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Incorporating Suborganismal Processes into Dynamic Energy Budget Models for Ecological Risk Assessment

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EDITOR'S NOTE:

This article was generated from the session "Predictive models in ecotoxicology: bridging the gap between scientific progress and regulatory applicability," presented at the 27th SETAC Europe Annual Meeting (May 2017, Brussels, Belgium). The session considered approaches used in ecotoxicology for understanding and predicting the effects of chemicals, from QSAR to ecological modelling. This series aims to critically analyze and debate application examples and future developments to increase the acceptability of predictive models by regulators, managers, NGOs, and other stakeholders.

ABSTRACT

A working group at the National Institute for Mathematical and Biological Synthesis (NIMBioS) explored the feasibility of integrating 2 complementary approaches relevant to ecological risk assessment. Adverse outcome pathway (AOP) models provide "bottom-up" mechanisms to predict specific toxicological effects that could affect an individual's ability to grow, reproduce, and/or survive from a molecular initiating event. Dynamic energy budget (DEB) models offer a "top-down" approach that reverse engineers stressor effects on growth, reproduction, and/or survival into modular characterizations related to the acquisition and processing of energy resources. Thus, AOP models quantify linkages between measurable molecular, cellular, or organ-level events, but they do not offer an explicit route to integratively characterize stressor effects at higher levels of organization. While DEB models provide the inherent basis to link effects on individuals to those at the population and ecosystem levels, their use of abstract variables obscures mechanistic connections to suborganismal biology. To take advantage of both approaches, we developed a conceptual model to link DEB and AOP models by interpreting AOP key events as measures of damage-inducing processes affecting DEB variables and rates. We report on the type and structure of data that are generated for AOP models that may also be useful for DEB models. We also report on case studies under development that merge information collected for AOPs with DEB models and highlight some of the challenges. Finally, we discuss how the linkage of these 2 approaches can improve ecological risk assessment, with possibilities for progress in predicting population responses to toxicant exposures within realistic environments. *Integr Environ Assess Manag* 2018;14:615–624. © 2018 SETAC

Keywords: Adverse outcome pathways Dynamic energy budgets Ecological risk assessment Suborganismal processes Mechanistic

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INTRODUCTION

Ecological risk assessment is often based on estimates of biological effect (or hazard), but in conjunction with exposure it is intended to support decisions regarding the risks of introduced chemicals on populations, communities, and ecosystems (Landis et al. 2003). However, if we were to require natural population- or community-level data on the biological effect of chemicals, we would likely be estimating the effect too late—usually after the exposure and potential population decline has occurred. Thus, prospective ecological risk assessment inevitably involves integrating information at multiple levels of biological organization. There is no ideal resolution of this problem—all approaches have pros and cons (Rohr et al. 2016)—but to be effective and proactive, we must measure the effect of a chemical and its breakdown products over a range of doses in controlled laboratory experiments. With over 80 000 chemicals and millions of species, many of which are endangered (Zimmerman and Anastas 2015), toxicity testing at the whole-organism level for each species, each compound, and every potential combination is infeasible. Further, there are increasing concerns surrounding animal welfare and regulations surrounding animal testing. In 2007 in the US, the National Research Council recognized the limitations in the current status of toxicological testing and called for action to radically improve toxicological evaluation for individuals. The shift required much of the science that is focused on testing the harmful effects of chemicals to move toward development of techniques that are focused on in vitro and in silico testing to extrapolate molecular- and cellular-level effects to the whole organism (National Research Council 2007). This new framework, designed for the protection of human health, was adopted by environmental scientists and managers to assess the risks of environmental chemicals to fish, wildlife, and ultimately ecosystem health, and the framework that originally extrapolated from the molecular to the individual had to extend to the population level for ecological risk assessment (Villeneuve and Garcia-Reyero 2011). In 2016, in support of this new framework, the US passed bipartisan reforms to the Toxic Substance Control Act that embraced alternative testing strategies that include in vitro assays and in silico approaches (Nel and Malloy 2017).

We propose a conceptual model that would allow for the extrapolation of toxicological effects to populations from molecular-level responses to chemicals. This concept will integrate 2 complementary modeling approaches relevant to ecological risk assessment—adverse outcome pathway (AOP) and dynamic energy budget (DEB) models. AOP models provide "bottom-up" mechanisms to predict specific toxicological effects that could affect an individual's ability to grow, reproduce, and/or survive from a molecular initiating event (Ankley et al. 2010). Dynamic energy budget models offer a "top-down" approach that reverse engineers stressor effects on growth, reproduction, and/or survival by compartmentalizing resources based on their acquisition and processing.

ADVERSE OUTCOME PATHWAYS AND ECOTOXICOLOGY

An important bottom-up approach is called the AOP framework (Ankley et al. 2010). The AOP framework was conceptualized by integrating concepts across (eco)toxicology (including the mode-of-action framework), which originally included chemical-specific mechanistic information, into hazard assessment to aid in risk assessment and understanding of stressor-mediated adverse outcomes (Meek et al. 2003; Seed et al. 2005; Boobis et al. 2006, 2008). The AOP, however, is chemically agnostic and conceptualizes the cause–effect relationships from the (molecular) initiating event to higher-level adverse outcomes, including changes at the population level, as a first step to inform human and ecological risk assessment (Ankley et al. 2010; Villeneuve et al. 2014). The AOP framework has been used as an effective tool for arranging information at the suborganismal levels of organization and as an aid in interpreting data from high-throughput screening methods for the purpose of risk assessment. With these methods, the potential for thousands of chemicals to interact with molecular and cellular processes can be determined rapidly and cost efficiently. For human health risk assessments, which focus on the protection of the individual, AOPs that link lower-level events to organismal level responses may be sufficient to indicate risk. For example, adverse outcomes may include cellular endpoints such as skin sensitization or abnormal cell proliferation (Nel and Malloy 2017), but how those interactions translate into effects on organismal performance and ecological processes remains uncertain (Margiotta-Casaluci et al. 2016). Ecological risk assessments are generally concerned with the protection of populations, food webs, or ecosystems, and there is a critical need to develop AOPs that inform these higher levels of biological organization (Rohr et al. 2017). Cellular and molecular data may never be sufficient for prediction of ecological effects, but they could be used to predict, for example, diverse outcomes on individuals that relate to the general processes of growth, survival, and reproduction, processes that are included in population-level assessments (Kramer et al. 2011).

To improve the use of AOPs in ecological risk assessment and to provide more accurate predictive models, we must develop biologically based, quantitative extrapolation tools or models that allow us to extrapolate cell- or tissue-level data to organism-level endpoints by creating quantitative AOPs (qAOPs). A qAOP can describe mathematically some or all of the cause–effect relationships within a given AOP from a molecular initiating event (MIE) to an adverse outcome. qAOPs can be a series of quantitative response-response relationships that describe transitions between key events (KEs) and, ideally, stem from a mechanistic model, but correlative information can also be useful (Conolly et al. 2017).

Quantifying the detailed mechanistic information necessary to develop qAOPs on a single species is challenging, expensive, and labor and time intensive (Margiotta-Casaluci

et al. 2016), and it is impractical to repeat for thousands of species. Existing AOPs generally converge on single biological endpoints as if independent (e.g., growth or reproduction) and thus ignore the trade-offs implied by resource limitation, e.g., through the competition for energy among physiological processes. These trade-offs are particularly important for supply-type organisms such as fish, reptiles, and insects. In supply-type organisms, which are the majority of species, physiological processes such as growth and reproduction are heavily dependent on environmental conditions, such as food availability and temperature. Therefore, for these organisms, if an AOP converged on feeding rates, maintenance, or growth, the energetic trade-offs between physiological processes would become more important (Jager 2016). This is in contrast to demand-type organisms, such as mammals and birds, in which growth and reproduction schedules are preprogrammed, and it is up to the organism to find appropriate resources to meet the demand (Lika et al. 2014; Jager 2016). Furthermore, important feedbacks exist at each level of organization. Exposure to toxicants commonly triggers protective physiological responses, such as enhanced synthesis of antioxidant compounds, in response to oxidative stress (Klanjscek et al. 2012, 2016). Individual organisms may also induce protective (or other) changes in their physicochemical environment (Stevenson et al. 2013) or their food environment (Martin et al. 2013b, 2014). Furthermore, some species may be capable of relatively rapid adaptation to chronic chemical stress (Di Giulio and Clark 2015; Nacci et al. 2016; Reid et al. 2016; Du et al. 2015). In short, the AOP framework, in its current form, is unable to provide a suitable framework for predictive modeling and hence improved risk assessment. A suitable framework should be able to explain the effects of toxicants on an organism's acquisition of resources from the environment and the consequences for energy-demanding traits such as growth and reproduction (Jager et al. 2016). Because of these limitations and the uncertainty surrounding the quantitative linkages, the use of the AOP framework in regulatory policy has been limited. However, where it can make an effect is in "win–win" situations, such as screening of potential new chemicals and prioritization of chemicals for further testing (Elliott et al. 2017). It is also likely to gain traction when development and acceptance of AOPs are done with full transparency and engagement with stakeholders (Elliott et al. 2017).

DYNAMIC ENERGY BUDGETS AND ECOTOXICOLOGY

In contrast with the "bottom-up" AOP approach, DEB theory (Jusup et al. 2017; Kooijman 1986, 2010; Nisbet et al. 2000) offers a "top-down" mechanistic conceptual framework for connecting suborganismal to organismal processes. The starting point for any study is a multicompartment dynamical systems model of the performance (growth, development, reproduction, mortality risk) in arbitrary environments. Although core themes of DEB models are conservation of energy and elemental matter, the theory also makes intimate connections with physiology through assumptions on homeostasis. The end result is that the DEB approach offers unifying metabolic theory that can be applied to any species with a small number of parameters (Kearney et al. 2015). In addition, the theory can be used to model effects of chemicals on individual organisms (e.g., Jager et al. 2006, 2011; Muller et al. 2010) and effects of other environmental drivers and stressors in a single integrative modeling framework (e.g., Muller and Nisbet 2014; Pieters et al. 2006) Moreover, the approach offers the possibility to extrapolate population-level and higher dynamics from an individual-level energy budget by individual-based modeling (Martin et al. 2013a, 2014; Gergs et al. 2014, 2016). This suggests that there is potential to provide a connection from an AOP to ecologically important levels of organization, but only if we have some quantitative approach for relating qAOP and DEB models.

Kooijman's DEB theory captures the metabolic dynamics of an individual organism through its entire life cycle, be it ectothermic or endothermic, autotrophic or heterotrophic, and is explicitly tied to food or substrate availability and temperature. The life cycle of an individual is the primary focus, from which sub- and supraorganismic levels are considered. Thus, DEB theory can serve as a pivotal framework for building process-based models that link molecular-, cellular-, and tissue-level responses to apical endpoints, such as survival, growth, and reproduction (Murphy et al. 2018), and subsequently to those at higher levels of ecological organization (Martin et al. 2013a,b; Forbes et al. 2017; Gergs et al. 2014, 2016).

The first systematic body of work using DEB models to interpreting toxicity data involved a suite of models (Kooijman and Bedaux 1996) that established methodology for using information from standardized toxicity tests to obtain biology-based measures of no-effect concentrations and other metrics that were independent of experimental protocols (see Baas et al. [2010]). Wider applications of the approach, now generally called DEBtox (for a freely available introduction, see https://leanpub.com/debtox_book), followed, as well as an Organization for Economic Cooperation and Development (OECD) guidance document (OECD 2006). However, this guidance document appears to be the extent of the incorporation of DEB into regulatory practices to date.

The key to DEBtox is the concept of "physiological mode of action" (pMoA) that summarizes how a stressor affects parameters associated with processes involving energy acquisition and use. For example, Kooijman's standard model contains parameters characterizing rates of resource assimilation, maintenance, turnover of energy reserves (linked to homeostasis), energy allocation priorities, and "efficiencies" or "yield coefficients" characterizing the consequences of biochemical or thermodynamic constraints. Absent other information, any of these physiological parameters could change in response to within-organism levels of contaminant. Modeling toxicity requires coupling the DEB representation of physiological processes to toxicokinetic (TK)

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and toxicodynamic (TD) submodels (Ashauer et al. 2011; Ashauer and Escher 2010). TK models describe the dynamics of bioaccumulation, elimination, and chemical transformations of chemical contaminants within an organism. Toxicodynamic (TD), sometimes called "toxic effect," models describe processes leading from toxicant interaction with a biological target to effects, which is achieved by making assumptions on the dominant pMoAs. TK-TD modeling, in general, has been included into the European Food Safety Authority (EFSA) guidance documents for risk assessment (EFSA 2013).

DEBtox models have been used to analyze chronic toxicity data under (assumed) constant exposure conditions (e.g., Jager et al. 2006; Jager and Selck 2011; Goussen et al. 2015) or time-varying exposure (Pieters et al. 2006) and to analyze effects resulting from chemical mixtures (Jager et al. 2010). These models have usually been applied to organism growth and reproduction data to identify the likeliest pMoA. However, in many cases, when the available data is limited in diversity (e.g., only reproduction data at the end of a standardized toxicity test), those data could be equally well described by several pMoA candidates. Identification of a pMoA typically requires a data set including time-resolved measurements and multiple endpoints (Muller et al. 2010; Jager et al. 2016). Furthermore, sublethal chemical effects might not be adequately described by a single pMoA; rather, it is conceivable that some chemicals affect multiple pMoAs (e.g., maintenance and feeding), and DEBtox integrates the affected pMoA. This is in contrast to qAOPs or qAOP networks that converge on a single adverse outcome.

In parallel with advances relating to chronic toxicity via DEBtox, there have been advances in modeling survival that use toxicokinetic-toxicodynamic (TK-TD) approaches but do not consider detailed physiological processes that cause mortality. These have, to a great extent, been reconciled within the General Unified Threshold model for Survival (GUTS; Jager et al. 2011), which invokes a dose metric, assumed proportional to the hazard (i.e., per capita mortality) rate, and may include processes such as bioaccumulation, distribution within the organism, biotransformation and elimination, damage accrual and recovery, and physiological compensation processes. Of particular importance for this paper is the abstract dynamic variable damage, described by Ankley et al. (1995) and related to the physiogically determined component of mortality. The definition of "damage" is context specific, but common examples would be damaged membranes or organelles, "wrong" proteins, or DNA damage (Kooijman 2010, chapter 6). The complexity of the dynamic equations for damage differs among studies, depending on data availability and the processes considered in the approach. Approaches can include a 1 parameter-scaled damage model and damage accrual and recovery (Jager et al. 2011; Ashauer et al. 2007; Klanjscek et al. 2016).

Previous work suggests that the damage concept may have value for linking qAOP and DEB. An early study of receptor kinetics by Jager and Kooijman (2005) analyzed survival of organisms exposed to organophosphorus pesticides. This remarkably simple model assumed that functional receptors are knocked out by the chemical, and functional receptors are turned into nonfunctional ones. Veltman et al. (2014) extended this approach to predict Na loss and acute mortality in several aquatic species. Enzyme (acetylcholinesterase) inhibition was also considered in the time-dependent accrual of damage on the molecular level to explain differential sensitivity at the organism level in *Daphnia magna* (Kretschmann et al. 2011, 2012).

The appealing simplicity and generality of DEB theory comes with a price; model quantities and processes have a relatively high level of abstraction. Auxiliary assumptions (that may be organism specific) link abstract variables to quantities that can be measured directly such as length, wet or dry weight, respiration, time to/length at first brood, egg output, and so on (Lika et al. 2011). Yet there is a large body of literature on methods for estimating DEB model parameters, including routine multivariate, nonlinear regression (or analogous likelihood) methods (Kooijman et al. 2008), a computer-intensive state-space method (Fujiwara et al. 2005), a Bayesian approach (Johnson et al. 2013), and an innovative, heuristic "pseudo-Bayesian" approach (Lika et al. 2011) that is currently the most widely used approach to the estimation of DEB parameters (called AmP, http://www.bio.vu.nl/thb/deb/deblab/add_my_pet/).

LINKING AOP TO DEB

qAOP and DEB models have contrasting strength and weaknesses. The approach presented here attempts to reduce the weaknesses and strengthen the advantages of either method. Both the AOP and the DEB approaches have the ultimate goal of informing predictions on ecologically important processes, most of which occur at population, community, or ecosystem levels (Fig. 1). The DEB modeling framework has the potential to integrate suborganismal processes from the AOP framework, to fine tune the mechanism by which a stressor operates, and to potentially incorporate high-throughput testing, potentially from previously developed assays in ToxCast (https://www.epa.gov/chemical-research/toxcast-dashboard). The importance of our overarching goal of using information on suborganismal toxicity for population dynamic projections was highlighted by Martin et al. (2014) who constructed an individual-based population model for *Daphnia* interacting with their algal food. They found that the outcome commonly depends strongly on the suborganismal mode of action. Indeed, when consumer-resource interactions are considered, toxicity mediated by different physiological processes that lead to the same outcome in a standard reproduction test may cause drastically different effects at the population level. These ranged from almost no effect to extinction, with contrasting effects on total population and biomass of stressors that involve different pMoAs. For example, a direct effect on reproduction caused a decrease in equilibrium population abundance but little change in population biomass, whereas a pMoA that changed the growth efficiency led to a reduction in population biomass accompanied commonly by an increase in total population. Stressors that changed feeding

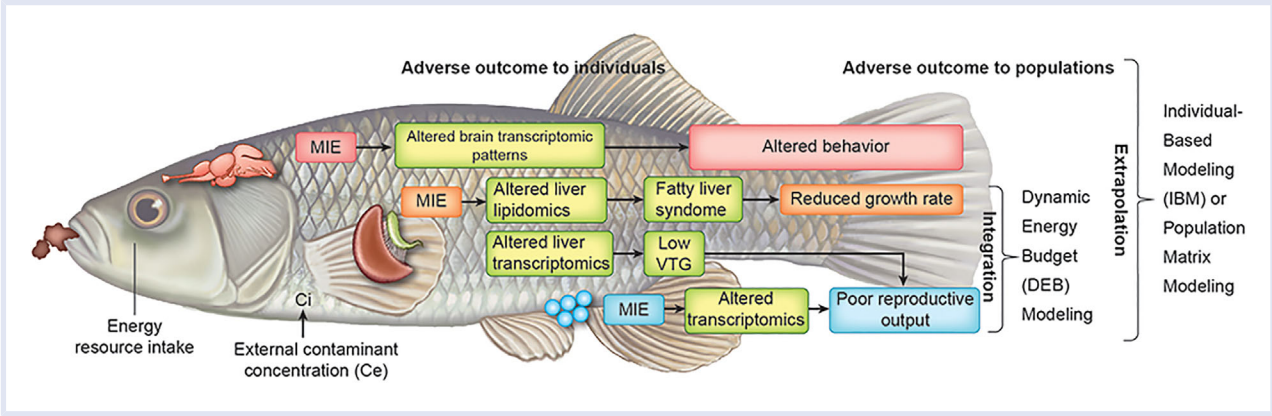

Figure 1. A conceptual model to link AOPs to DEB for a particular stressor or contaminant scenario will first require an inventory of the key events affected in different organs and at different levels of biological organization, such as molecular, cellular, organ-level responses. The dynamic energy budget integrates the key events and acts as a pivot to population-level outcomes. Ce = concentration of toxicant external to the organism; Ci = concentration inside the organism; MIE = molecular initiating event; VTG = vitellogenin.

and assimilation or maintenance concentration of toxicant external to the organism rates caused simultaneous reductions in abundance of biomass. These different responses were accompanied by substantial changes in size and structure, potentially of ecological importance for zooplankters in a food web.

Essentially, an AOP represents a pathway that is integral to a DEB, and we propose that translations from one to another are possible by using the damage variable introduced in the preceding section. Within the AOP, KEs and adverse outcomes that occur at the molecular, cellular, organ, and whole-organism level can influence DEB parameters and hence have a significant effect on the damage variable(s). A theory that offers semimechanistic submodels for connecting damage to fluxes was recently developed (Muller et al. 2018) and provides a flexible framework. This potentially integrates the AOP into a more holistic DEB model that uses a relatively small number of variables and parameters to integrate all metabolic processes, that allows for trade-offs of energy between growth, reproduction, and survival in multivariate environments, and thus generates population-relevant outputs.

Murphy et al. (2018) proposed a conceptual model for mechanistically linking AOP to DEB (Table 1; Figure 2). The mathematical structure, shown in Table 1, recognizes five types of variables and their causal connections. KEs are measurable endpoints in an AOP (e.g., Figure 2). These KEs either represent, or are themselves, measures of damage that is manifest at the level of cells, organs, or of the entire organism. Damage is caused, directly or indirectly, by internal toxicant concentrations—the accumulation of damage being described by some TK/TD representation. Damage affects the processes in a bioenergetic model. This flow of causal connections restricts the form of dynamic equations we can use (see Table 1 for a list of variables and functional dependencies).

In applications that we can currently envision, the model of the complete organism will use dynamic (differential or difference) equations to describe "performance"—growth, development, reproduction, damage, and risk of mortality. These are described by the DEB model, which is tightly coupled to the body burden dynamics (growth affects internal toxicant concentration and vice versa), and also to

Table 1. Proposed variables and equations used to characterize response to toxicity within an organism (from Murphy et al. 2018)

Variables
$\mathbf{K} = \{K_1, K_2, \ldots\}$ = set of suborganismal key events from AOP
$\mathbf{R} = \{R_1, R_2, \ldots\}$ = set of damage-related variables—may overlap with $\mathbf{K}$
$\mathbf{Q} = \{Q_1, Q_2, \ldots\}$ = set of internal toxicant-related concentrations
$\mathbf{B} = \{B_1, B_2, \ldots\}$ = set of DEB model variables
$\mathbf{E} = \{E_1, E_2, \ldots\}$ = set of environment variables
Dynamics
$\frac{d\mathbf{B}}{dt}$ = functions of $\mathbf{B}, \mathbf{R}, \mathbf{E}, \mathbf{Q}$
$\frac{d\mathbf{R}}{dt}$ = functions of $\mathbf{Q}, \mathbf{R}, \mathbf{K}$ (only occasionally $\mathbf{B}$)
$\frac{d\mathbf{Q}}{dt}$ = functions of $\mathbf{B}, \mathbf{Q}$ – TK model

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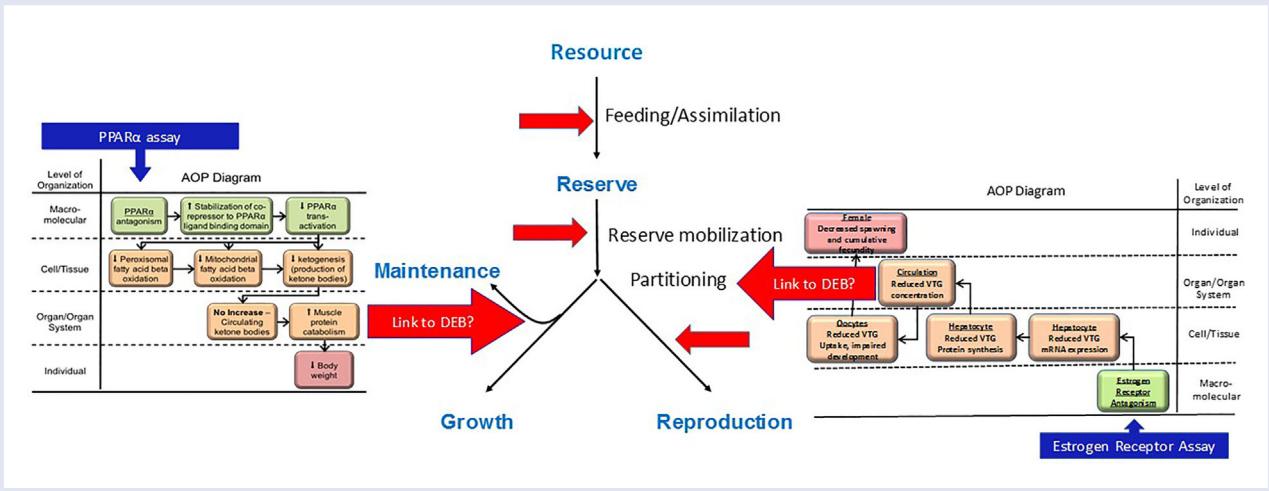

Figure 2. Key event network (mode of action) translates to some measure of damage that would have to be related to key rates or allocations (indicated by red arrows) in dynamic energy budget models. Here, 2 different AOPs are represented, the first related to reproduction and the second related to growth. While more complex relationships are likely, we show here for clarity a simple 1-to-1 relationship between an MIE and a DEB parameter (indicated by larger red arrows). Estrogen receptor assays (e.g., those used in screening programs like ToxCast, https://www.epa.gov/chemical-research/toxcast-dashboard) can inform vitellogenesis, egg production, and population trajectories (https://aopwiki.org/aops/30) and could potentially link to the partitioning rule in DEB that determines what proportion of energy goes toward reproduction or growth but also to the utilization of material in the reproductive buffer. Similarly, information from PPARα assays (such as those included in ToxCast screening—Toxcast lists 3 assays, 2 are human liver cell based [for cis and trans config] and one is cell free). PPARα antagonism leads to reduced ability to obtain energy from fatty acids and reduces the production of ketone bodies and ultimately results in increased muscle protein catabolism that reduces body weight in an individual (https://aopwiki.org/aops/6). Again, 1 AOP may actually link to more than 1 flux in DEB.

the damage dynamics. Changes in internal concentrations are described by a TK submodel. The damage variables will commonly be conceptually different from the higher-level processes with the causal link unknown or only vaguely understood, but explicit connection is possible, e.g., oxidative stress involves production of reactive oxygen species from both "routine" metabolism and from toxicity.

We see potential in using statistical methodologies to identify appropriate connections in Figure 2, recognizing that information on pathways effected by toxicants comes increasingly from 'omics (e.g., transcriptomics, proteomics, lipidomics, metabolomics)-based data (Murphy et al. 2018). Lists of features such as genes, metabolites, or proteins that are significantly changed as a result of a given perturbation can be used to identify higher-level biological processes that are potentially affected in response to exposure. Combinations of these biological processes are represented by DEB rates and fluxes. With small model organisms such as *Daphnia*, this may be the only approach available, as data on the effects of contaminants is measured at either the molecular level or the whole-organism level, and to link AOP to DEB the molecular information would have to be directly translated to DEB rates.

For larger organisms such as fish, organ-level data are available and in our NIMBioS working group, we are exploring mechanistic approaches to link qAOPs to DEBs via endocrine disruption as the stressor, and rainbow trout (*Oncorhynchus mykiss*) as the target species. We were fortunate to have access to a rich data set on rainbow trout hormones, egg production, weights, as well as a well-developed physiologically-based model (Gillies et al. 2016). Endocrine disruption is perhaps the most studied and best understood system in ecotoxicology, and extensive qAOPs have been developed to link MIE to adverse outcomes such as egg production in a few fish species (Conolly et al. 2017; Gillies et al. 2016). Therefore, we focused on endocrine disruption for these case studies, but ideally, the linkage between qAOPs that converge on maintenance and growth adverse outcomes, once developed, should also be explored as they connect to pMoA in DEB because then the full implication of energetic trade-offs could be realized. For this study, we found 2 ways to link existing qAOPs related to endocrine disruption to DEB and discuss them briefly here.

One method is to change the assumptions on the rules for energy allocation between growth and reproduction in the DEB framework to create demand-driven feedback mechanisms that can be exerted by the gonads on the allocation of resources to production of reproductive matter. With this approach, species- and sex-specific characteristics of endocrine regulation can be modified, while keeping the remainder of the DEB core intact. Ongoing work indicates that this modeling approach successfully describes the time-resolved measurement of body weight, ovaries, and liver, as well as the egg diameter of spawned eggs and plasma levels of estradiol and vitellogenin in rainbow trout. The approach is flexible and can be adjusted for different reproductive strategies such as iteroparity, semelparity, and batch-mode reproduction, depending on species and available data. The next step in this approach will be to add toxicokinetics and simulate endocrine disruption.

Another approach is to add an egg module to the standard DEB model, allowing for hormone dynamics to control conversion of material in the DEB "reproduction buffer" into eggs (Figure 3). In this approach, well-developed physiologically-based models (e.g., Conolly et al. 2017; Gillies et al. 2016; Li et al. 2011; Murphy et al. 2009;

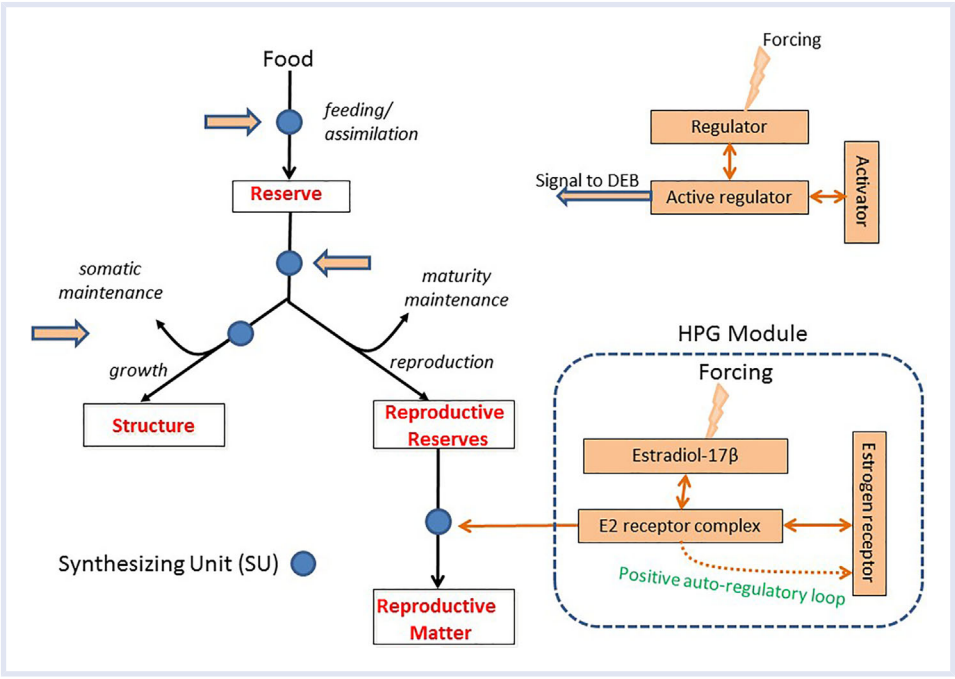

Figure 3. One approach to link qAOPs to DEB is to create a simplified submodel of the physiological processes (in this case, the hormones involved in the fish hypothalamic–pituitary–gonadal axis [HPG axis]); this submodel can regulate model fluxes within the standard DEB by interfacing with synthesizing units (SU; blue dot). The model of the HPG axis is reduced into forcing function, regulator, activator, and active regulator.

Watanabe et al. 2009) are collapsed into simple forcing functions, regulators, activators, and active-regulator dynamics and incorporated into the standard DEB through simple characterizations of networks as "generalized enzymes" or "synthesizing units"; Kooijman 2010). Although this example is restricted to reproduction, forcing functions may be useful for other stressors and physiological modes of action in DEB, possibly by utilizing the functions from Muller et al. (2018).

DISCUSSION

In this paper we described a conceptual model for linking AOP to DEB. Our working group has developed 2 case studies based on *Daphnia* and rainbow trout that merge information collected for AOPs with DEB. Our ongoing work on rainbow trout that focuses on endocrine disruption for which there are existing quantitative AOPs that integrate molecular-, cellular-, and organ-level responses to predict effects on reproduction. We are investigating approaches in which connecting with a DEB representation is achieved either by modifying the "standard" DEB model to include feedbacks that characterize the integrated effects of hormonal control mechanisms or by adding a module to standard DEB. With *Daphnia*, there is little organ-level data, so we are seeking correlative connections with transcriptomic data (work in progress). In both studies, our goal is to identify a set of KEs or a key event network leading to measures of "damage" that affect specified DEB parameters and rates. Eventually we envision a system where AOPs link to DEB rates, and the DEB is then used within the construct of a whole organism where energetic trade-offs between physiological processes are considered. Such a system would improve the predictive power of suborganismal key events, by placing such KEs into a framework that would allow for extrapolation to population (via IBMs) and up to population, community, and ecosystem effects (Figure 4).

Ashauer and Jager (2018) hypothesized that "chemicals of the same class," i.e., triggering the same MIE and hence having the same adverse outcome, should it exhibit the same pMoA when toxicity data are analyzed with DEBtox methods. They found very limited evidence to support this hypothesis; indeed, "baseline toxicants" apparently exhibit different dominant pMoAs for *Daphnia magna*. They cautioned that unambiguous identification of pMoA is remarkably challenging and discussed data requirements to resolve this. A more fundamental issue is that all DEBtox analyses known to us assume that there are is a single dominant pMoA, or occasionally 2 pMoAs, but the energy fluxes in a DEB model represent flows of abstractly defined generalized compounds. As recognized earlier in this paper, 1 AOP might correspond to a combination of several DEB fluxes and apparent differences in dominant pMoA may simply reflect different relative weights for each DEB flux. We hope that our approach may help resolve the apparent dichotomy identified by Ashauer and Jager (2017).

In recent years, with the advent of 'omics technologies and their ever-decreasing cost, it has become feasible to more comprehensively integrate subcellular effects with higher levels of biological organization. While the computational approaches for true integration of multilevel data are still scarce, a simpler integration across these levels has been approached in a number of publications (Rohart et al. 2017; Van Aggelen et al. 2010; Williams et al. 2011; Joyce and Palsson 2006). Looking forward, AOPs in particular would benefit greatly from multilevel analysis approaches as an AOP inherently represents the combination of many biological

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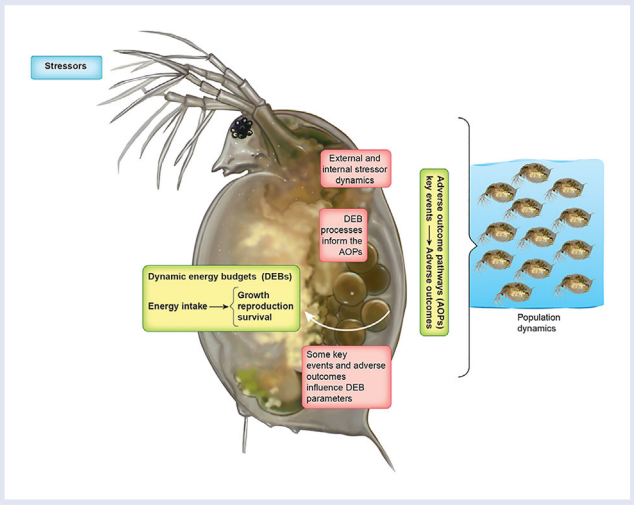

Figure 4. Schematic relating parallel descriptions of suborganismal processes (AOP and DEB) and how they can interact to improve predictions of how whole organisms respond to stressors.

levels. Metaboanalyst 3.0 (http://www.metaboanalyst.ca/faces/home.xhtml) is an excellent example of the current state of multilevel integration, allowing for metabolite and gene expression level integration to understand the possible affected pathways within a given organism. With the increase of available metabolic models for more and more species, a more integrated methodology could be developed for understanding effects across multiple levels. The approach described in this paper represents a first cut at such integration.

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Data Accessibility—This is an overview paper, and as such, references no data which would need to be provided.

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