Capturing ecology in modelling approaches applied to environmental risk assessment of endocrine disrupting chemicals in fish

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Abstract

Endocrine disrupting chemicals (EDCs) are widespread in freshwater environments and both laboratory and field based studies have shown reproductive effects in fish including for 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 is growing and recently this has resulted in the EU Commission stating that results derived from reliable models may be considered when assessing the relevance of adverse effects of EDCs at the population level (European Commission 2016). 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 enabling capturing both direct and indirect population-level effects.

Keywords: environmental risk assessment; endocrine disrupting chemicals; population sensitivity; population resilience; life-history strategy; density dependence; population models; individual-based models

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1. Introduction

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 & 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 & Weis 1974; Mathers et al. 1985; Brown et al. 1987; Saglio & 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 estrogens 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 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 & 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. 2015). 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 estrogens (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 assessment. 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)estrogens, (anti)androgens, aromatase inhibitors, and progestins (Tyler et al. 1998; Hutchinson et al. 2006; Goodhead & 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 & 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 estrogenic EDCs within sewage effluents and surface waters are widely documented. One of the more potent synthetic estrogens, 17α-ethinylestradiol (EE_2), 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}. Reproductive impairments, including feminization of male fish and reduced reproductive success, have been demonstrated in the lab after exposure to concentrations of steroid estrogens within environmentally relevant ranges (e.g. EE_2 ranging from < 1 ng L^{-1} up to 5 ng L^{-1} (Nash et al. 2004; Parrott & 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 estrogens (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 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 μg 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 & 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 estrogens (Hamilton et al. 2015).

An experimental study has shown population level effects of EE_2 in a Canadian lake that was dosed at 4–6 ng EE_2 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 EE_2. Evidence for indirect effects of EE_2 were also seen in the Canadian lake study with subsequent declines in the predatory lake trout (*Salvelinus*

201 *namaycush*) as well as increases in the zooplankton and emerging insects (e.g. Chaoborus) on
202 which FHM prey (Kidd et al. 2014). These findings constitute an ecosystem level effect of EE_2,
203 however, it should be emphasized that the dosing level adopted (4–6 ng EE_2 L^{-1}) is higher than
204 occurs for most undiluted wastewater treatment works (WwTW) effluent discharges (Desbrow
205 et al. 1998; Belfroid et al. 1999; Larsson et al. 1999; Ternes et al. 1999).

206 Although single chemical exposures give a good indication of potential effects based on the
207 mode of action of that chemical, surface waters generally receive inputs of mixtures of EDCs,
208 and numerous studies have established that the combined effects of mixtures of EDCs can be
209 additive (Silva et al. 2002; Brian et al. 2005; Correia et al. 2007). Furthermore, mixed chemical
210 exposure effects outcomes can differ significantly than for single class of EDCs. As an example
211 of this, in laboratory based exposures of roach the feminizing effects of a mixture of
212 antiandrogens and ethinyloestradiol in combination was far greater than that for either the
213 antiandrogens or EE2 separately - Lange et al., 2011). The interactive effects of chemicals are
214 now being measured directly in an increasing number of research studies [e.g. exposure to
215 sewage effluents (Lange et al. 2011; Hamilton et al. 2015) and risk assessment schemes for
216 pesticides now consider the potential cumulative effects of similarly acting compounds
217 [Regulation (2013a) No 284/2013]. Detecting the effects of low-dose exposure is another major
218 issue in the study of EACs. Low-dose effects can be defined as any biological changes which
219 occur at doses lower than those typically used in standard testing protocols (Melnick et al.
220 2002); consequently, effects at these concentrations are easily overlooked in traditional risk
221 assessments. In order to capture low-dose mixture effects, it has been suggested that regulatory
222 testing needs to incorporate biomarker endpoints rather than traditional dose-response
223 relationships alone (Kortenkamp 2008). The US EPA requested the development of a strategy
224 to address the current issues associated with detecting low-dose effects for EACs (National
225 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 modeling for EAC effects has not yet received major attention due to uncertainties into where these effects may occur and for what EACs.

Collectively, laboratory and (limited) field studies for selected environmental estrogens suggest that they can have adverse impacts on some wild fish at the individual level with potential for impacts on the population. Quantifying the effects of EDC exposure at the population-level more generally, however, is extremely challenging. Challenges in EDC 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. 2015).

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 & 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 & 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 & 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 EE_2 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 estrogens 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 ethinyloestradiol 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 EE_2 (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 Regulation (2009) No 1107/2009 and Regulation (2013) No 283/2013) but they are resource and animal intensive and are rarely used in the routine testing of EDCs (Ankley & 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 & 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 & Bradshaw 2006). This regulation underlies the management of fish populations (Rose et al. 2001) and is exploited throughout fisheries worldwide to permit sustainable yields.

Depensatory density dependence, on the other hand, results in a reduced per capita population growth at low densities (Liermann & Hilborn 2001) as, for example, a result of reduced rates of survival and reproduction (Allee & Alle 1958; Wood 1987; Fowler & Baker 1991). Fish schooling is an example of a depensatory mechanism at low densities as it relies on the congregation of numerous fish to increase survival or reproductive success (Marsh & Ribbink 1986). As such depensatory 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 depensation could reduce population growth rates during EDC exposure, by reducing schooling behavior. Although there is evidence for the occurrence of depensation in fish populations (Wood 1987; Myers et al. 1995), it's possible role in exacerbating the effects of chemical exposure has received very little study. This is because depensation 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 & 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, toxico-

dynamic/ toxico-kinetic (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).

Correlative modelling has been used in ecology since the 1700s (Malthus 1926). Correlative models have a wide application within fisheries to estimate population recruitment (Ricker 1954; Beverton & Holt 1957), growth rates (Von Bertalanffy 1957) and fecundity (Carlander 1997), and are relatively easily adapted for use in chemical assessments. However, correlative 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 correlative models. They also benefit from their low data requirements and are therefore relatively easy to parameterize. However, in a similar way to correlative 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 & Ankley 2004; Hayashi et al. 2009; Brown et al. 2014; Ibrahim et al. 2014;). Furthermore, matrix models do not incorporate density dependent processes or individual variability and thus the level of realism is relatively low.

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 & 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 & 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

426 with their surrounding environment (including chemical contaminants). IBMs are a pragmatic
427 approach towards more complex population modelling, as they bridge the gap between
428 individual effects observed in toxicity studies and the potential consequences on wild
429 populations. The implementation of additional sub-models into IBMs is an approach often
430 adopted to develop greater realism (accuracy). These sub-models include TK/TD models,
431 which allow incorporation of ADME (absorption, distribution, metabolism, and excretion) and
432 internal damage and repair processes into environmental risk assessment (Liu et al. 2014); fate
433 models, which can be used to predict the fate of chemicals within aquatic water bodies (Focks
434 et al. 2014); and matrix models, which can predict the effects of chemicals on population
435 dynamics (Meli et al. 2014).

436 Population models require guidance and standardization for their development and
437 validation, and communication for their subsequent uptake and acceptance into ERA
438 (Schmolke et al. 2010b). This has been facilitated by a European funded project, CREAM
439 (Mechanistic Effect Models for Ecological Risk Assessment of Chemicals) that has produced
440 several IBMs assessing the effects of various chemicals on a range of taxa (Gabsi et al. 2014;
441 Kułakowska et al. 2014; Liu et al. 2014). However, IBMs which assess chemical effects on
442 fish populations are relatively few compared to other (invertebrate) taxa (Focks et al. 2014;
443 Gabsi et al. 2014; Johnston et al. 2014; Meli et al. 2014). This is likely because life-history data
444 for shorter-lived invertebrates are relatively easy to obtain, making model development and
445 validation more tractable. However, given that population models might be used to help inform
446 on the environmental risk and identification of EDCs (European Commission 2016), in
447 combination with experimental evidence of individual and population-level effects in fish
448 (Huestis et al. 1996; Jobling et al 1998; Kidd et al. 2007), practical challenges for the
449 development of IBMs for fish need to be addressed: i.e. selection of appropriate species and
450 populations, data availability for parameterization and validation.

451 The relevance and reliability of population models can be established through appropriate
452 model evaluation. Methods of evaluation include model verification (model outputs compared
453 to data used for parameterization), sensitivity analysis (testing the influence of input parameters
454 on model outputs), and validation (model predictions compared to empirical laboratory and/or
455 field data) (Schmolke et al. 2010a). Validation is of particular importance because it
456 demonstrates the structural realism of the model as well as the accuracy of parameterization
457 (Schmolke et al. 2010a). However, validation is not always straightforward because empirical
458 data are not always available. In a study which evaluated 62 models dealing with toxicant
459 effects for a range of taxa, Schmolke et al. (2010a) found that only 3% of models were validated
460 against independent empirical data. Validation of fish models is restricted by the fact that there
461 are very few datasets which provide long term information on fish populations and their natural
462 fluctuations, and even less data for chemical effects exposures (Hamilton et al. 2015). In the
463 absence of long-term population dynamics, data validation may be permitted using population
464 census data which provide a snapshot of a population size/ age distribution, as demonstrated
465 by Hazlerigg et al. (2014). This, however, does not account for variation in year class strength.
466 In cases where data on population dynamics do not exist, Augusiak et al. (2014) suggest that a
467 thorough evaluation, including validation of sub-models, can in some cases be sufficient to
468 assess a model's realism in the absence of a full validation. However, this is debatable, and
469 access to population dynamics data for wild fish populations needs to be a key priority when
470 assessing the realism of IBMs.

471 There is a trade-off between more general models which incorporate a greater range of
472 processes and interactions (i.e. community or ecosystem models), and models which do not
473 necessarily represent a whole system but provide more specific outputs. Population models are
474 constrained by their ability to represent only single species populations i.e. a discrete
475 population within a defined waterbody or an interconnected meta-population in a larger

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

491 ***4.1.1. Incorporating behavioral effects into the assessment of EDCs***

492 In the context of fish and EDCs, a strong feature of IBMs is their ability to incorporate aspects
493 of an individual's behavior. However, despite experimental evidence documenting the effects
494 of chemical exposure on fish behavior and possible impacts on individual fitness (Scott &
495 Sloman 2004; Valenti Jr et al. 2012; Brodin et al. 2014; Dzieweczynski et al. 2014; Klaminder
496 et al. 2014), environmental risk assessment schemes have not yet begun to explicitly measure
497 the effects of behavioral changes in fish as an endpoint for chemical effects. Territoriality,
498 courtship and guarding of eggs and fry within fish are characteristics seen for a number of
499 species (e.g. three-spined stickleback, *Gasterosteus aculeatus*; fathead minnow, *Pimephales*
500 *promelas*; sand goby, *Pomatoschistus minutus*) and chemical-induced behavioral impairments

501 of these traits can have significant impacts on young survival rates (Wibe et al. 2002; Brian et
502 al. 2006; Sebire et al. 2008; Saaristo et al. 2010). This, in turn, could have population-level
503 effects, however, the actual relevance or impacts of such effects at the population level have
504 received very little study. This is particularly relevant in the assessment of EDCs because of
505 their reported effects on reproductive behavior (Weis & Weis 1974; Mathers et al. 1985; Scholz
506 & Gutzeit 2000; Balch et al. 2004; Bell 2004; Sebire et al. 2008; Söffker & Tyler 2012).
507 Similarly, anti-predator behavior is a vital survival trait in virtually every species, and
508 impairments have been documented as a result of EDC exposure, for example in killifish (Weis
509 et al. 2001). Reported declines in fish schooling behavior have also been observed in several
510 fish species after exposure to various EDCs (Ward et al. 2006, Xia et al. 2010) and population
511 declines could emerge from these effects *via* increased predation/ reduced feeding success.

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

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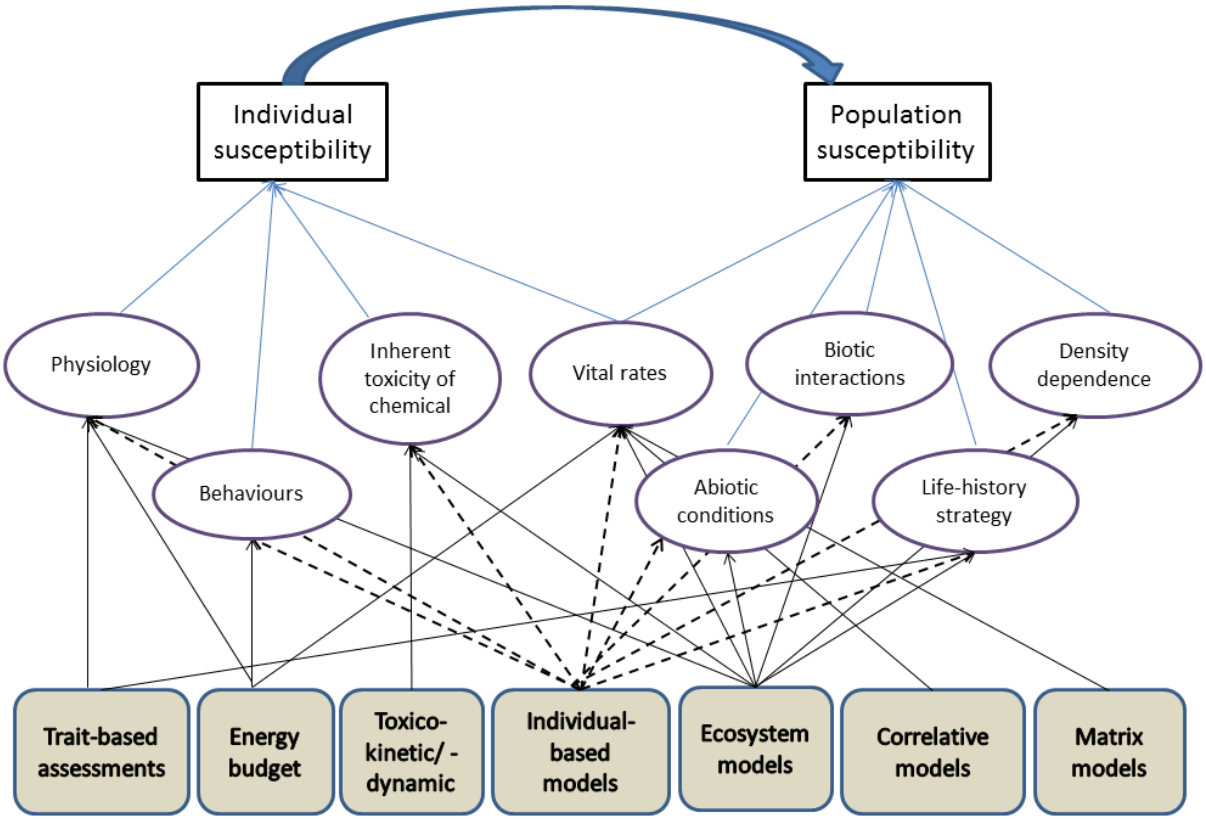

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Figure 1. Conceptualisation of the factors / processes which affect individual- and population-level sensitivity to toxicant exposure (blue arrows) and the category of model which incorporates each of these factors/ processes (black arrows). Dotted arrows highlight the factors which are incorporated within IBMs.