Accounting for transgenerational effects of toxicant exposure in population models alters the predicted long-term population status

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Abstract

Acute environmental stressors such as short-term exposure to pollutants can have lasting effects on organisms, potentially impacting future generations. Parental exposure to toxicants can result in changes to the epigenome (e.g., DNA methylation) that are passed down to subsequent, unexposed generations. However, it is difficult to gauge the cumulative population-scale impacts of epigenetic effects from laboratory experiments alone. Here, we developed a size- and age-structured delay-coordinate population model to evaluate the long-term consequences of epigenetic modifications on population sustainability. The model emulated changes in growth, mortality, and fecundity in the F0, F1, and F2 generations observed in experiments in which larval *Menidia beryllina* were exposed to environmentally relevant concentrations of bifenthrin (Bif), ethinylestradiol (EE2), levonorgestrel (LV), or trenbolone (TB) in the parent generation (F0) and reared in clean water up to the F2 generation. Our analysis suggests potentially dramatic population-level effects of repeated, chronic exposures of early-life stage fish that are not captured by models not accounting for those effects. Simulated exposures led to substantial declines in population abundance (LV and Bif) or near-extinction (EE2 and TB) with the exact trajectory and timeline of population decline dependent on the combination of F0, F1, and F2 effects produced by each compound. Even acute one-time exposures of each compound led to declines and recovery over multiple years due to lagged epigenetic effects. These results demonstrate the potential for environmentally relevant concentrations of commonly used compounds to impact the population dynamics and sustainability of an ecologically relevant species and model organism.

Key words: endocrine disruption; delay-coordinate model; age-structured integral projection model; fish; contaminants of emerging concern

Introduction

The result of exposure to environmental stress, even if occurring only for a brief period of time, is not dissimilar in nature to the ripples created by tossing a pebble in a pond. A seemingly small disruption to a biological system is rarely limited to one receptor or gene. The influence of a single short-term exposure to a pollutant, for example, can cause persistent changes that alter the biological make-up and function of cells, tissues, organs, and entire organisms (e.g. [1, 2]), sometimes via epigenetic changes, and in many cases across generations [4–6]. The epigenome is a suite of chemical compounds that modulates the conformation of histones and chromatin, which in turn dictate the structure and accessibility of DNA open reading frames, thus controlling the accessibility of genes for transcription and directing gene expression ([5, 7], Fig. 1). Ultimately these non-sequence altering modifications are responsible for a multitude of internal and external responses, including cellular differentiation and development, life-stage and tissue-specific gene expression, and response and rapid adaptation to an organism's external environment [8–11].

Epigenetic mechanisms include the addition of functional groups to histones, such as acetylation or phosphorylation, DNA methylation, and interactions of non-coding RNA during transcription (e.g. silencing), all of which contribute to the patterns of gene and eventual protein expression experienced by an individual organism [12–14]. These mechanisms are generally conserved across taxonomic groups, with some differences as to how much one particular mechanism is relied upon compared to others. In fishes, for example, CpG-site-specific methylation may be as much as three times higher than what has been measured in the mouse model [10]. There are also indications that the amount of within-gene-body methylation may be greater and play a larger role in influencing the gene expression in teleosts [7, 15],
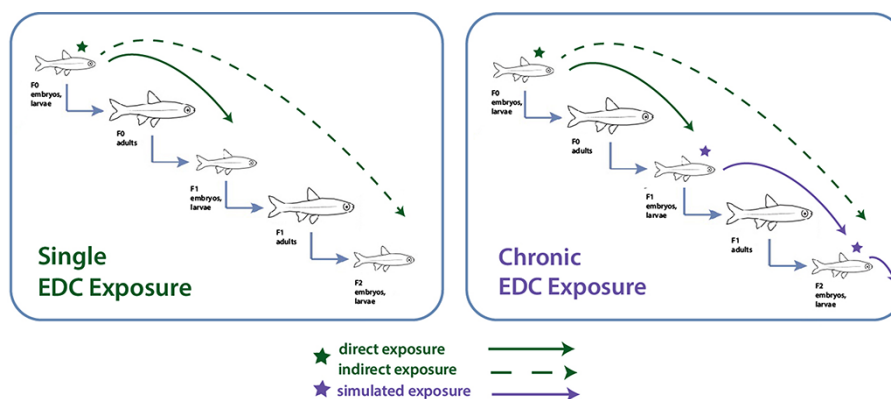
Figure 1: Conceptual figure illustrating the structure of the population model, both acute (single exposure in parent generation) and chronic (repeated exposures in each generation) were simulated using an age-structured delay-coordinate model

in comparison to mammals. As such, it is apparent that DNA methylation and its influence on downstream changes in cellular function and ultimately organism performance and fitness are critical to responses to environmental stress in fishes both within and across generations [16].

Although a great deal of work on epigenetic mechanisms and on their role in responses to environmental perturbations, such as pollutants, across generations has been done in commonly used fish models such as zebrafish, *Danio rerio*, and Japanese medaka, *Oryzias latipes* (e.g. [12, 15, 17–19]), the decreased expense of sequencing and the need to better understand how multi- and transgenerational responses are conserved across teleosts has spurred work in species traditionally considered to be non-model organisms, such as the inland silverside (*Menidia beryllina*). Inland silversides are an annual euryhaline species native to the Atlantic and Gulf coasts of North America and introduced to the US Pacific coast in the 1960s [20, 21]. They have temperature-sensitive sex determination, which may make them more sensitive to endocrine disruption exposure and to climate change [22, 23]. With a recently sequenced genome, several longer-term (2-year) studies encompassing the measurement of epigenetic modifications, as well as observations at the organism level, such as embryonic development, hatching success, growth, and fecundity, have been completed in the silverside [6, 7, 22, 23]. Notably, following the exposure to endocrine disrupting compounds (EDCs) with various mechanisms of action, alterations in the methylation of genes across three generations, including those involved in development, endocrine function, and immune response, among others, were correlated with abnormal development in early-life stages, altered immune response, skewed sex ratio, and reduced egg production [6, 7].

Given that disrupted egg production, decreased hatching rates, or lowered quality of offspring all directly contribute to population fitness [24–26], the observed impact to silverside fecundity and thus fitness at environmentally relevant exposure levels (ng/l) in the above-described works calls for a better understanding of the longer-term implications. Furthermore, as a bioindicator species [21, 27], measured impacts to the silverside can be extrapolated to fishes with similar life histories, exposure risks, and/or habitat usage. For these reasons, we used data acquired from earlier empirical work to parameterize a single-species population model to predict the impact of a single exposure to EDC representative of those detected in aquatic ecosystems globally, as well as to extrapolate to estimate cumulative effects from a more realistic continuous exposure scenario. We used a delay-coordinate population

modeling approach that tracks age, parental and grandparental age, and sex (male/female) to predict population densities for each generation and chemical for single (empirically derived) and continuous (modeled) exposures. We hypothesized that the perturbations to fitness-relevant endpoints that appeared following these brief exposures to single chemicals, across generational time and not necessarily within the same generation, are additive with each subsequent generation and that both single and continuous exposures would lead to gradual population decline, with the latter being more detrimental.

Methods
Exposure

In the experiment used to parameterize the model, inland silversides were exposed from 10 to 12 h post fertilization, as embryos, singly to four different EDCs: two predicted to be estrogenic—bifenthrin (Bif) and ethinylestradiol (EE2), and two predicted to be androgenic—levonorgestrel (LV) and trenbolone (TB), at 3–9 ng/l (measured concentrations). Exposures performed at ambient temperatures (22–23°C) ended at 21 days post hatch [6]. Fish were then reared in clean water to the larval stage of the F2 generation. This means that parents were directly exposed as larvae, F1s were indirectly exposed, and F2s were not exposed to the four chemicals. Data from [22] were also included. In this work, silversides were exposed to two estrogenic EDCs—Bif and EE2 (1 ng/l for both chemicals), as adults for 14 days prior to spawning, and then reared for two subsequent generations, at ambient (22°C) and high temperatures (28°C). The F1 larvae were exposed until 21 days post hatch. Thus, in this experiment, the F0 and F1 generations were directly exposed, as adult fish and as larvae, respectively, and the F2 generation was only indirectly exposed. Responses to EDCs across both publications included changes in larval survival, larval size alteration, altered fecundity, reduced hatching success, and altered sex ratio. We also have included a summary of results in Table 1. More details on exposure conditions and results can be found in these publications.

Model Description

The premise of the population model is that the demographic rates (growth, survival, and reproduction) experienced by a fish at time t depend on whether that fish was exposed to toxicants earlier in life (direct exposure), whether its parents were exposed to toxicants prior to reproducing (when the focal fish would have been germinal tissue in the mother; indirect exposure), or whether

Table 1: Parameter values (mean $\pm$ standard deviation) used to characterize transgenerational effects of toxicants on demographic rates. Literature source is indicated

		Chemical		
	EE2	Bif	TB	LV
Mortality (proportional change)	F0: na	F0: 1.50 $\pm$ 0.27 [22]	F0: na	F0: na
	F1: 10.00 $\pm$ 0.82 [6, 22]	F1: 2.00 $\pm$ 1.40 [22]	F1: 3.00 $\pm$ 0.33 [6]	F1: na
	F2: na	F2: na	F2: na	F2: 2.5 $\pm$ 0.29 [6]
Larval survival (proportional change)	F0: na	F0: 1.15 $\pm$ 0.08 [6]	F0: na	F0: na
	F1: na	F1: na	F1: na	F1: na
	F2: 0.88 $\pm$ 0.20 [6, 22]	F2: na	F2: na	F2: 0.76 $\pm$ 0.08 [6]
Larval size (increase, mm)	F0: na	F0: na	F0: na	F0: na
	F1: 1.0 $\pm$ 0.1 [6]	F1: na	F1: 1.0 $\pm$ 0.1 [6]	F1: 1.0 $\pm$ 0.1 [6]
	F2: 0.5 $\pm$ 0.1 [6]	F2: na	F2: na	F2: na
Fecundity (proportional change)	F0: 0.44 $\pm$ 0.22 [6]	F0: 0.39 $\pm$ 0.64 [6]	F0: na	F0: na
	F1: 0.29 $\pm$ 0.83 [6]	F1: 0.38 $\pm$ 0.63 [6]	F1: 0.10 $\pm$ 5.00 [6]	F1: 0.43 $\pm$ 0.56 [6]
	F2: na	F2: na	F2: na	F2: na
Hatching success (proportional change)	F0: na	F0: na	F0: 0.85 $\pm$ 0.14 [6]	F0: na
	F1: 2.26 $\pm$ 0.09 [6]	F1: 2.31 $\pm$ 0.06 [6]	F1: 2.23 $\pm$ 0.13 [6]	F1: 2.31 $\pm$ 0.14 [6]
	F2: na	F2: na	F2: na	F2: na
Sex ratio (% reduction in proportion male, or reduction in temperature at 50:50 sex ratio, °C)	F0: 17% [6]	F0: na	F0: na	F0: na
	F1: 5°C [22]	F1: 2°C [22]	F1: na	F1: na
	F2: na	F2: na	F2: na	F2: na

its grandparents were exposed to toxicants before they had reproduced (epigenetic exposure). We refer to these generations as F2, F1, and F0, respectively. We focus our investigation mainly on exposures occurring during the embryonic and larval stages. This is because environmentally relevant concentrations of toxicants generally have their greatest effect on the embryonic stage; due to its rapid development [28], the rationale used by the studies that generated the experimental data we used to parameterize the model [6, 22, 23].

Tal *et al.* [29] point out that an offspring's phenotype depends on both the transmissibility of epigenetic markers (i.e. the probability of transmission of an epigenetic tag) and the effect of the epigenetic marker (if present) in that organism. Experimental quantification of epigenetic effects (such as by [6]) necessarily includes the net combined effect of both of those processes. Separate experiments would be needed to measure covariances in epigenetic expression among related individuals in order to explicitly estimate the transmissibility alone (as [29] suggests). Therefore, the magnitude of our modeled epigenetic effects implicitly combines both the transmissibility and the effect of a given epigenetic state.

The base model, independent of any transgenerational effects, is an integral projection model (IPM [30]). This is a size-structured integrodifference model, meaning that the population is represented as a continuous size distribution, with discrete time step. This is appropriate because in fish, many demographic rates are size-dependent, including growth, maturity, fecundity, and mortality due to predation. In some cases, demography depends on both size and age, so it is necessary to use a model that tracks both structuring dimensions [31, 32]. In that case, the abundance (or density) of fish of size x and age a at time t, $n(x, a, t)$ would depend on the density of fish of size y and age $a-1$ in the prior time step, $t-1$, and the probability density of transitioning from size y to size x as the fish ages from $a-1$ to a, $K(x, y, a-1)$. For ages $a>2$ in our model, this relationship is

$$n(x, a, t) = \int_\Omega K(x, y, a-1)\, n(y, a-1, t-1)\, dy \tag{1}$$

where Ω denotes that the integration is over all possible sizes x, representing the probability of fish of any size at age $a-1$ being size x one time step later. K is termed the kernel and can be both size- and age-dependent.

There is a separate equation governing the production of new age $a=1$ individuals that includes density-dependent survival of juvenile fish, $\phi[n(t)]$:

$$n(x, 1, t) = \phi\left[\sum_{a=2}^{A} \int_\Omega Q(x, y, a)\, n(y, a, t-1)\, dy\right] \tag{2}$$

where the kernel $Q(x, y, a)$ describes the size-dependent production of new offspring, and the production of each age cohort is summed. Density-dependent mortality followed the Beverton–Holt relationship,

$$\phi[S] = \frac{\alpha_1 S}{1 + \frac{\alpha_1 S}{\alpha_2}} \tag{3}$$

where α_1 is survival at low offspring densities, S is the total abundance of the offspring cohort (the term in brackets in Equation (2)), and α_2 is the maximum density of age-1 offspring. The latter is essentially an arbitrary scaling term for the purposes of our analysis. The value of α_1 was based on literature estimates for fish similar to silversides (as in [26]).

We expanded this model structure to include several additional structuring dimensions and delay coordinates in order to represent sex-specific transgenerational effects. We describe each in turn. All model parameters and literature sources for their values are given in Table 1.

Growth and Mortality

We assumed growth was indeterminate and asymptotic and follows a von Bertalanffy relationship. Accordingly, the mean change in growth during one time step, Δt, for a fish of size x is $\mu_x = (L_\infty - x) e^{-k\Delta t}$, with asymptotic maximum size L_∞ and instantaneous growth rate k. Variability in growth about that mean follows a normal distribution with coefficient of variation CV_L. Fish have

an instantaneous natural mortality rate M. Both processes can be altered by toxicant exposure, as we describe below in Transgenerational Effects of Toxicants, but in the base model the growth and mortality kernel giving the probability density of transition from size x to y during interval Δt have the structure

$$K(y,x,a) = N(y,\mu_x,CV_L\mu_x)\,e^{-M\Delta t}$$

where $N(x,\mu,\sigma)$ represents the probability density of a normal distribution with mean μ and standard deviation σ evaluated at x. Because inland silversides are short-lived and have within-year changes in sex ratio, we used a time step of $\Delta t = 2$ months.

Reproduction and Sex-specific Dynamics

The model explicitly tracks the abundance of male and female fish, adding the indexing dimension s ($s = m$ or f) to the state variable. Including sex is necessary because female reproductive success depends on the operational sex ratio at the time of spawning [26]. Maturity is size dependent, and the probability of maturity of a fish of size x and sex s, $p_m(x, s)$ follows a cumulative normal distribution with a sex-specific mean and standard deviation. Egg production by females is related to biomass, which we represented as a power function of length, with parameters γ and δ: γx^δ. We modeled the effect of the sex ratio on mating success as in [26], where the relationship between sex ratio X_t and fertilization success follows a cumulative beta distribution G with parameters β_1 and β_2. Finally, the size distribution of offspring follows a normal distribution with mean μ_ρ and standard deviation σ_ρ. Combining these factors we obtain the component of the kernel representing the probability density of a female of size x producing offspring of size y:

$$Q_t(y,x,a) = N(y,\mu_\rho,\sigma_\rho)\,G(2X_t,\beta_1,\beta_2)\,\gamma x^\delta$$

Note that evaluating the beta distribution G at $2X_t$ ensures maximum fertilization at a sex ratio of 0.5. Sex ratio was calculated at each time step as the total number of mature males in the population divided by the total number of mature males and females.

Sex determination in inland silversides is temperature-dependent, and this is represented in the model by setting the distribution of age-1 male and female individuals at the time of reproduction. The proportion of age-1 males follows a cumulative normal distribution on temperature, with mean μ_{temp} (the temperature at which the offspring sex ratio is 0.5) and standard deviation σ_{temp}. We modeled seasonal variation in temperature as a sine wave with a period of 1 year and a temperature range of 8–30°C, respectively, representing typically annual conditions in a temperate estuary.

Age-dependent Dynamics

Demographic rates in inland silversides are primarily size-dependent, but we also tracked age structure in the model, for two reasons. First, it facilitated tracking the seasonal shifts in offspring sex ratio described in the previous section. Second, it allowed the model to track different age cohorts in the model and assign different growth, mortality, and fecundity parameters if a given cohort had been exposed to a toxicant as juveniles. We tracked age classes up to 24 months, which is beyond the typical age of a fish in the wild.

Separately, we tracked the ages of the parents and grandparents contributing to each age-1 cohort, in order to determine whether that cohort should have life history traits reflecting transgenerational transmission following early-life exposure of

either or both of those two ancestral generations. Unfortunately, dimensional limitations on computer memory make it impractical to uniquely track all permutations of paternal, maternal, and grandparental ages contributing to a cohort. Therefore, because in many fish species the primary transmission of the methylome (and thus epigenetic effects) is via the father [33], we focused on tracking paternal transmission, thus expanding the state variable (and associated kernel) to be $N(x,\, a,\, a_p,\, a_{gp},\, t)$ with a_p and a_{gp} indexing paternal and paternal grandpaternal age, respectively.

However, evidence from medaka (*O. latipes*) suggests that epigenetic transmission from both parents could be important [33], particularly in atherinid species, which include both medaka and silversides. Because we could not separately index parental and grandparental ages on the maternal side, we compromised by estimating the age distribution of the mature mothers contributing to each age-1 cohort. Specifically, for the age-1 offspring cohort in a given time step, we calculated the distribution of both maternal ages present in the population at that time (i.e. the proportion of 8-month-old, 10-month-old, etc. mothers). We calculated the maternal grandparent age distribution by weighting the distribution of grandparental ages contributing to each maternal cohort by the relative abundance of that maternal cohort. Then, for a past exposure τ years in the past, we calculated the proportion of reproductive mothers that age τ at time t, and scale the effect on the age-1 cohort by that proportion. For example, if 40% of the possible mothers of a given offspring cohort were exposed to a chemical, then the indirect effect of that exposure on the offspring cohort was set to be 40% of the empirically measured effect, effectively representing the probability that the average member of the cohort would express epigenetic effects (note that this works because all of the effects we simulate are linear rather than nonlinear dose–responses; the latter would require a more sophisticated approach). The effects of maternal grandparents were similar, but depended on the age of the grandmother generation at the times when they spawned the different cohorts in the maternal generation, rather than at time t. Note that in this formulation it is possible for one age class to contribute to the age-1 cohort F2 as both parent (F1) and grandparent (F0), although this is likely to be rare given the short lifespan of the fish.

Transgenerational Effects of Toxicants

Transgenerational effects of toxicants were represented as those that were detected in [6] and [22]. The four compounds examined were EE2, TB, Bif, and LV. We organize our explanations of these effects by demographic rate below. In each case, changes to baseline model parameters are given as proportional increases or decreases, based on the observed statistically significant effects relative to control treatments in those two studies. For the results from [22], we only used values from the "present day" 22°C treatments rather than the warming treatments. The proportions and the uncertainty bounds on them are given in Table 1. In all model simulations we assume that exposures are at the same ecologically relevant nominal concentrations as those used in [6] and [22]: Bif (5 ng/l), EE2 (5 ng/l), TB (10 ng/l), and LV (10 ng/l).

Survival

The experiments in [6, 22] used sublethal exposure concentrations, so mortality was not a direct endpoint. However, they detected increases in the rate of larval deformities (craniofacial,

Table 2: Parameter values used in the model

Parameter	Description	Value	Source
α_1	Slope of Beverton–Holt relationship	0.31	Mean of values reported by [86] for ecologically similar species, as in [26]
L_∞	Asymptotic maximum length	13 cm	[87], for female *Menidia menidia* in a Massachusetts estuary
k	von Bertalanffy growth rate	1.39 y^{-1}	[87], for female *M. menidia* in a Massachusetts estuary
M	Adult mortality rate	2.10 y^{-1}	[87], for female *M. menidia* in a Massachusetts estuary
γ	Biomass-length coefficient	1 g cm^{-3}	[26]
δ	Biomass-length exponent	3.0	[26]
μ_p	Mean offspring size	9.0 mm	S.M. Brander, unpublished data
σ_p	Std. dev. of offspring size	1.0 mm	S.M. Brander, unpublished data
β_1	Mating function parameter	1.0	[26] for laboratory-reared *M. beryllina*
β_2	Mating function parameter	1.3	[26] for laboratory-reared *M. beryllina*
μ_{temp}	Temperature at 1:1 offspring sex ratio	25°C	[22] for laboratory-reared *M. beryllina*
σ_{temp}	Std. dev. of temperature sex determination function	8°C	[22] for laboratory-reared *M. beryllina*

cardiovascular, and skeletal), which would be expected to result in heightened mortality risk in the field because of impaired locomotion and thus impaired predator evasion [34, 35]. Therefore, we translated the proportional increase in the proportion of deformed fish in exposure treatments to a proportional increase in the mortality rate M. Both EE2 and Bif caused increased deformities as a direct effect in the F0 generation ([22]; Table 2), EE2 and TB caused increased deformities as an indirect effect in the F1 generation, and LV produced increased deformities in the F2 generation ([6]; Table 1). Additionally, exposure to TB reduced survival of exposed larvae in the F0 generation, which we modeled as a proportionate reduction in the abundance of exposed cohorts.

Larval Size

DeCourten *et al.* [6] detected a positive effect of EE2, TB, and LV on larval size in the F0 generation, as well as a positive effect of EE2 in the F2 generation. This was represented by adjusting the mean size of new offspring entering the population, μ_ρ. Because maturity is size-dependent, those fish would mature faster and contribute more to reproduction later in life.

Reproduction

Multiple experimental endpoints measured in [6] translate into aspects of reproductive success in the model context. In mature F0 females, EE2 and Bif increased the incidence of ovarian atresia, which we modeled as a proportional reduction in fecundity of those fish by reducing the proportionality constant for fecundity, γ; EE2 also reduced the overall egg production. However, all four compounds had a positive effect on the hatching success of F0 progeny, which we also modeled as proportional (upward) adjustments to γ. Conversely, all four compounds had negative effects on egg production in F1 females, modeled as reductions in γ. Finally, both EE2 and LV had negative effects on larval survival of F1 progeny, which we also modeled as a proportional reduction in the γ parameter.

Sex Ratio

We set the parameter μ_{temp}, which determines the temperature at which sex ratio is 0.5, such that sex ratios at 22°C and 28°C would match the results observed in control treatments in [22], which was 25°C. We then modeled the reductions in male sex ratio they observed by adjusting the μ_{temp} parameter downward to produce the observed changes in sex ratio with Bif and EE2 exposure. These changes were applied to the F1 generation. Separately we

also modeled the 17% reduction in the probability of being female that [6] observed in F0 fish exposed to EE2.

Model Analysis

We quantified the expected population effects of each toxicant by simulating the dynamics of inland silverside populations in which new offspring experience a pulsed exposure to one of the four toxicants in early life, just as in the experiments used to parameterize the model (and assuming the same concentrations of each [6, 22]). We compared the resulting trajectory of population density relative to that expected for an unexposed population. We also compared the effects of an "acute" exposure of only one generation of offspring in a single model "summer" (the three 2-month time steps corresponding to the warm months in a single year) to a "chronic" exposure in which each generation of offspring are exposed in every time step. We examined each toxicant's effects separately. For each one we isolated the relative types of direct versus transgenerational effects by conducting simulations in which we "turned off" different combinations of effects. Specifically, we simulated (i) direct effects on the exposed F0 generation only; (ii) effects on F0 and their F1 offspring only; and (iii) effects on the F0, F1, and F2 generations.

All simulations were initialized at the stable age distribution and at a steady state and then iterated for 72 time steps (12 years) to build up a history of parental and grandparental generations in the state variable N prior to simulating either acute or chronic exposures. We then simulated dynamics for 24 time steps (4 years) after the initial exposure, which was sufficient time for transgenerational effects to completely propagate through the population in the acute exposure scenarios. In order to represent uncertainty in exposure effects, we performed 1000 simulations for each scenario, and in each simulation the parameters describing toxicant effects were drawn from the distributions given in Table 1. Simulations were performed using Matlab R2020b (9.9.0.1524771, Mathworks, Natick, MA).
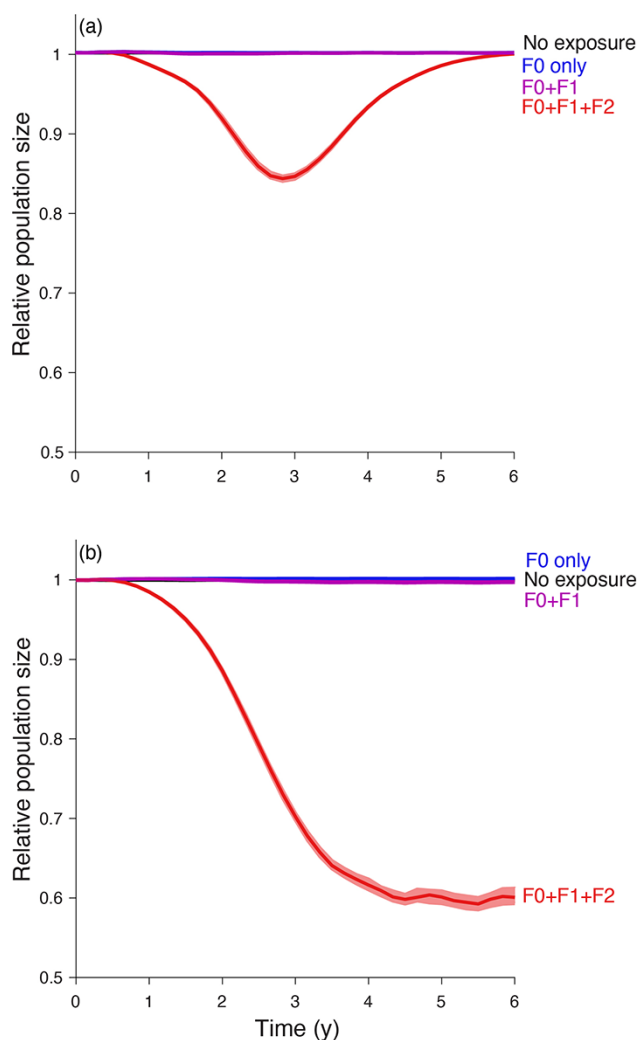
Results

In all model scenarios, the baseline no-exposure simulations exhibited a slight annual oscillation in total population abundance. This was a realistic effect of temperature on the sex ratio; the offspring sex ratio becomes female-biased in cold temperatures, leading to a female-biased sex ratio and slightly higher total population reproductive output when those offspring mature ~6 months later. The oscillation is dampened by density-dependent mortality in the no-exposure simulations, but

when population densities are reduced by toxicant effects, the oscillations become more pronounced. We consider the predicted population effects of each toxicant in turn; in each case we express population abundance relative to the no-exposure case.

Levonorgestrel

The simulated acute exposure to LV, a synthetic progestin used in birth control pills, produced the greatest difference in predictions that did and did not include transgenerational effects (Fig. 2a). The acute exposure led to a small increase in relative population abundance 1 year after the exposure due to the increased hatching success of F0 progeny. That bump in population size then diminished due to natural mortality of the short-lived fish, with the F0-only model returning to baseline conditions by the second year.

Figure 2: Model results showing changes in predicted relative population size for inland silversides exposed in the larval stage to 10 ng/l LV in (a) a single spawning season, starting at time = 0, or (b) chronically for every time step. Population abundance is expressed relative to abundance at $t = 0$. The curve labeled 'No exposure' is a deterministic simulation with no chemical exposure, as a reference. The other curves are labeled to indicate the model structure in each set of simulations: direct effects on the exposed F0 generation only, effects on both F0 and F1 generations, and full transgenerational effects on F0, F1, and F2 generations. Shading around each curve represents the range of model runs that include observed variability in exposure effects; the shading contains 95% of simulated trajectories

The model that also included F1 effects declined slightly faster due to the reduction in F1 egg production and survival of larval progeny of the F1 fish, but the difference was very small. However, when the transgenerational F2 effects of reduced larval survival were included, the simulated population declined to nearly 85% of its original size during the second and third years after exposure, as the F2 generation was smaller and then consequently spawned fewer total F3 offspring. Only by the fourth year after exposure was the population returning toward initial conditions (Fig. 2a).

Similar trends held in the chronic exposure scenario (Fig. 2b). Simulations that only included F0 effects reached new, slightly higher steady-state abundances due to the positive effect of hatching success of F0 progeny. Including both F0 and F1 effects led to steady-state abundances slightly lower than the baseline conditions. However, the simulation that also included F2 effects experienced the same initial increase but once the first F2 generation was spawned, the population began to decline to nearly 60% of its original abundance due to the ongoing effects of low larval survival in F2 cohorts.

Bifenthrin

In all transgenerational scenarios, simulated acute exposure to the pesticide Bif produced an immediate decline of relative population abundance to ∼80% of the baseline within 1 year of exposure (Fig. 3a). This decline was due to the combined effects of reduced larval survival in the exposed F0 larvae and later reductions in F0 fecundity, but buffered somewhat by a female-biased F0 sex ratio and higher hatching success of F0 progeny. The population then began recovery and returned to its pre-exposure state within 3 years. The recovery was somewhat slower in the simulations that included F1 effects because of reduced hatching success in F1 progeny. Bif did not induce any additional F2 effects, so the simulations that included those effects were identical to the F0 + F1 simulations. Similar trends held in the chronic exposure scenario (Fig. 3b). Simulations that included F0 effects reached a new steady state ∼20% lower than initial conditions, while simulations that also included F1 (or F1 + F2) effects were projected to have a new steady state >25% lower than initial conditions.

Trenbolone

Simulated exposures to TB followed a pattern similar in many ways as that for LV. In the acute exposure scenario, all exposed simulations had an initial increase in relative population size due to the positive effect of TB on hatching success of F0 progeny, despite the reduced survival of F0 larvae (Fig. 4a). In the simulations with only F0 effects, the population then returned to baseline conditions within 2 years of exposure. Simulations that included F1 effects declined sharply after the F0 cohort began reproducing, due to reduced egg production and larval mortality in the F1 generation. The population reached a maximum decline of nearly 15% in the second year before recovering within nearly 4 years of exposure. As with Bif, there were no additional F2 effects so the F0 + F1 + F2 simulations were identical to F0 + F1. Similar trends held in the chronic exposure scenario (Fig. 4b). As with levothyroxine, simulations that included only F0 effects reached a new steady state essentially identical to initial conditions, while simulations that also included F1 (or F2) effects declined to a new steady state less than half of the initial abundance due to the ongoing reductions in egg production.
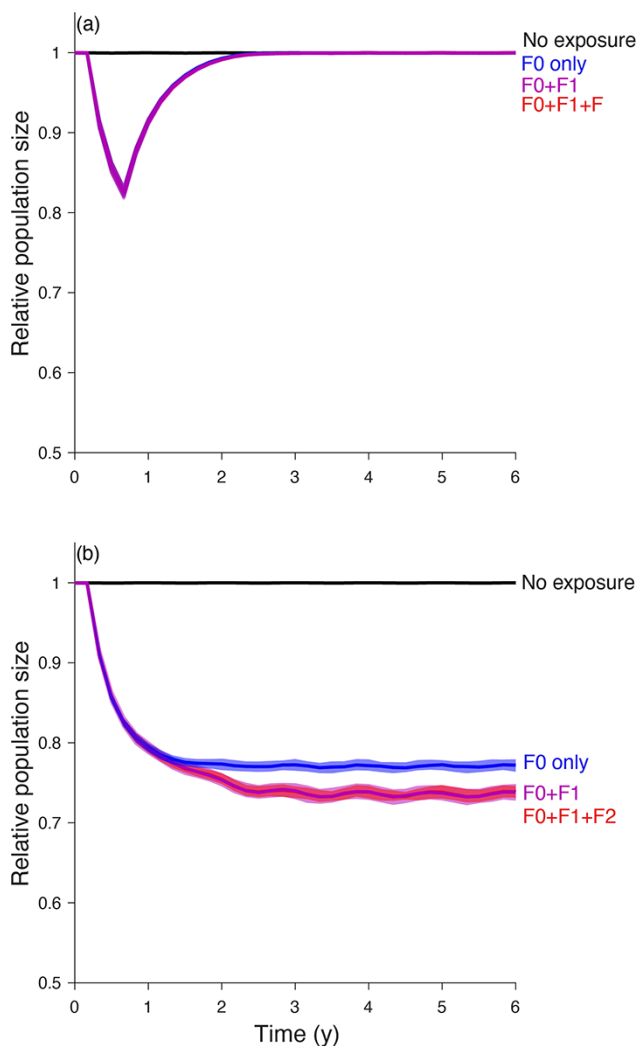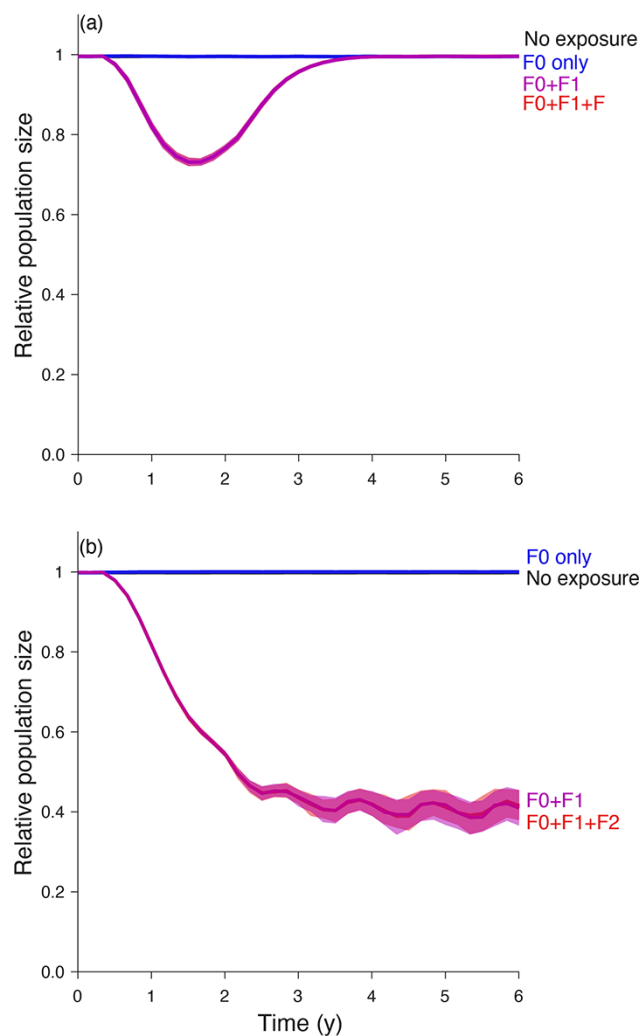
Figure 3: Model results showing changes in predicted relative population size for inland silversides exposed in the larval stage to 5 ng/l Bif in (a) a single spawning season, starting at time = 0, or (b) chronically for every time step. Population abundance is expressed relative to abundance at t = 0. The black curve labeled 'No exposure' is a deterministic simulation with no chemical exposure, as a reference. The other curves are labeled to indicate the model structure in each set of simulations: direct effects on the exposed F0 generation only, effects on both F0 and F1 generations, and full transgenerational effects on F0, F1, and F2 generations. Shading around each curve represents the range of model runs that include observed variability in exposure effects; the shading contains 95% of simulated trajectories

Figure 4: Model results showing changes in predicted relative population size for inland silversides exposed in the larval stage to 10 ng/l TB in (a) a single spawning season, starting at time = 0, or (b) chronically for every time step. Population abundance is expressed relative to abundance at t = 0. The black curve labeled 'No exposure' is a deterministic simulation with no chemical exposure, as a reference. The other curves are labeled to indicate the model structure in each set of simulations: direct effects on the exposed F0 generation only, effects on both F0 and F1 generations, and full transgenerational effects on F0, F1, and F2 generations. Shading around each curve represents the range of model runs that include observed variability in exposure effects; the shading contains 95% of simulated trajectories

Ethinylestradiol

The results of both simulated acute and chronic exposure to EE2 mirrored those of TB (Fig. 5a and b). This reflects the similar positive effects on hatch success in F0 progeny (buffered by reduced survival of F1 larvae), followed by reduced egg production in F1 females and higher larval mortality in the F1 generation. EE2 also had a small positive effect on larval size in the F2 generation but this did not appear to affect population dynamics substantively.

Discussion

EDCs can exert adverse but often subtle or difficult to detect effects in fishes that are relevant to the population level at extremely low concentrations [3, 6, 36, 37]. They are known to occur at sublethally toxic levels in estuaries and are demonstrated

to induce alterations in sex ratio, fecundity, and thus population size across species [2, 21, 38, 39]. Although EDCs have now been studied for decades, the introduction of hundreds of new chemicals each year, many of which are understudied but likely also interfere with endocrine function, compounds an already formidable challenge [40, 41]. This is especially true considering that climate change can exacerbate the impacts of EDCs [23]. Here we have shown that direct and transgenerational effects of low, environmentally relevant exposure to several EDCs could cause dramatic reductions in fish population abundance. Additionally, failure to account for the transgenerational effect could lead to a severe underestimate of those negative consequences.

We chose to focus our study on exposure to single chemicals, modeling each EDC alone rather than considering the complexities of simulating mixture exposures. This is a common
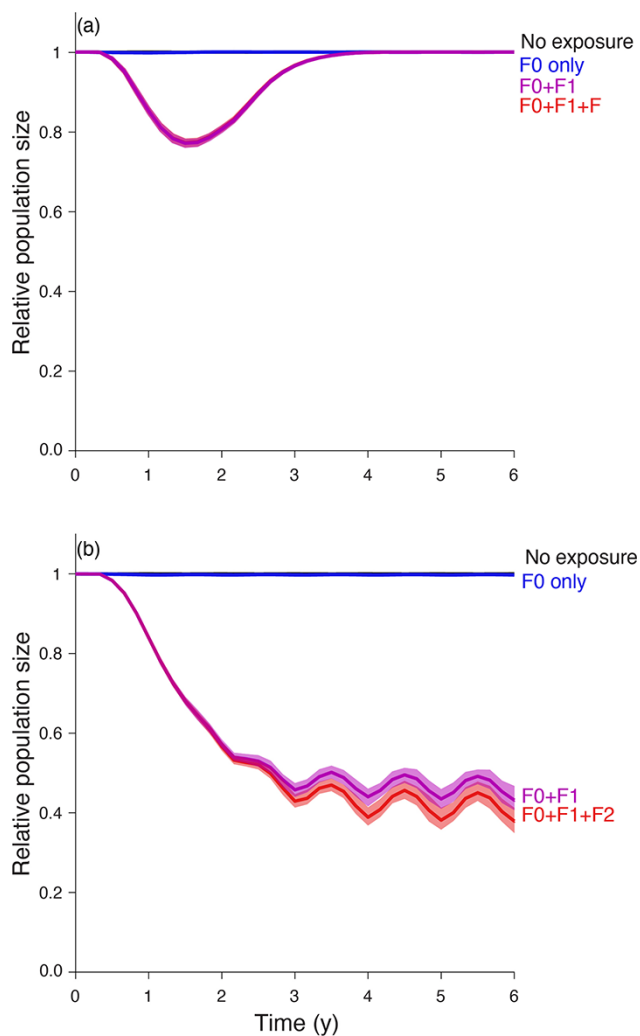
Figure 5: Model results showing changes in predicted relative population size for inland silversides exposed in the larval stage to 5 ng/l EE2 in (a) a single spawning season, starting at time = 0, or (b) chronically for every time step. Population abundance is expressed relative to abundance at t = 0. The black curve labeled 'No exposure' is a deterministic simulation with no chemical exposure, as a reference. The other curves are labeled to indicate the model structure in each set of simulations: direct effects on the exposed F0 generation only, effects on both F0 and F1 generations, and full transgenerational effects on F0, F1, and F2 generations. Shading around each curve represents the range of model runs that include observed variability in exposure effects; the shading contains 95% of simulated trajectories

approach when analyzing ecotoxicological population models (e.g. [24, 25, 42]), despite mixtures being commonplace in nature, particularly in estuaries. There are examples of theoretical studies that examine mixture exposures, but this requires assumptions about the potential for synergistic or antagonistic interactions among the compounds in question. For example, Schipper *et al.* [43] modeled the effects of combined DDE and PDBE exposure on peregrine falcon (*Falco peregrinus*) populations and simply assumed that the effects of the two compounds were additive based on two single-chemical dose–response curves. In their case they were able to validate that assumption because they could compare the model output to a long-term record of both population abundance and approximate exposure. In the case of epigenetic exposures, we suspect there is greater potential for nonlinear, nonadditive

interactions among exposed chemicals because they act via different mechanisms and thus responses can be non-monotonic, and thus synergistic, or more rarely antagonistic depending on concentration (e.g. [44–46]), so were conservative in only examining single-exposure outcomes.

To our knowledge, this is the first example of a population model that attempts to represent epigenetic, transgenerational effects of toxicant exposures. The modeling approach we took, using time-lagged state variables to represent the past effects of parental exposures on current demographic rates, has been used in a more limited form in past studies. The most common application is in stage-structured models of freshwater crustaceans in which development time depends on environmental conditions (e.g. water temperature) and thus varies through the year (e.g. delay differential equation models by [47, 48]). More recently, Amarasekare and Coutinho [49] used this approach to model the effects of climate warming on development times and population dynamics of invertebrates and reptiles. Our approach is novel in also extending backward in time to the grandparental generation, although as we mentioned this effort quickly encounters the curse of dimensionality because there are so many unique possible combinations of parental and grandparental ages. That issue could be relaxed somewhat if one simply assumes that there is a constant exposure concentration across all times and ages. A completely alternative modeling approach that could be attempted is an individual-based model [50] that tracks every individual in a population, along with the specific ancestry and exposure history of that individual, as opposed to the distribution of individuals with the same history (as we did). This approach presents its own dimensionality and computational issues because of the large number of individuals one would need to track and account for, but is a promising approach [51].

Small oscillations in population abundance are common in fishes, particularly in short-lived species with temperature-sensitive sex determination, such as the inland silverside ([22, 52, 53]; described above in Results). Those high-frequency fluctuations were apparent in our model results, but were small in comparison to the multiyear, multigeneration fluctuations induced by simulated exposure to EDCs. The model results indicate that exposure to each of the EDCs of portend population decline to varying extents and across different timescales. We describe the specific results for each individual chemical in turn below.

Levonorgestrel

LV is specifically synthesized to interact with the progesterone receptor, as its intended purpose is to control monthly fluctuations in hormone production associated with ovulation in human females. It is commonly detected in the treated effluent discharged from secondary and tertiary wastewater treatment outfalls [54] and is demonstrated to disrupt sexual development in fishes. In several fish species it acts as an androgen, causing the development of masculine secondary sexual characteristics and limiting fecundity [38, 55–57]. In silversides, it did not produce responses clearly indicative of an androgenic EDC; however, exposure levels were in some cases an order of magnitude lower, or more, than those used in comparable exposures with other species, thus the responses reported here to a low ng/l concentration may be more indicative of those occurring in the environment. For LV, considerable negative impacts on silversides would not have been detected if empirical data and associated model predictions were not carried through the F2 generation, and thus the simulated acute exposure to LV produced the greatest difference in predictions between models that did versus those that did

not include transgenerational effects. This is particularly important because the net effect of low LV exposure in the first generation was slightly positive and because increased deformities and reduced egg production were not observed until the F1 and F2 generations. This highlights how current regulatory approaches that are heavily reliant on acute testing, especially those such as the Environmental Protection Agency (EPA) National Pollutant Discharge Elimination System [58] (NPDES), which determine the safety of treated wastewater effluent discharged to waterbodies in the USA, are likely missing the latent effects that low concentrations of endocrine-active compounds can have.

Mifenthrin

Bif, which is a commonly used insecticide and a member of the third most popular class of pesticides used globally (pyrethroids), is not designed to specifically interact with hormone receptors [27, 36]. However, a number of studies demonstrate that Bif can act as both an estrogen receptor (ER) agonist and antagonist in fishes and *in vitro*, depending upon metabolic state and concentration [36, 59–61]. The mechanisms involved are complex and appear to be dependent on metabolism to a more estrogenic metabolite, 4-OH Bif, in some fish species—including silversides [59, 62]. This can make Bif's mechanism of action in terms of endocrine disruption unpredictable and different from synthetic estrogens specifically designed to interact with ER, with lower concentrations often eliciting a larger estrogenic response than higher. This is possibly because the parent compound, which can act as an ER antagonist [59], is competing with a metabolite that likely acts as an ER agonist [60, 63]. In all transgenerational scenarios described in the modeling simulations above, acute exposure to Bif caused a pronounced and immediate decline in population size, within just 1 year of exposure, likely due to its endocrine disrupting propensity [64]. This is evidenced by the apical endpoints measured by DeCourten and colleagues [6, 22], which produced the data used to parameterize these models, demonstrating an effect of Bif on development and sex ratios following early-life exposure. Repeated exposures over time, which more closely mimic exposure scenarios in the wild, create an additive impact, potentially driving the population to eventual extinction. This is notable considering that additional studies using silversides and other fish species have observed similar impacts on fecundity and the underlying endocrine pathways involved in sexual determination empirically [23, 61, 65, 66] and that the concentrations used herein are regularly measured nationwide [67–69]. Given that other pyrethroid pesticides in addition to Bif are also known to cause endocrine disruption, concern is warranted over the continued use of pyrethroids for pest control, particularly for urban applications such as mosquito, termite, and ant abatement that are unregulated [36, 70, 71].

Trenbolone

TB is commonly used in livestock on feedlots and enters the aquatic environment through agricultural runoff. Metabolites of TB are frequently found in runoff and thus in waterways and sediments near agricultural activity and cause toxic effects in aquatic vertebrate species [72, 73]. TB metabolites act as endocrine disruptors and affect androgen receptor signaling and have been found to alter genes involved in hormone signaling and enzymes facilitating steroid synthesis [6, 74]. Masculinization of female fish exposed to TB has been documented in fishes; however, the potential effects on population stability have not been as well studied [75, 76]. In this study, simulated exposures to TB followed a pattern similar in many ways as that for LV. In the acute exposure scenario,

all exposed simulations had an initial increase in relative population size due to the positive effect of TB on hatching success of F0 progeny, despite the reduced survival of F0 larvae. Chronic exposure scenarios resulted in eventual extinction of the population caused by reduced egg production, which is consistent with the androgenic effect of TB in fishes. Similar to LV, current regulatory frameworks do not consider the potential for latent effects that clearly have an impact on fitness-relevant endpoints that dictate population persistence.

Ethinylestradiol

EE2, one of the active ingredients in the birth control pill, is one of the most studied pharmaceuticals in fish [77]. EE2 enters the aquatic environment through untreated wastewater effluent, similar to LV [78]. EE2 is a well-studied endocrine disruptor that has been shown to skew sex ratios, reduce egg production, and alter gene expression in exposed fishes [6, 79, 80]. The results of both simulated acute and chronic exposure to EE2 mirrored those of TB (Fig. 5a and b). This reflects the similar positive effects on hatch success in F0 progeny (buffered by reduced survival of F1 larvae), followed by reduced egg production in F1 females and higher larval mortality in the F1 generation. Our findings support previous studies that found deleterious population-level effects in fishes following *in situ*, mesocosm and laboratory exposures to EE2 [3, 81, 82]. These studies suggest that fish populations can be susceptible to localized extinction following EE2 exposure. These risks are important to consider when building regulatory framework and management strategies as EE2 is detected in surface waters worldwide [83].

Conclusion

The end result of exposure to what may amount to hundreds of small, brief encounters with EDCs during the short lifespan of fishes such as inland silversides can only realistically be described by using a combination of empirical and modeling approaches, as we have done here. These exposures are complex and dependent on life stage, weather patterns, reliant on changing land use, as well as the evolution of chemical usage. For commonly used pharmaceuticals such as LV and EE2, both common components of treated wastewater effluent, low concentrations can elicit latent effects that go undetected unless multiple generations are assessed. This is also true of TB, used globally to accelerate growth in livestock, which like other synthetic hormones tested and modeled in our work elicits impacts that escape the bounds of current regulatory toxicity frameworks, most of which only require exposures that last several days. Potentially even more concerning are the effects triggered by the pyrethroid pesticide Bif, which is only regulated for agricultural use, even though much of its presence in aquatic ecosystems is a result of residential use and subsequent runoff. Bif is only one of a large class of pesticides that disrupt endocrine function and fitness-relevant endpoints via a variety of different mechanisms, and thus the effects seen following exposure may be similar to those elicited by other pyrethroids, and in the wild are exacerbated by complex mixtures of compounds like these, and others, as well as climate change. While phenomena such as epigenetic buffering, in which epigenetic modifications can increase phenotypic resilience in the face of environmental change [84], should be taken into consideration when considering how DNA methylation may influence populations over ecological timeframes, this demonstration of the elevated risk of local extinction from exposure to environmentally detected levels of EDCs is alarming. Future empirical work on transgenerational inheritance in fishes should take complexities such as buffering and

heritability into account and may need to be run across additional generations using differing exposure lengths in order to account for potential recovery of impacted phenotypes or epigenetic washout [30, 84, 85]. However, at a minimum our results call for strengthening of regulatory frameworks and management strategies used globally to limit the entry of EDCs into aquatic ecosystems.

Data Availability

All data used in this study and all model code is available at GitHub, DOI 10.5281/zenodo.6229314.

Conflict of interest statement. None declared.

Author Contributions

S.M.B., J.W.W., R.E.C., and A.M. designed the study and obtained funding; S.M.B., B.M.D., and K.M. contributed data used in the model; J.W.W. performed model analysis; S.M.B., J.W.W., B.M.D., and S.J.H. wrote the manuscript with input from R.E.C. and A.M.

References

1. Brander S, Hecht S, Kuivila K. The challenge: "bridging the gap" with fish: advances in assessing exposure and effects across biological scales. *Environ Toxicol Chem* 2015;**34**:459.
2. Connon RE, Hasenbein S, Brander SM *et al.* Review of and recommendations for monitoring contaminants and their effects in the San Francisco Bay–Delta. *San Franc Estuary Watershed Sci* 2019;**17**:1–42.
3. Kidd KA, Blanchfield PJ, Mills KH *et al.* Collapse of a fish population after exposure to a synthetic estrogen. *Pnas* 2007;**104**:8897–901.
4. Vandegehuchte MB, Janssen CR. Epigenetics and its implications for ecotoxicology. *Ecotoxicology* 2011;**20**:607–24.
5. Brander SM, Biales AD, Connon RE. The role of epigenomics in aquatic toxicology. *Environ Toxicol Chem* 2017;**36**:2565–73.
6. DeCourten BM, Forbes JP, Roark HK *et al.* Multigenerational and transgenerational effects of environmentally relevant concentrations of endocrine disruptors in an estuarine fish model. *Environ Sci Tech* 2020;**54**:13849–60.
7. Major KM, DeCourten BM, Li J *et al.* Early life exposure to environmentally relevant levels of endocrine disruptors drive multigenerational and transgenerational epigenetic changes in a fish model. *Front Marine Sci* 2020;**7**:471.
8. Head JA. Patterns of DNA methylation in animals: an ecotoxicological perspective. *Integ Comp Biol* 2014;**54**:77–86.
9. Skinner MK. Environmental epigenetics and a unified theory of the molecular aspects of evolution: a neo-Lamarckian concept that facilitates neo-Darwinian evolution. *Genome Biol Evol* 2015;**7**:1296–302.
10. Best C, Ikert H, Kostyniuk DJ *et al.* Epigenetics in teleost fish: from molecular mechanisms to physiological phenotypes. *Comp Biochem B* 2018;**224**:210–44.
11. Crotti M, Yohannes E, Winfield IJ *et al.* Rapid adaptation through genomic and epigenomic responses following translocations in an endangered salmonid. *Evol Appl* 2021;**14**:2470–89.
12. Bhandari RK. Medaka as a model for studying environmentally induced epigenetic transgenerational inheritance of phenotypes. *Environ Epigen* 2016;**2**:1–9.
13. Nilsson EE, Sadler-Riggleman I, Skinner MK. Environmentally induced epigenetic transgenerational inheritance of disease. *Environ Epigen* 2018;**4**:dvy016.
14. Hutton SJ, Brander SM. Epigenetics in aquaculture. In: Frances P. and Hanping W. *Epigenetics in Aquatic Toxicology*. New York: Wiley, 2023.
15. McGaughey DM, Abaan HO, Miller RM *et al.* Genomics of CpG methylation in developing and developed zebrafish. *G3–Genes Genom Genet* 2014;**4**:861–9.
16. Skinner MK, Manikkam M, Guerrero-Bosagna C. Epigenetic transgenerational actions of endocrine disruptors. *Reprod Toxicol* 2011;**31**:337–43.
17. Bogdanović O, Veenstra GJC. DNA methylation and methyl-CpG binding proteins: developmental requirements and function. *Chromosoma* 2009;**118**:549–65.
18. Bhandari RK, Vom Saal FS, Tillitt DE. Transgenerational effects from early developmental exposures to bisphenol A or 17α-ethinylestradiol in medaka, *Oryzias latipes. Sci Rep* 2015;**5**:9303.
19. Wang X, Bhandari RK. The dynamics of DNA methylation during epigenetic reprogramming of primordial germ cells in medaka (*Oryzias latipes*). *Epigenetics* 2020;**15**:483–98.
20. Moyle PB. Fish introductions in California: history and impact on native fishes. *Biol Cons* 1976;**9**:101–18.
21. Brander SM, Connon RE, He G *et al.* From 'omics to otoliths: responses of an estuarine fish to endocrine disrupting compounds across biological scales. *PLoS One* 2013;**8**:e74251.
22. DeCourten BM, Brander SM. Combined effects of increased temperature and endocrine disrupting pollutants on sex determination, survival, and development across generations. *Sci Rep* 2017;**7**:9310.
23. DeCourten BM, Connon RE, Brander SM. Direct and indirect parental exposure to endocrine disruptors and elevated temperature influences gene expression across generations in a euryhaline model fish. *PeerJ* 2019;**7**:e6156.
24. Gurney WS. Modeling the demographic effects of endocrine disruptors. *Environ Health Persp* 2006;**114**:122–6.
25. Hazlerigg CR, Tyler CR, Lorenzen K *et al.* Population relevance of toxicant mediated changes in sex ratio in fish: an assessment using an individual-based zebrafish (*Danio rerio*) model. *Ecol Model* 2014;**280**:76–88.
26. White JW, Cole BJ, Cherr GN *et al.* Scaling up endocrine disruption effects from individuals to populations: outcomes depend on how many males a population needs. *Environ Sci Technol* 2017;**51**:1802–10.
27. Hutton SJ, St Romain SJ, Pedersen EI *et al.* Salinity alters toxicity of commonly used pesticides in a model euryhaline fish species (*Menidia beryllina*). *Toxics* 2021;**9**:114.
28. Villeneuve D, Volz DC, Embry MR *et al.* Investigating alternatives to the fish early-life stage test: a strategy for discovering and annotating adverse outcome pathways for early fish development. *Environ Toxicol Chem* 2014;**33**:158–69.
29. Tal O, Kisdi E, Jablonka E. Epigenetic contribution to covariance between relatives. *Genetics* 2010;**184**:1037–50.
30. Ellner SP, Childs DZ, Rees M. *Data-driven Modelling of Structured Populations*. Switzerland: Springer, 2016.
31. Chu C, Adler PB, de Kroon H. When should plant population models include age structure? *J Ecol* 2014;**102**:531–43.

32. Botsford LW, White JW, Hastings A. *Population Dynamics for Conservation*. Oxford: Oxford University Press, 2019.

33. Wang X, Bhandari RK. DNA methylation dynamics during epigenetic reprogramming of medaka embryo. *Epigenetics* 2019;**14**:611–22.

34. Jin M, Zhang X, Wang L *et al*. Developmental toxicity of bifenthrin in embryo-larval stages of zebrafish. *Aquatic Toxicol* 2009;**95**:347–54.

35. Linares-Casenave J, Werner I, Van Eenennaam JP *et al*. Temperature stress induces notochord abnormalities and heat shock proteins expression in larval green sturgeon (*Acipenser medirostris* Ayres 1854). *J Applied Ichthyol* 2019;**29**:958–67.

36. Brander SM, Gabler MK, Fowler NL *et al*. Pyrethroid pesticides as endocrine disruptors: molecular mechanisms in vertebrates with a focus on fishes. *Environ Sci Tech* 2016;**50**:8977–92.

37. Overturf MD, Overturf CL, Carty DR *et al*. Levonorgestrel exposure to fathead minnows (*Pimephales promelas*) alters survival, growth, steroidogenic gene expression and hormone production. *Aquatic Toxicol* 2014;**148**:152–61.

38. Kirby MF, Allen YT, Dyer RA *et al*. Surveys of plasma vitellogenin and intersex in male flounder (*Platichthys flesus*) as measures of endocrine disruption by estrogenic contamination in United Kingdom estuaries: temporal trends, 1996 to 2001. *Environ Toxicol Chem* 2004;**23**:748–58.

39. Blazer VS, Gordon S, Jones DK *et al*. Retrospective analysis of estrogenic endocrine disruption and land-use influences in the Chesapeake Bay watershed. *Chemosphere* 2021;**266**:129009.

40. Carnevali O, Santangeli S, Forner-Piquer I *et al*. Endocrine-disrupting chemicals in aquatic environment: what are the risks for fish gametes? *Fish Physiol Biochem* 2018;**44**:1561–76.

41. Kristiansson E, Coria J, Gunnarsson L *et al*. Does the scientific knowledge reflect the chemical diversity of environmental pollution? – a twenty-year perspective. *Environ Sci Pol* 2021;**126**:90–8.

42. Miller DH, Jensen KM, Villeneuve DL *et al*. Linkage of biochemical responses to population-level effects: a case study with vitellogenin in the fathead minnow (*Pimephales promelas*). *Environ Toxicol Chem* 2007;**26**:521–7.

43. Schipper AM, Hendriks HW, Kauffman MJ *et al*. Modelling interactions of toxicants and density dependence in wildlife populations. *J Appl Ecol* 2013;**50**:1469–78.

44. Welshons WV, Thayer KA, Judy BM *et al*. Large effects from small exposures. I. Mechanisms for endocrine-disrupting chemicals with estrogenic activity. *Environ Health Perspect* 2003;**111**:994–1006.

45. Vandenberg LN, Colborn T, Hayes TB *et al*. Hormones and endocrine-disrupting chemicals: low-dose effects and nonmonotonic dose responses. *Endocr Rev* 2012;**33**:378–455.

46. Brander SM. Thinking outside the box: assessing endocrine disruption in aquatic life. In: Ahuja S (ed.), *Monitoring Water Quality: Pollution Assessment, Analysis, and Remediation*. Amsterdam: Elsevier, 2013, 103–47.

47. Nisbet RM. Delay-differential equations for structured populations. In: Tuljapurkar S, Caswell H (eds), *Structured-Population Models in Marine, Terrestrial, and Freshwater Systems*. New York: Chapman & Hall, 1997, 89–118.

48. de Roos AM. A gentle introduction to physiologically structured population models. In: Tuljapurkar S, Caswell H (eds), *Structured-Population Models in Marine, Terrestrial, and Fresh-Water Systems*. New York: Chapman & Hall, 1997, 199–204.

49. Amarasekare P, Coutinho RM. The intrinsic growth rate as a predictor of population viability under climate warming. *J Animal Ecol* 2013;**82**:1240–53.

50. Grimm V, Railsback SF. *Individual Based Modeling and Ecology*. Princeton: Princeton University Press, 2005.

51. Jager T, Barsi A, Hamda NT *et al*. Dynamic energy budgets in population ecotoxicology: applications and outlook. *Ecol Model* 2014;**280**:140–7.

52. Brown EE, Baumann H, Conover DO. Temperature and photoperiod effects on sex determination in a fish. *J Exp Mar Biol Ecol* 2014;**461**:39–43.

53. Conover DO, Kynard BE. Environmental sex determination: interaction of temperature and genotype in a fish. *Science* 1981;**213**:577–9.

54. Kolodziej EP, Gray JL, Sedlak DL. Quantification of steroid hormones with pheromonal properties in municipal wastewater effluent. *Environ Toxicol Chem* 2009;**22**:2622–9.

55. Zeilinger J, Steger-Hartmann T, Maser E *et al*. Effects of synthetic gestagens on fish reproduction. *Environ Toxicol Chem* 2009;**28**:2663–70.

56. Zucchi S, Castiglioni S, Fent K. Progestins and antiprogestins affect gene expression in early development in zebrafish (*Danio rerio*) at environmental concentrations. *Environ Sci Technol* 2012;**46**:5183–92.

57. Svensson J, Fick J, Brandt I *et al*. The synthetic progestin levonorgestrel is a potent androgen in the three-spined stickleback (*Gasterosteus aculeatus*). *Environ Sci Tech* 2013;**47**:2043–51.

58. United States Environmental Protection Agency. *EPA-821-R-02-012: Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms*. 5th Edition. https://www.epa.gov/sites/default/files/2015-08/documents/acute-freshwater-and-marine-wet-manual_2002.pdf (25 October 2022, date last accessed).

59. Brander SM, He G, Smalling KL *et al*. The in vivo estrogenic and in vitro anti-estrogenic activity of permethrin and bifenthrin. *Environ Toxicol Chem* 2012;**31**:2848–55.

60. DeGroot BC, Brander SM. The role of P450 metabolism in the estrogenic activity of bifenthrin in fish. *Aquatic Toxicol* 2014;**156**:17–20.

61. Bertotto LB, Bruce R, Li S *et al*. Effects of bifenthrin on sex differentiation in Japanese medaka (*Oryzias latipes*). *Environ Res* 2019;**177**:108564.

62. Li S, Hu T, Bertotto LB *et al*. Pesticide and surfactant mixtures alter sexual differentiation in Japanese medaka (*Oryzias latipes*). *Environ Sci Tech Water* 2021;**1**:1533–40.

63. Tyler CR, Beresford N, Van der Woning M *et al*. Metabolism and environmental degradation of pyrethroid insecticides produce compounds with endocrine activities. *Environ Toxicol Chem* 2000;**19**:801–9.

64. Magnuson JT, Huff Hartz KE, Fulton CA *et al*. Transcriptomic and histopathological effects of bifenthrin to the brain of juvenile rainbow trout (*Oncorhynchus mykiss*). *Toxics* 2021;**9**:48.

65. Brander SM, Jeffries KM, Cole BJ *et al*. Transcriptomic changes underlie altered egg protein production and reduced fecundity in an estuarine model fish exposed to bifenthrin. *Aquatic Tox* 2016;**174**:247–60.

66. Ligocki IY, Munson A, Farrar V *et al*. Environmentally relevant concentrations of bifenthrin affect the expression of estrogen and glucocorticoid receptors in brains of female western mosquitofish. *Aquatic Toxicol* 2019;**209**:121–31.

67. Kuivila KM, Hladik ML, Ingersoll CG *et al*. Occurrence and potential sources of pyrethroid insecticides in stream sediments from seven US metropolitan areas. *Environ Sci Tech* 2012;**46**:4297–303.

68. Weston DP, Holmes RW, Lydy MJ. Residential runoff as a source of pyrethroid pesticides to urban creeks. *Environ Pollut* 2009;**157**:287–94.

69. Weston DP, Lydy MJ. Urban and agricultural sources of pyrethroid insecticides to the Sacramento-San Joaquin Delta of California. *Environ Sci Technol* 2010;**44**:1833–40.

70. Major KM, Brander SM. The ecological and evolutionary implications of pyrethroid exposure: a new perspective on aquatic ecotoxicity. In: Eljarrat E (ed.), *Pyrethroid Insecticides. The Handbook of Environmental Chemistry*, Vol. 92. Switzerland: Springer, 2020, 109–48.

71. Weston DP, Moschet C, Young T *et al.* Chemical and toxicological effects on Cache Slough after storm-driven contaminant inputs. *San Franc Estuary Watershed Sci* 2019;**17**:3.

72. Sellin Jeffries MK, Abbott KI, Cowman T *et al.* Occurrence and endocrine effects of agrichemicals in a small Nebraska, USA, watershed. *Environ Toxicol Chem* 2011;**30**:2253–60.

73. Ojoghoro J, Scrimshaw M, Sumpter J. Steroid hormones in the aquatic environment. *Sci Total Environ* 2021;**792**: 148306.

74. Ekman DR, Villeneuve DL, Teng Q *et al.* Use of gene expression, biochemical and metabolite profiles to enhance exposure and effects assessment of the model androgen 17β-trenbolone in fish. *Environ Toxicol Chem* 2011;**30**:319–29.

75. Morthorst JE, Holbech H, Bjerregaard P. Trenbolone causes irreversible masculinization of zebrafish at environmentally relevant concentrations. *Aquatic Toxicol* 2010;**98**: 336–43.

76. Ankley GT, Coady KK, Gross M *et al.* A critical review of the environmental occurrence and potential effects in aquatic vertebrates of the potent androgen receptor agonist 17β-trenbolone. *Environ Toxicol Chem* 2018;**37**:2064–78.

77. Martyniuk CJ, Feswick A, Munkittrick KR *et al.* Twenty years of transcriptomics, 17alpha-ethinylestradiol, and fish. *Gen Comp Endocrinol* 2020;**286**:113325.

78. Hintemann T, Schneider C, Schöler HF *et al.* Field study using two immunoassays for the determination of estradiol and ethinylestradiol in the aquatic environment. *Water Res* 2006;**40**:2287–94.

79. Filby AL, Horpe KL, Maack G *et al.* Gene expression profiles revealing the mechanisms of anti-androgen-and estrogen-induced feminization in fish. *Aquatic Toxicol* 2007;**81**:219–31.

80. Duffy TA, McElroy AE, Conover DO. Variable susceptibility and response to estrogenic chemicals in *Menidia menidia*. *Mar Ecol Prog Ser* 2009;**380**:245–54.

81. Jackson L, Klerks P. Effects of the synthetic estrogen 17α-ethinylestradiol on *Heterandria formosa* populations: does matrotrophy circumvent population collapse? *Aquatic Toxicol* 2020;**229**:105659.

82. Schwindt AR, Winkelman DL, Keteles K *et al.* An environmental oestrogen disrupts fish population dynamics through direct and transgenerational effects on survival and fecundity. *J Appl Ecol* 2014;**51**:582–91.

83. Tang Z, Liu ZH, Wang H *et al.* A review of 17α-ethynylestradiol (EE2) in surface water across 32 countries: sources, concentrations, and potential estrogenic effects. *J Environ Manag* 2021;**292**:112804.

84. O'Dea RE, Noble DWA, Johnson SL *et al.* The role of non-genetic inheritance in evolutionary rescue: epigenetic buffering, heritable bet hedging and epigenetic traps. *Environ Epigen* 2016;**2**:dvv014.

85. Burggren WW. Dynamics of epigenetic phenomena: intergenerational and intragenerational phenotype "washout." *J Experiment Biol* 2015;**218**:80–7.

86. Myers RA, Bowen KG, Barrowman NJ. Maximum reproductive rate of fish at low population sizes. *Can J Fish and Aquatic Sci* 1999;**56**:2404–19.

87. Conover DO, Ross MR. Patterns in seasonal abundance, growth and biomass of the Atlantic silverside, *Menidia menidia* in a New England estuary. *Estuaries* 1982;**5**:275–86.