive effects of multiple stressors on fish populations. Furthermore, it is one of very few studies which explores interactive effects of chemical exposure and food limitation; two stressors which fish are likely to encounter in the wild. The energy budget-IBM (Chapters 4 and 5) provides a tool to further explore these two stressors at the population level; this model has good potential to simulate multiple stressor scenarios which cannot feasibly be undertaken in the lab or field, as demonstrated in Chapter 4.

The work presented in this this thesis has demonstrated that population modelling can increase the realism of exposure scenarios for use in ERAs in three key ways: (1) providing an ecologically relevant method for extrapolating from individual- to population-level effects (2) identifying factors which affect population susceptibility to chemical exposures and (3) incorporating multiple stressor scenarios. The IBMs can use ecologically relevant scenarios to

determine risk and thus provide more targeted protection for vulnerable populations.

3. Recommendations for future research in the consideration for adoption of modelling approaches in ERA

A criticism of IBMs is that they produce highly specific outputs for only a single species. If IBMs are to be taken up within ERA then future model development needs to be carefully prioritised.

Since it is unfeasible to produce population models for every fish species, future model development would be well placed to focus on categorising species based on their life-history traits and subsequently developing robust and reliable models for representative species within each of these categories. The choice of focal species can be further prioritised to focus on those identified as being potentially vulnerable to the effects chemical exposures (Ibrahim et al., 2013; 2014), as well as their amenability for such work (availability of data requirements, relevance for different ecosystems etc.). The development of environmental scenarios using global- to catchment-scale spatially explicit models is a method by which to identify areas of higher exposure risk and generate ecologically relevant exposure information (Franco et al., 2017). This would aid in identifying vulnerable species and predicting relevant ecological scenarios. Developing reliable population models for a range of model species could significantly increase the predictability of trait-based risk assessments (Van den Brink et al., 2011). Although this method would create uncertainties associated with extrapolating across species, it is the only feasible way for population models to serve their full potential within ERA.

The stickleback models presented here are based on the available data from the published literature, personal communications and from my own lab studies. However, there are areas where the model could be developed further in order to expand its application. Firstly, the current modelled system in the IBM represents a small static system (20m²) meaning that movement decisions are unrestricted by distance. The model could be expanded to represent a larger static system or a river so that spatial, as well as temporal, exposure regimes of chemical exposures can be accounted for (e.g. sheltered patches with low concentrations vs patches close to an effluent source with high concentrations).

Movement decisions could then determine the likelihood of exposure of individuals. It should be taken into account, however, that a smaller static system was chosen here because it significantly reduced the run time of the model and this approach would therefore be somewhat limited by computer power. Secondly, the possibility for incorporating toxicokinetic-toxicodynamic (TKTD) models could also be explored, so that effects on life-cycle traits relate to the behaviour of the chemical within the organism (mode of action) and the internal concentration (uptake, distribution, clearance), rather than the concentration within the water body (Ashauer et al., 2013).

As environmental issues are becoming increasingly complex, the drive for computational models to aid decision making for wildlife conservation is growing. The development of mechanistic effect models has soared in recent years as a result of this demand (e.g. Grimm et al., 2009) and this has been accelerated by a drive to reduce the number of animals in research (Hutchinson et al., 2016). Unfortunately, however, models are only as robust as the data and knowledge available for their construction; it is therefore vital to carefully prioritise future research if they are to be used in regulatory decision making. Experimental efforts should focus on filling identified data and knowledge gaps to aid model parameterisation and validation. In compliance with the 3Rs, this does not necessarily involve undertaking animal intensive *in vivo* studies; rather, enhancing and refining general frameworks, such as AOPs (Ankley et al., 2010), would provide tools to obtain extensive information for model development e.g. inferring mechanistic pathways of chemicals for parameterisation of energy budget-IBMs (Goodchild et al., 2018).

This thesis demonstrates the potential for mechanistic population models to provide realistic predictions of EDC effects on fish and to explore uncertainties relating to fish population susceptibility to these chemicals. In order to ensure that chemical exposures (and other anthropogenic stressors) do not cause adverse effects on fish populations, ERAs would benefit from employing mechanistic population models.

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