Connecting Suborganismal Data to Bioenergetic Processes: Killifish Embryos Exposed to a Dioxin-Like Compound

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Abstract: A core challenge for ecological risk assessment is to integrate molecular responses into a chain of causality to organismal or population-level outcomes. Bioenergetic theory may be a useful approach for integrating suborganismal responses to predict organismal responses that influence population dynamics. We describe a novel application of dynamic energy budget (DEB) theory in the context of a toxicity framework (adverse outcome pathways [AOPs]) to make quantitative predictions of chemical exposures to individuals, starting from suborganismal data. We use early-life stage exposure of Fundulus heteroclitus to dioxin-like chemicals (DLCs) and connect AOP key events to DEB processes through “damage” that is produced at a rate proportional to the internal toxicant concentration. We use transcriptomic data of fish embryos exposed to DLCs to translate molecular indicators of damage into changes in DEB parameters (damage increases somatic maintenance costs) and DEB models to predict sublethal and lethal effects on young fish. By changing a small subset of model parameters, we predict the evolved tolerance to DLCs in some wild F. heteroclitus populations, a data set not used in model parameterization. The differences in model parameters point to reduced sensitivity and altered damage repair dynamics as contributing to this evolved resistance. Our methodology has potential extrapolation to untested chemicals of ecological concern. Environ Toxicol Chem 2023;42:2040–2053. © 2023 Oak Ridge National Laboratory and The Authors. Environmental Toxicology and Chemistry published by Wiley Periodicals LLC on behalf of SETAC.

Keywords: Adverse outcome pathway; Environmental modeling; Dioxins; Ecological risk assessment; Predictive toxicology

INTRODUCTION

Recent advances in connecting organism-level responses to contaminant exposures to higher levels of biological organization, such as ecosystem services (Forbes et al., 2017), hold promise in extrapolating the impact of an environmental contaminant across levels of biological organization. However there remains a lack of methodology and few studies (Ankley et al., 2009) that relate suborganismal (molecular) data to the ecologically important apical endpoints for a whole organism. The present study aims to use a framework proposed by Murphy et al. (2018) to extrapolate impacts observed at the suborganismal level to organism-level response by connecting contaminant impacts on suborganismal function to bioenergetic processes. Murphy et al. proposed the integration of dynamic energy budget (DEB) theory (Kooijman & Kooijman, 2010) and the adverse outcome pathway (AOP; Ankley et al., 2010) approach to inform ecological risk assessments of chemical exposures.

An AOP (Ankley et al., 2010) provides one option of a “bottom-up framework” to summarize molecular information to connect to an adverse outcome (AO) at a relevant level of biological organization for risk assessment (Murphy et al., 2018). An AOP is pathway-, but not chemical-, specific: A chemical binds to its specific biological target (molecular initiating event [MIE]), which starts a cascade of key events (KEs) that span...
increasing levels of biological organization until reaching an AO at the level of the whole organism. However, precision is lost with progress through the KEs, and quantitative prediction of important AOs (quantitative AOPs [qAOPs]; Conolly et al., 2017) is rare; but this is a developing field.

By contrast, DEB theory offers a top-down approach that relates physiological processes to organismal performance (Jusup et al., 2017; Kooijman & Kooijman, 2010; Nisbet et al., 2000). A small number of differential equations describe the rates at which an organism assimilates and utilizes energy and nutrients from food for its maintenance, growth, reproduction, and development. Applications to ecotoxicology require additional toxicokinetic and toxicodynamic (TK-TD) modules (Jager, 2017, 2020; Jager et al., 2006; Jager & Zimmer, 2012) that describe, respectively, toxicant exchange with the environment and chemical transformations within an organism. The scope for this combination was recognized in an opinion from the European Food Safety Authority (EFSA Panel on Plant Protection Products and Their Residues et al., 2018). Because important details of KEs are often not known, many applications utilize an abstract quantity denoting cellular damage, which links the level of toxicant inside the organism directly to organism-level impacts (Jager, 2020).

Damage-based modeling has been applied in ecotoxicology to model survival (Jager et al., 2011) and sublethal effects (Klanjscek et al., 2016) as well as in epidemiology (Civitello et al., 2018, 2022). The feasibility of integrating the top-down and bottom-up approaches rests on the insight from systems biology that the complex, multicomponent networks underlying regulatory processes within a cell behave like a relatively small number of interacting aggregate processes regulated through feedback mechanisms (see Doyle & Stelling, 2006). In short, the AOP and DEB approaches offer complementary schemes for connecting suborganisinal endpoints to organismal processes, and integrating these two established frameworks may offer opportunities for predictive effect modeling of organisms exposed to contaminants. Connecting these levels of biological organization could leverage the expanding availability of suborganisinal data thanks, in part, to the rapid development of high-throughput assays and technologies by quantitatively linking changes observed on the molecular scale to organismal effects. This could also allow for extrapolation between species that are closely related, reducing the number of animals needed for chemical testing, a current goal in the field. The present study demonstrates the connection of these two frameworks using a data-rich case study on the effect of dioxin-like chemicals (DLCs) on Fundulus heteroclitus, the Atlantic killifish. To our knowledge, this is the first attempt to explicitly link a qAOP and DEB.

Dioxins are a group of polyhalogenated aromatic hydrocarbons consisting of polychlorinated dibenzo-p-dioxins or dioxins proper, polychlorinated dibenzofurans or furans, and dioxin-like polychlorinated biphenyls (PCBs; Kulkarni et al., 2008). They are persistent organic pollutants with a global environmental distribution. Dioxin-like chemicals are carcinogenic and can impact various neurological, immunological, developmental, and reproductive systems in humans and wildlife (Kulkarni et al., 2008). They have been studied extensively in numerous animal models (Doering et al., 2018; Fernandez-Salgueiro et al., 1996; Mimura et al., 1997; Prasch et al., 2003), and a putative AOP was published as part of the seminal AOP paper by Ankley et al. (2010). Many of the canonical toxic outcomes following exposures to DLCs are mediated through the aryl hydrocarbon receptor (AhR) signaling pathway, and the MIE for DLCs is the binding of a DLC to the AhR (Ankley et al., 2010). This is based on extensive experimental research that establishes causal linkages between AhR activation and DLC toxicity (Fernandez-Salgueiro et al., 1996; Mimura et al., 1997; Prasch et al., 2003). Further, a recent qAOP developed using data from nine fishes correctly predicted activation of AhR (specifically AHR2) and early/life stage mortality for numerous bird and fish species (Doering et al., 2018). However, while the binding of DLCs to AhR leads to dioxin toxicity, the cellular and physiological KEs that connect AhR activation to eventual toxic outcomes are unknown (Ankley et al., 2010), although many have been proposed (Goldstone & Stegeman, 2006). In the AOP wiki, AOP21 describes the connection of AhR activation (the MIE) with early/life stage mortality through the cyclooxygenase-2 expression, for example (Doering et al., n.d.). There may be multiple different cascades of KEs that are initiated by the binding of DLCs to the AhR and result in larval mortality.

Along the East Coast of the United States, several semiclosed killifish populations have been exposed to high levels of DLCs for decades (Nacci et al., 2009; Van Veld & Nacci, 2008; Whitehead et al., 2012). As a result, these populations have evolved adaptations to DLC toxicity (Whitehead et al., 2012). Killifish populations that live and thrive in habitats with high concentrations of DLC contamination (resistant populations) have converged on similar mechanisms to desensitize AhR pathway activation, allowing these fish to tolerate concentrations of DLCs three orders of magnitude greater than those in fish from sensitive populations (Reid et al., 2016; Whitehead et al., 2012). The DLC tolerance range of F. heteroclitus is the widest known and exceeds the range of sensitivity of even broad taxonomic groups such as birds, invertebrates, mammals, or fishes (Whitehead et al., 2012). We incorporate data from both sensitive and resistant fish in our experiments and model analysis to parameterize and then test the model framework developed in the present study.

We developed a model framework connecting suborganisinal (gene expression and AhR induction) and organismal biological data (embryonic development and survival, larval growth) to describe the effect of PCB126, a model DLC, on developing F. heteroclitus embryos and newly hatched larvae from sensitive populations. We tested the model by changing a small subset of parameters and predicting the effect of PCB126 exposure on larvae from tolerant populations.

**METHODS**

**Model description**

The framework described in the present study involves three interconnected modules (Figure 1): (1) bioenergetic module describing embryonic development and larval growth (DEBkiss); (2) toxicokinetic module describing dynamics of PCB
concentrations; and (3) toxicodynamic module of damage accumulation and regulation describing sublethal effects on both growth and mortality. We briefly describe each module below, and the model equations and variables are defined in Table 1 (see Supporting Information for a full description).

Bioenergetic (DEB) module. We used a version of DEBkiss, a simplified DEB model developed by Jager et al. (2013), because its description of embryonic development matches the resolution of the available data on killifish development and larval growth. The specific version of DEBkiss we used assumed V1-morphic (instead of isomorphic) growth, a common choice to describe growth of fish posthatching because of their accelerated larval growth (Augustine et al., 2011). Embryos assimilate energy-rich material from an “egg buffer” (yolk) and use it for growth and maintenance. Eggs hatch when the buffer is completely assimilated. Larvae start feeding immediately on hatching and utilize assimilates to support growth and maintenance. A yield coefficient (y_G) takes account of overheads on growth for both life stages. Our two bioenergetic state variables thus represent egg buffer (M_E) and biomass (M_t).

TK module. Because the killifish were exposed to PCB126 for 7 days during embryonic development and then transferred to clean water until hatch, we assumed that (1) during embryonic exposure, PCB enters eggs at a rate proportional to (invariant) egg size and to the solubilized PCB concentration; (2) PCB inside the eggs partitions instantaneously between the lipid fractions of yolk and embryo; and (3) PCB inside the eggs is neither excreted nor metabolized, which is supported by experimental data (Matthews & Dedrick, 1984). We modeled both the total mass of PCBs within the egg (Q(t)) and the total mass of PCBs in the two compartments within the egg. See further details on the TK module in the Supporting Information.

TD module. Our TK module relates the abstract damage variable to internal toxicant concentrations (calculated in the TK module) and was used to describe both sublethal and lethal impacts of the toxicant on young fish. Damage is produced by accumulated toxicant within the organism and impacts bioenergetic processes (Jager, 2017). We assumed that damage is produced at a rate determined by the density of DLCs in live biomass and is repaired at a rate which saturates at high damage densities. We parameterized the damage production function (see Table 1) with data on gene expression levels (see section Linking TD and bioenergetic modules—Gene expression functional enrichment analysis methods).

Damage is an abstraction that is not defined operationally, with the implication that no parameters with units involving damage are estimable. We therefore recast all TD equations to only include ratios of damage-related parameters, with the state variable Δ(t) representing scaled damage density at time t (see Table 1 and model description in Supporting Information).

Data and model parameterization

We have data sets of *F. heteroclitus* from DLC-sensitive and -resistant populations exposed to PCB126. We initially used data on *F. heteroclitus* only from sensitive populations to parameterize all modules constituting our full model. Later, we used a complementary data set on the responses of developing killifish from resistant populations (discussed in the section Model verification of response of fish from resistant populations to DLC exposure). The combined modules need values for 20 parameters in total. Below we outline the parameterization methods used for each module, including descriptions of the data sets used.

Bioenergetic module (DEBkiss).

Data. This module uses embryo-larval survival and larval growth data from laboratory assays using killifish embryos exposed to PCB126. Early-life stage responsiveness to exposures of solubilized PCB126 was assessed using standardized methods (Nacci et al., 2005, 2010), briefly described below. Embryo-larval survival data used for modeling were derived from assays using 11 wild killifish populations and used to characterize seven of these wild killifish populations as PCB126-sensitive and four as PCB126-tolerant (Nacci et al., 2002, 2010). Killifish embryos were exposed from 1 to 7 days postfertilization (dpf) to three...
TABLE 1: Model equations of the full model, including the toxicokinetic, toxicodynamic, and bioenergetic modules

State variables

\[
\begin{align*}
M_V(t) & \quad \text{Biomass at time } t \text{ (mg fish)} \\
M_E(t) & \quad \text{Egg reserve at time } t \text{ (mg assimilates)} \\
Q(t) & \quad \text{Total mass of PCB within the egg } (M_V + M_E) \text{ at time } t \text{ (nanograms of PCB)} \\
Q_V(t) & \quad \text{Total mass of PCB within fish biomass } (M_V) \text{ at time } t \text{ (nanograms of PCB)} \\
q_V(t) & \quad \text{PCB concentration in fish biomass } (M_V q_v = \frac{Q_v}{V} \text{ at time } t \text{ (nanograms of PCB per mg fish)}) \\
\Delta(t) & \quad \text{Scaled damage density at time } t \Delta = k_{MD} \Delta (\text{dimensionless)} \\
St & \quad \text{Survival to time } t \text{ (probability of surviving)}
\end{align*}
\]

Fluxes

\[
\begin{align*}
J_A & \quad \text{Assimilation rate (mg assimilates/day)} \\
J_M & \quad \text{Somatic maintenance rate (mg assimilates/day)} \\
J_S & \quad \text{Biomass growth rate (mg fish/day)} \\
k_M & \quad \text{Maintenance (mg assimilates/mg fish per day)} \\
r & \quad \text{Repair of scaled damage (1/day)} \\
p & \quad \text{Production of scaled damage from AOP (1/day)}
\end{align*}
\]

Functions defining rates

\[
\begin{align*}
J_A & = \begin{cases} 
J_{A\text{emb}} M_V & \text{if } M_E < 0 \text{ (embryos)} \\
J_{A\text{lar}} M_V & \text{if } M_E = 0 \text{ (larvae)}
\end{cases} \\
J_M & = k_M M_V \\
J_S & = \gamma_0 (J_M - J_A) \\
p & = \frac{k_{MD} \theta + \Delta}{\theta + \Delta} \\
r & = \frac{\alpha \theta}{\theta + \Delta}
\end{align*}
\]

Sublethal effect of damage

\[
k_{MD} = k_{MD}(1 + \Gamma \Delta) \quad \text{Somatic maintenance rate is assumed to increase linearly with scaled damage density}
\]

Toxicokinetics

\[
C_W = \begin{cases} 
C_W(t) & \text{if } t < T_{\text{exp}} \\
0 & \text{otherwise}
\end{cases}
\]

\[
Q_V = \frac{M_V}{M_E + PM_E} Q \text{ with } p = \frac{PE}{P_V}
\]

Dynamic equations

\[
\begin{align*}
\frac{dM_V}{dt} & = J_A \\
\frac{dM_E}{dt} & = \begin{cases} 
-J_M & \text{if } M_E > 0 \\
0 & \text{otherwise}
\end{cases} \\
\frac{dQ}{dt} & = UC_W \\
\frac{d\Delta}{dt} & = p - r - \frac{\Delta}{\theta} \frac{dM_V}{dt} \\
\frac{dS}{dt} & = -S (\mu_E + \Phi \Delta)
\end{align*}
\]

Initial values

\[
M_V = 0.3; \; M_E = M_{E0}; \; Q = 0; \; Q_V = 0; \; q_V = 0; \; \delta = 0; \; \Delta = 0; \; S = 1
\]

Conversion assumption

\[
L = \frac{M_V}{V}
\]

L is standard length in millimeters, \( M_V \) is dry weight in milligrams, constants b and c and relationship from Kneib & Stiven (1978)

PCB = polychlorinated biphenyl.

concentrations of PCB126, a solvent (acetone), or no-solvent control treatments. Data from the acetone and no-solvent controls did not differ, so data were pooled from these two treatments as “controls” (Supporting Information). After 7 days of exposure, embryos were transferred to individual vials of uncontaminated seawater; observations were made daily, and seawater was renewed on alternate days. At 10 dpf, a subset of embryos was flash-frozen and archived for transcriptomic analysis (Reid et al., 2016; Whitehead et al., 2010). After hatching (~14 dpf), larva were fed Artemia daily and observed through 7 days posthatching (dph). For a subset of killifish populations (two PCB126-sensitive and two PCB126-tolerant), living larvae were observed microscopically and photographed on 0 and 7 dpf. Measurements of larval length were made from these photos using ImageJ software. All procedures involving fish were conducted in accordance with approved US Environmental Protection Agency Office of Research and Development Institutional Animal Care and Use Committee protocols and carried out in accordance with the relevant guidelines and regulations.
Parametrization.

Maintenance. We assumed that starving *F. heteroclitus* lose weight at an exponential rate. We used body weight data from food restriction studies on adult killifish from a PCB126-sensitive population to estimate $k_{M_0}$ (see details on restricted feeding experiments and parameterization in the Supporting Information).

Growth efficiency, function response, energy allocation. The parameter $y_{GC}$ cannot be estimated without data on composition of egg buffer and food. We set this value to 1. Because the fish were fed ad libitum, we set $f$ to 1 and used the default value suggested by Jager (2015) for $\kappa$.

Remaining DEBkiss parameters. For fitting, we used the DEBkiss package (Ver 2.0) in Bring Your Own Model (Ver 5.1) developed by Tjalling Jager (DEBtox, n.d.) for Matlab for fitting and added a few model elaborations. In the published DEBkiss model (Jager, 2015), the maximum size-specific assimilation rates for embryos and larvae are assumed to be equal. Our data indicate that in killifish the rate in embryos is 7 times larger than that in larvae (see Supporting Information). We fit $J_{Mede}$, $J_{AMe}$, $M_{ED}$, and $\mu$ to the control data set, using initial values based on the literature for similar species or previous information. The initial value of the egg buffer, $M_{ED}$, is particularly impactful to the model simulations, and thus other parameter values, so we calculated an initial value and range to control the area of parameter values searched (see Supporting Information). Best-fit model parameters are calculated based on maximum likelihood estimation, and details on the fitting routines in this software can be found in Jager (2021).

Negative log likelihood values for each model fit are provided in the figure legends. Confidence intervals (Table 2) were calculated from asymptotic standard errors. Details on the fitting strategy for each module are described in the Supporting Information.

Experimental parameters. The concentration of PCB126 in the waterborne exposures, $C_{W_0}$, and the experimental exposure time, $T_{exp}$, were defined by the experimental setup.

Length. Most of our data were measurements of fish length, but DEBkiss rates and parameters are based on the dry weight of the body mass and assimilates. Length to dry weight conversions were obtained with the allometric equation of Kneib and Stiven (1978; see Table 1, parameters b and c).

Parametrizing the TK module. There are two parameters: the uptake rate of PCB126 into the egg ($u$; see Supporting Information for details) and the partitioning coefficient of PCB126 between buffer (yolk) and structure ($p = \frac{F_{v}}{F_{p}}$). The uptake rate was estimated using data on embryonic concentrations of PCB126 produced by embryo-larval exposures described in the previous section, which was measured in replicate assays (Nacci et al., 1999, 2002). For the latter, we used data from Binder et al. (1985), which measured the distribution of Aroclor 1254 (a commercial mixture of PCBs that includes PCB126; Kodavanti et al., 2001) between egg and embryo in *F. heteroclitus*.

Parameterizing the TD module. The TD module requires values for parameters describing damage production ($h$ and $\theta$), damage repair ($a$ and $k$), and the lethal and sublethal impacts of DLC-induced damage on *F. heteroclitus* ($\phi$ and $\gamma$, respectively). We used data on cytochrome P450 1A (CYP1A) gene expression (Whitehead et al., 2012) as a proxy for damage production (see Supporting Information) because CYP1A is the hallmark of AhR pathway activation and because DLC toxicity is largely mediated through exposure-induced AhR pathway activation (Andreasen et al., 2002; Prasch et al., 2003). We used a Hill function to relate internal PCB concentrations and damage levels because this dose–response relationship has been shown to satisfactorily describe DLC levels and CYP1A induction (Simon et al., 2015). We used transcriptomic data from Whitehead et al. (2012), which reported the relative changes in CYP1A expression in *F. heteroclitus* embryos 7 dpf across increasing concentrations of PCB126. These authors collected data on embryos from sensitive and resistant populations, and the shape of the dose–response relationship of PCB126 to CYP1A induction was similar in embryos from all populations, the crucial difference being that embryos from resistant populations showed an equivalent response to sensitive fish at PCB126 doses at least 2 orders of magnitude higher (Whitehead et al., 2012). The fit for sensitive and resistant populations is shown in Figure 2, and further details on this parameterization are in the Supporting Information.

The parameters describing damage repair and lethal and sublethal impacts were estimated from the whole data set on the effect of PCB126 on developing embryos and larval fish from sensitive populations (see details in Supporting Information).

Linking TD and bioenergetic modules—Gene expression functional enrichment analysis methods

We connected DEBkiss to the damage modules via transcriptomic responses of embryos from sensitive and resistant killifish populations exposed to PCB126 at effects-matched doses (Whitehead et al., 2012). Effects-matched doses for these two populations are 2, 20, and 200 ng/L for fish from sensitive populations and 200, 2000, and 20,000 ng/L PCB126 for fish from resistant populations, which matches the dose response between the concentration of PCB126 and the impact on mortality in both populations (Whitehead et al., 2012). These concentration ranges cause expression changes in a suite of genes with increasing dose (including CYP1A) that is parallel between populations, and at higher concentrations developmental abnormalities (cardiovascular system defects) start to emerge, in 10-dpf embryos. Embryos were exposed for 7 days during development and then snap-frozen on postfertilization day 10. Whole embryos were homogenized, and total RNA was extracted. Further methods details are in Whitehead et al. (2012).

Transcriptomics analysis following exposures to a range of PCB126 concentrations identified hundreds of up-
TABLE 2: Model parameters: the parameter name, if the parameter values were fixed (0) or fit (1) to the full data set, the parameter’s units, the source of the parameter value (either the reference if from the literature or the data set it was fit to), and the confidence interval of the estimate, if applicable

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Parameter value</th>
<th>Parameter value source</th>
<th>Confidence interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>$b$</td>
<td>3.29 (0)</td>
<td>Units: 1/day</td>
<td></td>
</tr>
<tr>
<td>$c$</td>
<td>-6.18 (0)</td>
<td>Units: mg assimilates/mg</td>
<td></td>
</tr>
<tr>
<td>$y_G$</td>
<td>1 (0)</td>
<td>Units: mg fish/mg assimilates</td>
<td></td>
</tr>
<tr>
<td>$k$</td>
<td>0.8 (0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$f$</td>
<td>1 (0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$k_{MO}$</td>
<td>0.023 (0)</td>
<td>Units: 1/day</td>
<td></td>
</tr>
<tr>
<td>$J_{Amp}$</td>
<td>0.91 (0)</td>
<td>Units: mg assimilates/(mg fish * day)</td>
<td></td>
</tr>
<tr>
<td>$J_{Aml}$</td>
<td>0.17 (0)</td>
<td>Units: mg assimilates/(mg fish * day)</td>
<td></td>
</tr>
<tr>
<td>$M_{EO}$</td>
<td>1.41 (0)</td>
<td>Units: mg assimilates</td>
<td></td>
</tr>
<tr>
<td>$\mu$</td>
<td>0.01 (0)</td>
<td>Units: 1/day</td>
<td></td>
</tr>
<tr>
<td>$C_{W0}$</td>
<td>0-20,000 (0)</td>
<td>Units: Nanograms/L</td>
<td></td>
</tr>
<tr>
<td>$T_{exp}$</td>
<td>7 Days</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\nu$</td>
<td>1.25e-4 (0)</td>
<td>Units: L/day</td>
<td></td>
</tr>
<tr>
<td>$\rho$</td>
<td>0.21 (0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$h$</td>
<td>1.45e-2 (sensitive; 0)</td>
<td>Units: Nanograms of PCB/mg fish</td>
<td></td>
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<tr>
<td>$\theta$</td>
<td>2.47 (resistant; 0)</td>
<td>Damage/(mg fish * day)</td>
<td></td>
</tr>
<tr>
<td>$a$</td>
<td>2.26e-2 (1)</td>
<td></td>
<td></td>
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<tr>
<td>$k$</td>
<td>3.65e-8 (sensitive; 1)</td>
<td>Units: 1/day</td>
<td></td>
</tr>
<tr>
<td>$\phi$</td>
<td>5.19e3 (1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$f$</td>
<td>9.64e4 (1)</td>
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</table>

*This interval is the range in which this parameter value could vary. This parameter was constrained because we started with a good estimate of its value (see Supporting Information). NA = not available (indicates that the parameter was not fit [parameter value was set to a value from the literature or is an experimental detail]). PCB = polychlorinated biphenyl; CYP1A = cytochrome P450 1A.*
In general, up-regulated genes are enriched for those related to xenobiotic metabolism and detoxification enzymes (Table 3; Supporting Information, Tables S3 and S4): CYP enzymes, which are highlighted in Cluster 3, are oxidoreductase enzymes (Cluster 1) and have alpha-helical transmembrane anchors (Cluster 4; Mustafa et al., 2019). Along with being evidence of detoxification, CYPs are also hallmarks of AhR pathway activation (Andreasen et al., 2002; Prash et al., 2003), the MIE of dioxin-like exposure—induced toxicity (Ankley et al., 2010). Cluster 2 suggests perturbation on cell-to-cell signaling, which, taken with the down-regulated clusters, could point to impacts on muscle structure and/or function and on mitochondrial energy metabolism.

The down-regulated clusters are less straightforward and had lower enrichment scores than the up-regulated clusters; Huang et al. (2009) recommend giving more attention to clusters with higher enrichment scores. We hypothesized that the down-regulated clusters together implicates on muscle signaling related to cardiovascular function (Table 3; Supporting Information, Tables S3 and S4). Exposure to PCB126 of fish and other vertebrates during development results in cardiovascular and branchial dysfunction (Grimes et al., 2008).

When considering these categories of functional enrichment together, we hypothesized that the core mechanism whereby DLC exposures impact bioenergetic processes is through increasing direct or indirect demands on somatic maintenance. This could be due to the increased energetic costs of detoxification enzymes and xenobiotic metabolism or of either repairing or struggling to meet basal somatic needs caused by impaired cardiovascular or branchial structures. We further assumed a direct effect on mortality. Both mechanisms are mediated by the damage variable, with different proportionality constants (Table 1).

### RESULTS/DISCUSSION

**Linking TD and bioenergetic modules—Gene expression functional enrichment analysis results**

The Functional Annotation Clustering of DAVID considered sets of genes that were dose-responsive and identified biological functions or pathways that were enriched for those sets. For up-regulated genes in response to exposure we detected 54 significantly enriched functional clusters and 65 clusters for down-regulated genes. All DAVID analysis outputs on the top 10 functionally enriched clusters and Kyoto Encyclopedia of Genes and Genomes pathways are included in the Supporting Information. The top five clusters are summarized in Table 3.

**Fit of model to fish from sensitive populations**

The fit of the full model to fish from sensitive populations is shown in Figure 3 and Supporting Information, Figure S5. The model in general fits well both the sublethal impacts (on length, Figure 3A, and time to hatch, shown through exhaustion of the egg reserve in Figure 3B) and lethal impacts (survival, Figure 3F) produced by the absorbed internal dose of PCB126. In assessing the goodness of fit, we aimed to minimize the minus log-likelihood value of the function (reported in the figure legends) and assessed the fit visually by comparing how
well the model predictions (lines) match the data (points); the latter is easier to assess using the individual panels displayed in Supporting Information, Figure S5. We did not have data for all state variables; either they are abstract concepts (scaled damage density, Figure 3E) or we did not have a matching data set (PCB concentrations, Figure 3C,D; we did use PCB data to parameterize the PCB uptake parameter from a separate experiment; see details in Supporting Information). Interestingly, the parameter estimates for damage repair do not seem to have a large impact on damage dynamics. The best-fit repair parameters for the sensitive data predict a very low maximum repair rate, with the principal regulator of damage for the sensitive populations being dilution of damage due to growth. This has implications for larger fish because their rate of dilution due to growth will be smaller than that for smaller, more rapidly growing fish.

Model verification of response of fish from resistant populations to DLC exposure

All of the parameterization for Figure 3 was conducted using data from sensitive F. heteroclitus populations. We attempted to predict the response to PCB126 in resistant populations by reestimating values of a small subset of the parameters for the sensitive populations. As an initial hypothesis, we assumed that the only parameter(s) that differed between the two populations related to damage production. We therefore reestimated one of the damage production parameters (h) for fish from resistant populations using only CYP1A data from resistant fish, as described in the section Parameterizing the TD module (see fit in Figure 2). The 3–order of magnitude difference between the two values of h largely accounts for the difference between the two populations: The internal concentration of PCB126 at which damage production is at its maximum rate is 3 orders of magnitude greater in fish from resistant populations compared with that of sensitive fish. We then predicted the responses of resistant fish by changing only this parameter, and the remaining parameters stayed the same as for the model run for sensitive populations (Figure 4). Visually, the simulation predicts the data reasonably well, but there is a mismatch at the highest concentrations where the model overpredicted the effect of PCB126 on larval growth at 210,000 ng/L (Figure 4A; Supporting Information, Figure S6) and on both embryonic and larval survival at 20,000 and 200,000 ng/L (Figure 4F; Supporting Information, Figure S6).

A hypothesis for the remaining mismatches at high concentrations is that the damage repair parameters differ between the populations. Therefore, we reestimated parameters relating to the repair process (a and k) for the tolerant fish. Changing the value of a did not alter the fit; however, allowing k to vary improved the fit dramatically. Therefore, after some preliminary fitting, we fixed the value of a at the value parameterized to the sensitive population data set and focused on...
only refitting $k$. Refitting this parameter in addition to the tolerant-specific value for $h$ yields a significantly improved fit to the sublethal and lethal impacts of DLCs on these fish (Figure 5; Supporting Information, Figure S7). We conducted a likelihood ratio test (LRT) between these two models to assess the goodness of fit and found that the model in which $k$ and $h$ are reestimated results in a statistically significantly ($\alpha = 0.05$) better fit compared with the reduced model in which only $h$ is reestimated between the tolerant and sensitive fish (see Supporting Information for details on LRT). Interestingly, the best-fit repair parameters for the tolerant populations led to a much smaller scaled half-saturation constant for repair compared with sensitive fish (parameter $k$, estimated as $3.65e-8$ for sensitive fish and $5.28e-9$ for tolerant fish) but no change to the maximum repair rate ($a$), implying that tolerant fish are able to repair DLC-specific damage at a much greater rate at a lower damage concentration than sensitive fish. Their overall maximum repair rates are the same; however, tolerant fish, with a lower half-saturation constant, reach half of the maximum repair rate at a lower damage concentration, which is consistent with a more efficient repair mechanism.

When considering the best-fit parameter values and simulations for both sensitive and tolerant populations, it is interesting to note that the regulatory feedback that controls damage production is primarily dilution due to growth rather than the explicit repair processes we incorporated into the model. Reestimating the repair-related parameters using the data set on tolerant fish yields a half-saturation constant an order of magnitude smaller than the value of this parameter for sensitive fish; however, the repair process still has a small effect on the damage dynamics even in tolerant fish. In our model simulations, damage only breaks completely from this regulatory feedback at the highest PCB126 concentrations (20,000 and 200,000 ng/g for sensitive fish, 200,000 ng/g for tolerant fish), leading to predicted mortality of all sensitive and 82% of tolerant larval fish at these exposures. Our model assumes that all damage produced by DLCs can be repaired and/or diluted with growth; however, this assumption may not be appropriate for all forms of DLC-produced damage. Specifically, DLC exposure can lead to malformed cardiovascular and branchial systems (Grimes et al., 2008), which may be irreversible. However, without data on relative proportions of irreversible versus reversible damage, we cannot separate out these reversible from irreversible damage components.

Connecting the suborganismal to the organismal level

The goal of the present study was to incorporate suborganismal information to the organismal level to take advantage of the wealth of information available in the literature on the impacts of stressors that alter molecular processes in organisms. We aimed to mechanistically link changes in gene
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expression during embryogenesis to impacts on growth and survival of larval fish up to 24 dpf (~7 dph). We were able to predict the impact of DLCs on larval growth and survival for this period (up to 1 week after hatching) based on data collected from embryos. With further study for other organisms and stressors, such mechanistic connections, if confirmed, could allow for reduced animal usage in toxicity testing through the design of scaled-down, more efficient tests and/or testing field-collected animals rather than conducting laboratory exposures. If we can connect suborganismal biomarkers to organismal responses that are currently used in standardized tests (i.e., if we can predict lethal or inhibitory concentrations that reduce survival, growth, or reproduction based on suborganismal signals), then smaller standardized tests, either in terms of the duration of the test, the number of treatments, or other reductions, could satisfy permit limits currently fulfilled using time-, animal-, and labor-intensive standardized tests. Furthermore, if we can demonstrate that certain molecular biomarkers connect to sublethal responses, we could sample organisms from the field and potentially predict their growth or reproductive capacity based on their current suborganismal profile, potentially making laboratory-based tests unnecessary. These goals are well beyond what we present in this article but are areas of future research and framework development.

Crucial information must be available to make these connections, some of which has been recently collected and organized in the AOP network (Knapen et al., 2018; Society for the Advancement of Adverse Outcome Pathways, 2020).

Traditionally, AOPs are not quantitative, although qAOPs (Conolly et al., 2017) have been developed more recently. An established AOP inherently bridges the connections necessary to identify ways in which we can connect suborganismal to organismal information in a mechanistic framework. However, the challenge remains to make these connections quantitative instead of qualitative.

One challenge in the present study was that although the MIE for DLCs has been identified and the AOP for DLCs was even included in the seminal paper on AOPs (Ankley et al., 2010), there is only a correlative linkage between the KEs of gene expression to yolk sac edema and hemorrhage due to the unknown mode of action of DLCs during disease progression. However, the information summarized in the AOP and identified elsewhere that CYP1A serves as a hallmark of AhR pathway activation (Andreasen et al., 2002; Prasch et al., 2003), and thus DLC toxicity (Andreasen et al., 2002; Fernandez-Salguero et al., 1996; Mimura et al., 1997; Prasch et al., 2003), allowed us to parameterize the damage module in the absence of a known mode of action. We further used suborganismal information to identify the bioenergetic mode of action through functional enrichment analysis of transcriptomics responses to DLC exposures from Whitehead et al. (2012). We hypothesized that this information implicates a bioenergetic impact of DLCs on developing *F. heteroclitus* embryos through increasing somatic maintenance costs, possibly due to increased energetic costs of detoxification enzyme production and xenobiotic metabolism and of either repairing

![FIGURE 5: Model testing by predicting growth (A), time to hatch (B), total concentration of polychlorinated biphenyls (PCBs; QV in C), concentration of PCbs per unit fish biomass (qV in D), scaled damage density (E), and survival (F) of fish from resistant populations re"
or struggling to meet basal somatic needs with cardiovascular or branchial structures that are impaired. In this way, using DAVID analysis on suborganismal data gave clues to identify the physiological mode of action (see Álvarez et al., 2006) of PCB126, integrating suborganismal processes with DEB.

The model has three constituent modules and consequently has many parameters. A key hypothesis underpinning our study is that the quantitative responses to exposure of sensitive and resistant fish to a stressor could largely be understood by considering changes in only a few of the parameters. We used suborganismal data on AhR activation to estimate a single parameter that distinguishes the production of DLC-specific damage from resistant populations from those from sensitive populations (parameter $h$ in the damage production function). We estimated this value to test if the model, parameterized using data from fish from sensitive populations, could predict the response of fish from resistant populations to DLCs as a model verification step. The model can predict the effect of PCB126 on resistant fish at all concentrations except the highest (Figure 4). Reestimating the value of one of the repair-associated parameters to the data on tolerant fish improved this fit dramatically (Figure 5).

In the DEB framework, response to toxic exposures is the net outcome of damage production minus repair. When comparing population-specific damage production using the same exposure scale, tolerant damage production is not only shifted to higher exposure concentrations but also attenuated or flattened in comparison with sensitive populations. Thus, clearly an important aspect of the PCB-protective response in tolerant relative to sensitive killifish is reduced damage production at any given exposure concentration. However, our models also explored protective differences in exposure-induced repair between populations. Maximal repair rates are similar between populations, but our best-fit tolerant model includes a lower estimator for repair half-saturation, consistent with relatively higher repair rates at lower damage levels in tolerant than in sensitive fish. Therefore, the evolved resistance observed in tolerant fish is a function not only of reduced sensitivity but also of modified damage repair dynamics.

More broadly, our ability to use the same model with minimal changes in parameters between populations with very different sensitivities to this stressor implies that this model framework may enable extrapolation to other species, especially considering the wide sensitivity range between these two populations of killifish. Indeed, that the intraspecific variation within killifish is greater than the interspecific variability of sensitivity between fish species to DLCs (Van Veld & Nacci, 2008).

Use of the “damage” variable in toxicity models

In our TD module, we use the abstract “damage” variable to characterize the lethal and sublethal impact of DLCs on F. heteroclitus. As discussed previously, many previous studies have advocated this approach. Jager’s most recent revision of DEB applied to toxic effects (Jager, 2020) emphasizes the power of damage in yielding a unified description of lethal and sublethal impacts of a contaminant in a framework amenable to data from standardized toxicity tests.

Our study expands on these previous uses of a damage module to explore the impacts of a stressor on an organism’s biology by incorporating suborganismal information. We use suborganismal data to parameterize the specific damage process of damage production (CYP1A expression data), minimizing the number of parameters we end up fitting to our larger data set. Further, this use of suborganismal data enabled us to test the model’s ability to predict the response of fish from resistant populations to DLC exposure.

The advantages of using a generalizable damage module are that it enables us to both predict the impact of toxicants with unknown modes of action (a common issue in ecotoxicology) and adapt the presented modeling framework to other stressors, organisms, and life stages. A researcher can adapt the damage module to nearly any stressor by identifying how damage is produced in an organism, either through an adaptation of the existing TK model to match the focal chemical or through a more qualitative understanding of how an external factor impacts organismal stress and estimating the damage module parameters. The damage module is able to predict the sublethal and lethal impacts of DLCs on F. heteroclitus from sensitive populations and to predict the lethal impact of DLCs on fish from resistant populations.

Remaining challenges connecting suborganismal, organismal, and higher levels

Many challenges remain when trying to connect suborganismal to organismal data, the most obvious of which is summarizing the extensive information available in suborganismal data such that it can be connected to a bioenergetic impact at the organism level. This is exemplified by the qualitative connection that we made between the molecular/cellular responses inferred from transcriptomic data from Whitehead et al. (2012) and the DEBkiss-based bioenergetic model. Functional enrichment analysis of transcriptomic responses to DLC exposures informed our hypothesis that DLC exposures caused bioenergetic impacts through impairment of somatic maintenance. Ideally, we would have made this connection via a more quantitative connection. We made such a connection through the use of CYP1A expression data to parameterize damage production, but quantitative connections between suborganismal data and broader processes to connect these biological levels would be more biologically justifiable and rigorous, which is of particular importance for usefulness in a risk-assessment framework in the future.

Implications for ecological risk assessment

The present study provides a framework to connect suborganismal exposure responses to responses that manifest at the whole-organism level. This approach leverages basic biological information about our model animal available in the literature and takes advantage of information-rich functional
genomics data that reveal exposure responses at the lower levels of biological organization. One of the motivations for the present study was to develop a broader framework in which the organism level is used as a “pivot” or summarizing point for data collected at lower levels to higher levels of biological organization, such as the population and community levels. Recently submitted work follows a similar approach to the present study, looking at PCB126 and methylmercury exposure to sensitive and resistant \emph{F. heteroclitus}, linking gene expression data to behavioral effects, and predicting larval survival and growth (Albers, 2022; J. L. Albers et al., Department of Fisheries and Wildlife, Michigan State University, East Lansing, Michigan, United States, unpublished data). Ecological risk assessment requires estimation of risk at these higher levels of biological organization. Excellent examples of the successful extrapolation of stressors to higher levels include from the organism to the population (Martin et al., 2013; Murphy et al., 2008) and community (Forbes et al., 2017, 2019; Galic et al., 2018) levels.

The framework we describe uses suborganismal data to predict organismal response to a stressor using a generalizable framework that may be applied to other case studies. We demonstrated the potential for this model to be applied to organisms with different stressor sensitivities by changing two parameters to predict the response of DLC-tolerant killifish populations based on mostly data from sensitive populations. The incorporation of an abstract “damage” variable further emphasizes the potential adaptability of this framework. Lastly, the use of a DEB-based bioenergetic model may further facilitate adaptation of the present model framework to other species and extrapolation to higher levels of biological organization. As well as other DEB model variants, including the standard model (Kooijman & Kooijman, 2010), DEBkiss (Jager, 2015) can be applied to other organisms by altering the species-specific parameters with minimal to no changes to the underlying model equations. Also, DEB models have facilitated extrapolation from the organism to higher levels of biological organization, via individual-based modeling (Galic et al., 2018; Martin et al., 2012) and matrix models (Klanjscek et al., 2006). We anticipate that this framework could be expanded from the suborganism to the population or even community level in future work, potentially making the wealth of suborganismal data available in the literature directly applicable for management by estimating effects at levels of biological organization relevant to ecological risk assessment.

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Data Availability Statement—All data and scripts are accessible: Transcriptomic data were reported in Whitehead et al. (2012), and raw microarray data are available at EBI ArrayExpress Accession E-MTAB-496. Fundulus data and Matlab model files are available at https://github.com/lstevenson09/KillifishDLC.

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