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Relating suborganismal processes to ecotoxicological and population level endpoints using a bioenergetic model

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Abstract. Ecological effects of environmental stressors are commonly evaluated using organismal or suborganismal data, such as standardized toxicity tests that characterize responses of individuals (e.g., mortality and reproduction) and a rapidly growing body of "omics" data. A key challenge for environmental risk assessment is relating such information to population dynamics. One approach uses dynamic energy budget (DEB) models that relate growth and reproduction of individuals to underlying flows of energy and elemental matter. We hypothesize that suborganismal information identifies DEB parameters that are most likely impacted by a particular stressor and that the DEB model can then project suborganismal effects on life history and population endpoints. We formulate and parameterize a model of growth and reproduction for the water flea *Daphnia magna*. Our model resembles previous generic bioenergetic models, but has explicit representation of discrete molts, an important feature of *Daphnia* life history. We test its ability to predict six endpoints commonly used in chronic toxicity studies in specified food environments. With just one adjustable parameter, the model successfully predicts growth and reproduction of individuals from a wide array of experiments performed in multiple laboratories using different clones of *D. magna* raised on different food sources. Fecundity is the most sensitive endpoint, and there is broad correlation between the sensitivities of fecundity and long-run growth rate, as is desirable for the default metric used in chronic toxicity tests. Under some assumptions, we can combine our DEB model with the Euler-Lotka equation to estimate long-run population growth rates at different food levels. A review of *Daphnia* gene-expression experiments on the effects of contaminant exposure reveals several connections to model parameters, in particular a general trend of increased transcript expression of genes involved in energy assimilation and utilization at concentrations affecting growth and reproduction. The sensitivity of fecundity to many model parameters was consistent with frequent generalized observations of decreased expression of genes involved in reproductive physiology, but interpretation of these observations requires further mechanistic modeling. We thus propose an approach based on generic DEB models incorporating few essential species-specific features for rapid extrapolation of ecotoxicogenomic assays for *Daphnia*-based population risk assessment.

Key words: bioenergetics; Daphnia magna; *dynamic energy budget model; individual growth and reproduction; molt dynamics; population growth rate; risk assessment; sensitivity analysis; standardized toxicity tests; toxicogenomics.*

Introduction

An increasingly important challenge in ecological risk assessment is to relate ecologically relevant processes to the growing body of suborganismal data (including high-throughput "omics" assays; "omics" refers to all types of omics, i.e., genomics, transcriptomics, proteomics, and metabolomics.), and thus discover mechanistic connections between biomolecular processes and subsequent whole organism, population, community, and ecosystem outcomes. This need to relate many levels of biological organization has been formally characterized through the adverse outcome pathways (AOP) paradigm (Ankley et al. 2010, Kramer et al. 2011). An AOP is defined as "a conceptual construct that portrays existing knowledge concerning the linkage between a direct molecular initiating event and an adverse outcome at a biological level of organization relevant to risk assessment" (Ankley et al. 2010). Kramer et al. (2011) discussed an AOP approach that connects mechanistic

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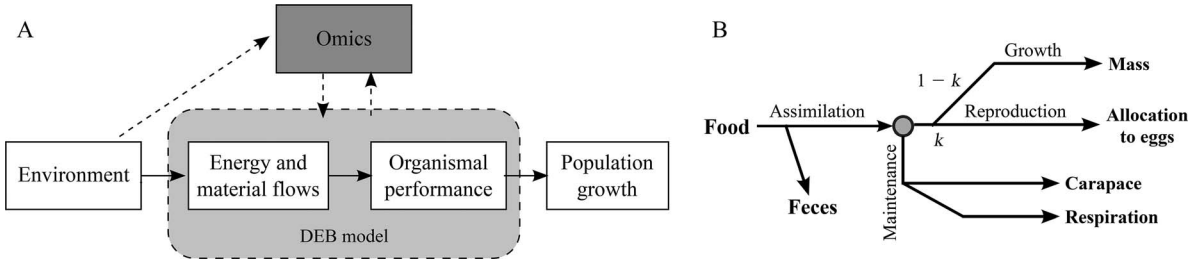

FIG. 1. (A) A dynamic energy budget (DEB) model relates the transformation of energy and material from the environment by an individual and thereby determines individual-level phenomena, such as growth and reproduction. With additional assumptions, this model also describes growth rates of a population sharing a common environment. From a molecular viewpoint, the DEB model equations are phenomenological with causal connections between environmental changes and DEB model parameters mediated by genomic, proteomic, and metabolomic processes (dashed arrows). "Omics" refers to all types of omics, i.e., genomics, transcriptomics, proteomics, and metabolomics. (B) The energy flow schematic for the molt-by-molt DEB model of an individual *Daphnia*. The energy source (food) and sinks are in bold and the processes are listed on the arrows; k is the energy allocation function.

suborganismal information to organismal-level measures of survival and reproduction, which in turn are quantitatively linked to population and community dynamics. The approach we adopt here, highlighted by Kramer et al. (2011), for making the connection from suborganismal effects of toxicants to population dynamics uses dynamic energy budget (DEB) models (Kooijman and Metz 1984, Kooijman and Bedaux 1996, Muller et al. 2009*a*, *b*, Baas et al. 2010, Jager and Zimmer 2012). Dynamic energy budget (DEB) model is used in the literature in two ways. The broad definition (Nisbet et al. 1996, 2004) used in this paper includes all dynamic models that describe the acquisition and utilization of energy by organisms and includes many so-called bioenergetic models. A narrower definition refers to one large family of models developed by Kooijman and collaborators (Kooijman 1986, 2000, 2010). The relationships between the different model approaches are discussed by Nisbet et al. (2012).

DEB models relate performance of individuals (e.g., growth and reproduction) to underlying flows of energy and elemental matter (e.g., feeding, maintenance costs) that, in turn, are impacted both directly and indirectly by environmental factors (e.g., temperature and food availability; see Fig. 1a). Physiological rates in individuals are described by mathematical functions with parameters that characterize combinations of suborganismal processes, whose values may change in response to environmental stressors, such as toxic substances. The effect of a stressor on a population of organisms sharing a common environment can be evaluated using individual-based (Grimm et al. 2005) or structured population models (Tuljapurkar and Caswell 1997). Additionally, in an ideal situation, where all organisms are assumed to experience the same mortality risk (hazard rate), the long-run population growth rate with stressors can be extrapolated from individual performance described by the DEB model using the Euler-Lotka equation (Kooijman and Metz 1984).

Daphnia is an ideal model organism for exploring gene-to-ecosystem connections (Miner et al. 2012). In brief, *Daphnia* has a pivotal role in freshwater foodwebs and is used in many standardized toxicity tests (U.S. Environmental Protection Agency 2002, American Society for Testing and Materials 2004, International Organization for Standardization 2012, OECD 2012). *Daphnia* typically reproduce parthenogenetically, thereby allowing the study of genomics, organismal performance, and population consequences independent of the effects of genetic variability, though the modeling approach will remain valid with multiple clones; see Nelson et al. (2007). There is a large body of literature covering previous studies of individuals and populations for the common *Daphnia* species (including *D. magna*), allowing much model development and parameter estimation without new empirical work. There is also a growing body of genomic information on *Daphnia* (e.g., Poynton et al. 2007, 2008*a*, *b*, Connon et al. 2008, Heckmann et al. 2008, Garcia-Reyero et al. 2009, 2012, Colbourne et al. 2011, Christie and McCoole 2012, Orsini et al. 2012). Finally, it is likely that our findings will generalize beyond this model organism, first because *Daphnia* is a good representative, both physiologically and ecologically, of freshwater pelagic zooplankters, but also because bioenergetic models have been shown capable of offering deep insight on individual-to-population connections in multiple ecological contexts (de Roos and Persson 2013).

We formulate and parameterize a detailed, empirically based model of growth and reproduction of individuals for the water flea *Daphnia magna* and evaluate its consistency with a large body of previously published data, as well as some new data generated at the U.S. Army Engineer Research and Development Center (ERDC) for this effort. We explicitly represent molting in our model because many toxicological endpoints and several life history measurements of interest in *Daphnia* are potentially affected by variability in molt duration (Münzinger 1990, Zou and Fingerman 1997, Zou 2005), molting is a vulnerable stage where mortality risk can be high (Lee and Buikema 1979, McCahon and Pascoe

1988, Baer and Owens 1999), and previous transcriptomic studies showed that expression of exoskeleton-related genes (built and discarded at the molt) change in response to metal contaminants, e.g., chitin and cuticle metabolism genes in Poynton et al. (2007).

Many previous bioenergetic models of growth and reproduction in *Daphnia* have been proposed and tested and differ in structure, complexity, and objectives. Much of the literature prior to 1989 was reviewed by McCauley et al. (1990). Many models use representations of growth where some measure of size, such as length or mass, is treated as a continuous variable (e.g., Paloheimo et al. 1982, Kooijman 1986, 2010, Nisbet et al. 2004 and many references therein, Preuss et al. 2009). Only a few previous models recognize that *Daphnia* growth proceeds through a sequence of discrete instars with abrupt changes in length and deposition of eggs in the brood chamber occurring at the molt (Gurney et al. 1990, Asaeda and Acharya 2000). Our model structure resembles that of Gurney et al. (1990) for *D. pulex*, but some important model assumptions differ on the basis of an extensive survey of data, much of which was published after 1990. The availability of new data removes the need for some nonmechanistic ad hoc assumptions in the model of Gurney et al. (1990).

We determine the sensitivity of several ecotoxicological endpoints and population growth rate to changes in model parameters that each represent specific physiological rate processes. The sensitivity patterns are summarized in heat maps (Figs. 4 and 5) in a manner analogous to the large-scale toxicogenomic profiling assays on *D. magna* (e.g., Table 1 in Poynton et al. [2007]). Such transcriptomic studies have the potential for rapid characterization of a large array of possible contaminants and toxicants. Equipped with our DEB model, we map literature-review-derived toxicogenomic results to model parameters in order to assess the potential of extrapolating pollutant-induced effects on gene expression identified in these toxicogenomic assays to consequences on individuals, thereby allowing predictions (not tested) on population growth rates. In particular, we demonstrate that the pathway- and process-level effects of natural and anthropogenic stressors on *Daphnia* characterized in an expanding suite of genomic investigations (Poynton et al. 2007, Garcia-Reyero et al. 2009, Poynton and Vulpe 2009, Colbourne et al. 2011) can be naturally mapped to model parameters that represent organismal processes.

Methods

Model specification

Our model describes growth, development, and reproduction of an individual animal in an arbitrary food and temperature environment. The model is deterministic; although variability in growth, reproduction, and survival of individuals due to clonal, environmental, and genetic differences are well documented, we disregarded these sources of "noise". In common with many previous models (Gurney et al. 1990, Noonburg et al. 1998, Nisbet et al. 2004, 2010), we chose carbon as the model currency. Carbon flows in the model are shown in Fig. 1, and the model equations are listed in Table 1. Net production, which is the assimilated energy in excess of the total maintenance costs, is allocated in a size-dependent manner between growth and reproduction. An individual's mass changes continuously, while its length changes only at the molts.

The model estimates the reproduction of an individual experiencing any given environment. When coupled with assumptions on mortality, the model thus predicts the lifetime reproductive output and the long-run growth rate of a population experiencing that environment. The key model assumptions are as follows.

The ingestion rate is the product of a length-dependent maximal feeding rate and a type 2 functional response that describes the dependence of ingestion rate on the algal food density. The assimilation efficiency is constant, independent of the state of the animal and its environment. Thus, a fixed fraction of ingested food is assimilated. The maintenance rate is the sum of a mass-dependent contribution to the respiration rate and a carapace construction cost that is distributed evenly over the duration of the current molt. Other contributions to respiration, such as those resulting from the transformation of food into biomass and specific feeding-related energetic costs, sometimes termed specific dynamic action (Bohrer and Lampert 1988, Nisbet et al. 2012), filtering, and swimming costs are lumped together into a reduced effective assimilation efficiency. Juveniles mature into adults when juveniles exceed a threshold length. Only adults allocate energy towards reproduction. Juveniles, starving adults (whose net production is negative), and adults (whose mass does not satisfy the minimum mass for their current length) all allocate the entire net production to growth. Otherwise, in adults whose mass exceeds the minimum mass for their current length, the distribution of net production between growth and reproduction is governed only by the length of the individual and hence, the distribution between growth and reproduction remains constant during the intermolt period.

The progress towards a molt is described by a molt development index that grows from zero and signals a molt on reaching a value one (Nisbet et al. 1989, McCauley et al. 1996). The rate of progress to the next molt (i.e., rate of increase of the molt development index) is a linear interpolation between the minimum rate (reciprocal of the maximum molt duration) and the maximum rate (reciprocal of the minimum molt duration) of progress. This food-dependent linear interpolation is determined by the ratio of the actual net production and the maximum (possible) net production under identical conditions. The minimum and maximum molt durations are size dependent.

TABLE 1. The complete description of the bioenergetic model of a *Daphnia magna* individual at different life history stages with population parameters estimated with the Euler-Lotka equation.

Parameter	Euler-Lotka equation	Units
Between molts (between molt $n-1$ and n)		
Food ingestion rate	$I(t) = I_{\max}(T)L^{\mu_I}\frac{F(t)}{F(t)+f_h}$	mg C/d
Maintenance rate	$M(t) = R(T)\left(W(t)-C(t)\right) + \left(\frac{W}{W_C}\right)^{\mu_C}\min\left(\frac{1}{t_{\min}}, \frac{q(t)}{t-t_{n-1}}\right)$	mg C/d
Respiration rate as function of temperature T	$R(T) = R_{20}\lambda(T)$	d^{-1}
Net production	$P(t) = \varepsilon I(t) - M(t)$	mg C/d
Allocation of net production to reproduction	$\kappa = 0,$ if $P(t)<0$ or $W(t)<W_{WFL}$ $\kappa = 1-\frac{1}{1+\gamma^{\mu_A}(L-L_{Th})_+^{\mu_A}},$ otherwise	
Molt development index	$\frac{dq}{dt} = \left[\frac{1}{t_{\min}} + \left(\frac{1}{t_{\min}}-\frac{1}{t_{\max}}\right)\frac{[P(t)]_+}{\varepsilon I_{\max}-M(t)}\right]\lambda(T)$ $t_{\min} = \frac{t_{\min}^{20}L^{\mu\min}}{\lambda(T)}$ and $t_{\max} = \frac{t_{\max}^{20}L^{\mu\max}}{\lambda(T)}$	d^{-1}
Mass gain	$\frac{dW}{dt} = (1-\kappa)P(t)$	mg C/d
Assimilate allocated to eggs	$\frac{dE}{dt} = \kappa P(t)$	mg C/d
Length	$\frac{dL}{dt} = 0$	mm/d
At each molt/when $q(t) = 1$ (at current molt n)		
Length corresponding to molt mass	$L(t_n) = L_W W(t_n)^{\left(\frac{1}{\mu_W}\right)},$ if $W(t_n) > W(t_{n-1})$	mm
Carapace weight at new mass	$C(t_n) = \left(\frac{W(t_n)}{W_C}\right)^{\mu_C},$ if $W(t_n) > W(t_{n-1})$	mg C
Minimum respiratory energy required to produce an egg of mass W_0	$W_{E0} = W_0 e^{R(T)t_0};\ W_0 = \left(\frac{L_0}{L_W}\right)^{\mu_W}$	mg C
Number of eggs in current molt	$N_E = \frac{E(t)}{W_{E0}}$	
Neonates produced from eggs in brood pouch	$J(n) = N_E$	
Time of current molt	$t_n = t$	d
Reset allocation to eggs	$E(t) = 0$	mg C
Reset molt development index	$q(t) = 0$	
Food dynamics		
In batch cultures, at each feeding	$F(t) = F_{\text{batch}}$	mg C
between feedings	$\frac{dF}{dt} = -\frac{I(t)N}{V}$	mg C/d
In a semi-chemostat	$\frac{dF}{dt} = D\left(F_{\text{chem}} - F(t)\right) - \frac{I(t)N}{V}$	mg C/d
Population parameters		
Lifetime reproduction	$P = \sum_k N_E(k)e^{-\delta t_k}$	
Population growth rate	$\sum_k N_E(k)e^{-(\rho+\delta)t_k} = 1,$ solved for ρ	d^{-1}

Notes: With the values for the parameters and the choice of temperature scaling function $\lambda(T)$ listed in Table 2, the model can be numerically solved. Units are given for the model variables. W is mass, L is length, C is carapace mass, and E is eggs. t_0 is time of birth of neonates, $t_{\min}$ is minimum molt duration, $t_{\max}$ is maximum molt duration, and W_{WFL} is mass for length at the previous molt.

At each molt, the length of the individual is updated using the mass–length relationship to correspond to its current mass. If the cumulative net production between molts is negative, individuals lose mass, but the length does not decrease, an assumption similar to that used by Gurney et al. (1990). The new carapace mass matches the current length of the individual and hence, never decreases during an individual's lifetime. Modeling starvation effects is beyond the scope of this work, and we considered only scenarios in which the net production is positive between successive molts. The eggs in the brood pouch are released as neonates at the molt and new eggs are deposited in the brood pouch by converting the carbon allocated to reproduction into

TABLE 2. The list of parameters (arranged in alphabetical order) in the model in Table 1 along with the nominal value for the parameters as estimated in Appendix A.

Symbol	Parameters	Value/range	Units
Model constants			
f_h	half-saturation constant of feeding rate	0.1	mg C
γ	allocation to reproduction scaling constant	1.1618	mm^{-1}
I_{max20}	maximum ingestion rate at 20°C at 1 mm	0.0111	mg C·d^{-1}·$mm^{-1.8159}$
$\frac{I_{max}(T)}{I_{max20}L^{\mu_I}}$	maximum ingestion rate temperature scaling	$-15.239 + 3.80589T - 0.33877T^2 + 0.013192T^3 - 0.000187T^4$	
κ	fractional energy allocation to reproduction		
L_{Th}	length at maturity of *Daphnia*	1.7	mm
L_W	mass for length scaling constant	10.857	mm/mg $C^{0.4337}$
$\lambda(T) = \frac{R(T)}{R_{20}}$	temperature scaling of maintenance and molt duration relative to 20°C	$0.055T - 0.1$	
μ_A	allocation to reproduction length exponent	2.486	
μ_C	carapace allometric relation exponent	1.259	
μ_I	maximum ingestion rate exponent	1.8159	
μ_{max}	maximum molt duration length exponent at 20°C	0.8305	
μ_{min}	minimum molt duration length exponent at 20°C	0.8305	
μ_W	mass for length exponent	2.306	
R_{20}	respiratory rate constant per unit mass at 20°C	0.11	d^{-1}
t^{20}_{max}	maximum molt duration of 1 mm *Daphnia* at 20°C	1.4853	d
t^{20}_{min}	minimum molt duration of 1 mm *Daphnia* at 20°C	1.1288	d
W_C	mass of *Daphnia* with 1 mg C carapace	2.8962	mg $C^{0.2057}$
Population constants			
δ	*Daphnia* survival rate for population parameter calculation (i.e., r and R)	0.03	d^{-1}
Experimental system parameters			
D	dilution rate in a semi-chemostat	8.64†	d^{-1}
ε	assimilation efficiency	0.5†	
F_{batch}	food added in batch cultures		mg C/L
F_{chem}	feeding level in semi-chemostat		mg C/L
L_0	size of neonates at birth	0.9†–1.05	mm
N	number of individuals per container	1†	
t_{batch}	intervals between feedings in batch cultures	1†	d
t_{end}	length of the experimental run	21†	d
T	temperature	16–25, 20†	°C
V	volume of experimental container	0.1†	L

Notes: The parameters are categorized into model constants (first group), population related constants (second group) and finally, system parameters that reflect experimental conditions (third group). Some parameters have fractional units, since allometric relationships are assumed between some physiological parameters.

† Default value used in simulations.

eggs. The carbon mass per egg is assumed to be constant, and its value is obtained from the mass of a neonate and the estimated maintenance energy cost through embryonic development. Eggs are produced only if there is sufficient allocation to produce at least one (complete) egg, as in McCauley et al. (1990). Finally, at each molt, the molt development index is reset to zero.

Lifetime reproduction in any specified food environment was calculated by assuming some age-independent mortality rate (see Table 2) and integrating survival-weighted reproduction. Population per capita growth

TABLE 3. Summary of literature studies synthesized in this work and used in Figs. 2 and 3.

Name of study	No. experiments	Experimental conditions						Source(s) within study
		t_{end} (d)	L_0 (mm)	N	V (l)	t_{batch} (d)	T (°C)	
ERDC data set 1	4†	21	0.95	1	0.04	1	25	this study
ERDC data set 2	7†	21	0.9	1	0.03	1	25	this study
Baillieul et al. (2005)	4†	15	0.91	200	3	1	20	Fig. 1A, C
Coors et al. (2004)	5†	19	1.10, 1.10, 1.10, 1.05, 1.05	1	0.04	1	20	Fig. 2A, B (control)
Duchet et al. (2011)	1	14	1.05	15	0.1	1	20	Table 2 (control), Table 3 (control)
Dzialowski et al. (2006)	1	9	0.9	1	0.025	1	25	Fig. 1A, B (control)
Gilbin et al. (2008)	1	22	1.02	1	0.05	1	20	Figs. 1 and 3 (controls)
Glazier (1992)	4†,‡	22	1.04, 0.93	1	0.12	1	20	Fig. 1
Haeba et al. (2008)	1	21	0.79	1	0.05	2	20	Fig. 3A, B, C (control)
Kamaya et al. (2005)	2	21	0.9	1	0.04	2.33	21	Tables 2 and 3 (control)
Lürling and Tolman (2010)	1	14	0.91	1	0.1	1	20	Figs. 1B and 2B, Table 3 (control)
Lürling et al. (2010)	1	15	0.91	1	0.01	1	20	Table 2, Fig. 2 (control)
Manar et al. (2009)	1	21	0.9	1	0.04	2	20	Table 1 (control)
Muyssen et al. (2010)	6‡	21	0.95	1	0.04	1	20, 24	Tables 1 and 2 (controls, three clones)
Palma et al. (2009)	1	21	0.95	1	0.05	1	20	Fig. 1 (ASTM)
Printes et al. (2008)	2	21	1.09, 1.03	15	0.75	1	20	Figs. 3 and 4 (control)
Sokull-Klüttgen (1998)	4†	17, 19, 18, 19	0.9	7	0.5	semi-chemostat	20	data from author
Viganò (1993)	5†	43.5, 42.4, 39.5, 38.3, 38	0.98, 0.99, 0.97, 0.97, 0.96	1	0.05	1	20	Table 1
Zeman et al. (2008)	1	21	1.04	1	0.05	1	20	Figs. 2 and 3A (control)

Notes: The parameters representing the experimental conditions in each study shown can be used to solve the model in Table 1 along with the estimated model parameters in Table 2. The effective feeding level is the only free model parameter that is estimated as described in *Methods*. Further, the sources of the experimentally measured endpoints used in Fig. 2 within each study are also provided. Our unpublished data is marked U.S. Army Engineer Research and Development Center (ERDC) data set. ASTM is American Society for Testing and Materials.

† Multiple food levels.

‡ Multiple clones.

rates were calculated using the Euler-Lotka equation. The equations for both calculations are in Table 1.

Parameter estimation

The model parameters, listed in Table 2, were estimated by synthesizing data on suborganismal *Daphnia* physiology in a large number of published reports on short-term physiological rates (respiration, molt duration, ingestion, and assimilation rates), relationships between size and stage, and short-term measurements of energy allocation between growth and reproduction. Details on the synthesized literature data and the methodology used for estimating model parameters are in Appendix A. The data used for parameter estimation in Appendix A is entirely distinct from that used for calibration and verification in the next section.

Model tests on endpoints related to growth and reproduction

In order to test the model predictions both across different studies and under different environmental conditions, we utilized 17 literature sources (Glazier 1992, Viganò 1993, Sokull-Klüttgen 1998, Coors et al. 2004, Baillieul et al. 2005, Kamaya et al. 2005, Dzialowski et al. 2006, Gilbin et al. 2008, Haeba et al. 2008, Printes et al. 2008, Zeman et al. 2008, Manar et al. 2009, Palma et al. 2009, Lürling and Tolman 2010, Lürling et al. 2010, Muyssen et al. 2010, Duchet et al. 2011) and data by one of us (A. J. Kennedy; termed ERDC data), summarized in Table 3. While most of the data synthesized conform to the prescription of standardized tests, we also included experiments running longer than 21 days (e.g., Viganò 1993) and experiments performed at higher temperatures (25°C for the ERDC data) for a more thorough verification of the model.

Largely motivated by ecotoxicological issues, we focused on endpoints commonly used in chronic toxicity tests, along with other measurements that can be made during such tests: length, cumulative reproduction, total number of broods, and total number of molts, and life history parameters (age at first reproduction or brood and length at first reproduction). We computed solutions to the model equations in Table 1 using MATLAB.

We measured goodness of fit of the model predictions using the approach proposed by Portilla and Tett (2007) based on linear regression. In this approach, a regression line $Y = \beta_1 X + \beta_0 + \varepsilon$ is fit, where Y is the data and X is the model prediction for one endpoint. We can then categorize the quality of the model fit for that endpoint into four categories: excellent ($\beta_1 = 1$, $\beta_0 = 0$), good ($\beta_1 = 1$, $\beta_0 \neq 0$) or ($\beta_1 \neq 1$, $\beta_0 = 0$), fair ($\beta_1 \neq 1$, $\beta_0 \neq 0$), and poor ($\beta_1 = 0$). The test for the coefficients being (or not being) significantly different from zero (or one) is performed using the t test at the 0.05 level.

Tuning feeding levels across studies

The experimental parameters, such as the feeding methodology (F_{batch}, t_{batch}, D, t_{end}) and experimental conditions (N, V, T), are study specific. Since experimental data on feeding/ingestion rate were hardest to reconcile (see f_h estimation in Appendix A) and there was seldom information that would allow comparison of feeding rations (and quality) of different algal species on a variety of *Daphnia* clones across multiple studies, we decided to make F_{batch}, the feeding ration in batch cultures (or feeding-level set-point in a semichemostat), a tunable parameter in our model.

The studies in the synthesized literature could be categorized into two types based on the number of feeding levels at which experiments were performed. In studies where experiments were performed at only one feeding level, we fitted the equivalent feeding level F_{batch} under our model formulation by minimizing the error between the measured and predicted cumulative reproduction endpoints. On the other hand, when data were available at multiple feeding levels from the same study, a stronger assessment of the model predictions was possible. In these cases, we chose one experimental feeding level (generally the second lowest) and fitted an equivalent feeding level F_{batch} under our model by minimizing the prediction error on the cumulative reproduction at that feeding level, as before. Importantly, the equivalent feeding levels at other experimental feeding levels (in the same study) were obtained by assuming that the equivalent and experimental feeding levels scale proportionally. For example, if an equivalent feeding level F_{eq} is fit at an experimental feeding level of f, then the equivalent feeding level for an experimental level of $0.5f$ in the same study is $0.5F_{\text{eq}}$.

Testing time course data on growth and reproduction

We compared the predicted time course of growth and reproduction against a 42-d experimental run at four different feeding levels under semichemostat conditions of growth and reproduction (Sokull-Klüttgen 1998). These four feeding levels were well characterized qualitatively as ranging from low to high. While the Sokull-Klüttgen (1998) data were published as standard 21-d toxicological tests (Preuss et al. 2009), we present the extended runs of 42 d, since the data were available to us. These longer runs enabled us to test our model outside the time span over which fitting was done.

We tested the model in two ways. First, in the spirit of the previous methodology that used a single tunable parameter, we estimated one equivalent feeding level by matching reproduction after 19 d for the 5000 cells/mL feeding level (a level we consider to be medium). The functional response for other treatments was then calculated using the half-saturation constant, f_h, shown in Table 2. We call this method 1. Second, we estimated the functional response for each treatment by matching reproduction after 17–19 d as specified in Table 3. This second method is similar to that used by Martin et al. (2013) when fitting a different model to the same data. We call this method 2.

Sensitivity analyses

The model in Table 1 relates initial conditions and process-specific parameters (listed in Table 2) under a variety of feeding and environmental conditions. Thus, it is possible to compute the sensitivity of the chosen endpoints to perturbations in the underlying physiological processes (by perturbing the corresponding the parameters). For each endpoint of interest and each physiological parameter, p, we define the normalized sensitivity, S, to be the (small) fractional change in the endpoint, y, for a corresponding (small) fractional change in the parameter, i.e.,

$$S = \frac{\left(\frac{dy}{y}\right)}{\frac{dp}{p}} = \frac{p}{y}\frac{dy}{dp} = \frac{d\log(y)}{d\log(p)}.$$

A negative value for S indicates that an increase in the parameter causes a decrease in the endpoint value and vice versa.

The model includes both continuously varying state variables (e.g., mass) and those that vary discretely at the molt (e.g., cumulative reproduction, length). Such systems have inherent discontinuities in their sensitivity scores, and such hybrid systems have been extensively studied (Barton and Lee 2002). In particular, the endpoints of interest show discontinuity with varying feeding levels (see Appendix B: Fig. B1). We partially smoothed endpoints only for the sensitivity analysis by converting mass and energy allocated to reproduction at the end of the simulation run to update length and cumulative reproduction, respec-

tively (as if a molt occurred), and defining a continuous total molt metric as the sum of total molts and the value of the molt-development index at the end of the simulation run. While a sensitivity score can also be constructed for discrete-valued number of broods, the perturbation-based sensitivity, as we computed, is only meaningful and nonzero if the endpoint happens to change value within that small perturbation. We therefore omitted the number of broods in our sensitivity analysis.

We produced heat maps describing the effects of all the parameters on the chosen toxicologically relevant endpoints. For specific assumed values of the age- and size-independent mortality rate (of 0.03 d^{-1}), we added rows to the heat maps to illustrate the sensitivity of lifetime reproductive output and long-run growth rate to the model parameters.

Most standardized tests on *Daphnia* are conducted in high-food environments, while many natural *Daphnia* populations live in low-food environments for much of the year (McCauley and Murdoch 1987, Murdoch et al. 1998). Moreover, individuals experiencing lower food environments may exhibit different sensitivities. We therefore performed sensitivity analyses at two food levels, low and high food, which were defined as feeding levels leading to population doubling times of ~25 days (population growth rate of 0.028) and ~2 days (population growth rate of 0.34), respectively, at the assumed mortality rate. These are reasonable representations of fast-growing and slow-growing *Daphnia* populations at more realistic lower feeding levels. All other experimental parameters were fixed at the nominal values listed in Table 2.

Links to genomic data

Our bioenergetic model relates changes in specific physiological processes or attributes (characterized by model parameters) to aggregated biological endpoints, such as growth or reproduction, that characterize the physiology of a complete organism. Such whole-organism outcomes are commonly called apical endpoints in the literature on ecotoxicology and ecological risk assessment (Villeneuve and Garcia-Reyero 2011, Villeneuve et al. 2012). Gene-enrichment analysis of global transcriptomic data sets (Subramanian et al. 2005) can, in turn, be used to identify physiological pathways affected by treatments of interest that underlie specific apical outcomes in individuals. We evaluated the potential use of our bioenergetic model for predicting possible adverse apical outcomes from physiological pathway-level effects diagnosed via transcriptomics analyses. Our approach was to identify genes affected within metabolic/functional pathways that logically mapped to the biological functions represented as parameters within the DEB model. We then used gene expression information and the heat maps of the model parameter sensitivity (described in *Methods: Sensitivity analyses*) to extrapolate effects on apical outcomes.

We reviewed the literature for studies investigating global gene expression in *Daphnia* species focusing primarily on studies that also found experimental connections between gene expression and apical outcomes of growth and reproduction (Appendix C). We summarized results across these studies to assess which model parameters are well represented by pathway level effects and ultimately, to evaluate the ability to use pathway level responses to predict effects on the apical outcomes of growth and reproduction.

The literature review represents studies collected from combinations of the following search terms: *Daphnia*, genomics, gene expression, transcript expression, and protein expression on the PubMed database (*available online*).[7] Criteria for study inclusion included: results must be published in peer-reviewed literature, studies must include a global gene-expression method, such as microarray or RNA-seq to provide pathway-level observations, and gene expression must be related to apical outcomes. The search returned literature primarily investigating transcript expression changes in response to environmental contaminant exposure (Appendix C), including exposures to various metals (Cd, Cu, Zn, and Ni), pharmaceuticals (ibuprophen, fenarimol, propiconazole), and industrial chemicals (polar and nonpolar narcotics). The majority of studies provided direct connections between gene-expression results and apical outcomes, including growth and reproduction. Three occurrences of hormesis were excluded as outlier observations for the purposes of this review (Campos et al. 2013, Stanley et al. 2013). Observations of differentially expressed genes were placed into five categories representing biological pathways/processes closely aligned to DEB model processes: four (energy utilization, oxygen transport, digestion, and molting) relate to suborganismal processes, while the last (reproduction) is a particularly important apical endpoint (Appendix C).

Results

Experimental endpoint measurements under a variety of conditions and across studies

In Fig. 2, we compare six experimentally measured endpoints against the predictions of the model with feeding levels fitted as described in *Methods: Tuning feeding levels across studies*. The main findings are as follows. Final length is predicted well (goodness of fit: good) by the model except at the smallest final lengths, which correspond to experiments with low food supply. Cumulative reproduction is also well predicted (goodness of fit: good), but there are two outlier points, both corresponding to experiments by Glazier

[7] http://www.ncbi.nlm.nih.gov/pubmed

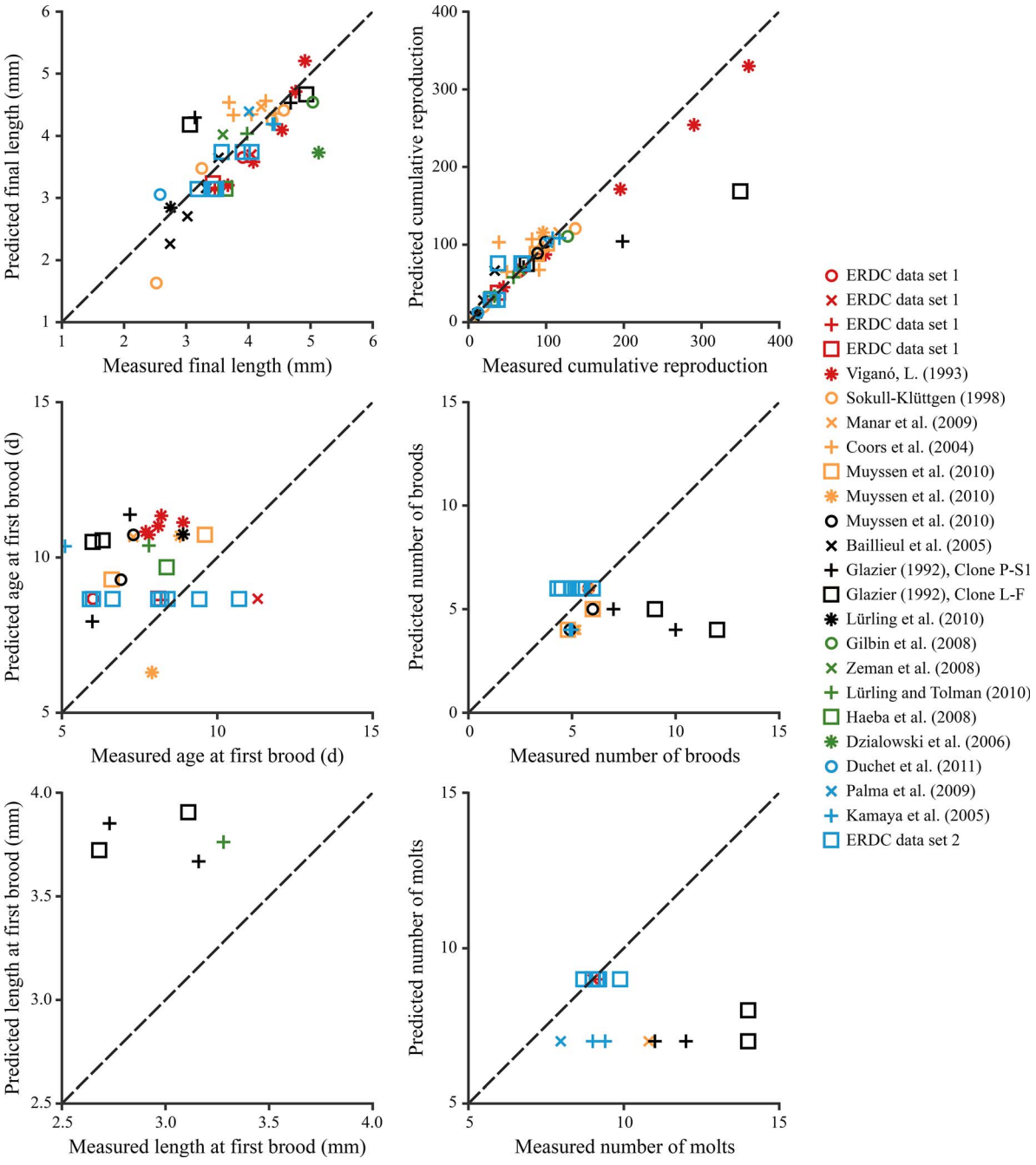

FIG. 2. Comparison of experimental data and model predictions on endpoints and life history parameters of individuals (symbol colors match data sources in legend). The normalized feeding level (only free parameter) was tuned for each data source as described in *Methods*. The data used for this synthesis are described in Table 3 with our unpublished data marked U.S. Army Engineer Research and Development Center (ERDC) data sets.

(1992). Age at first brood shows considerable variability among studies (goodness of fit: poor), although the number of studies reporting this endpoint is limited, and in some studies, the precise definition of age at first reproduction is unclear. The model prediction falls in the middle of the cluster of points, but the model has little predictive value for this endpoint. There were few data sources for length at first brood. The plot has five data points (goodness of fit: poor), one showing good agreement, the other four coming from Glazier (1992). Model predictions of number of molts (goodness of fit: fair) show broad consistency with data, except for data from Glazier (1992). The model slightly overestimates number of broods (good-

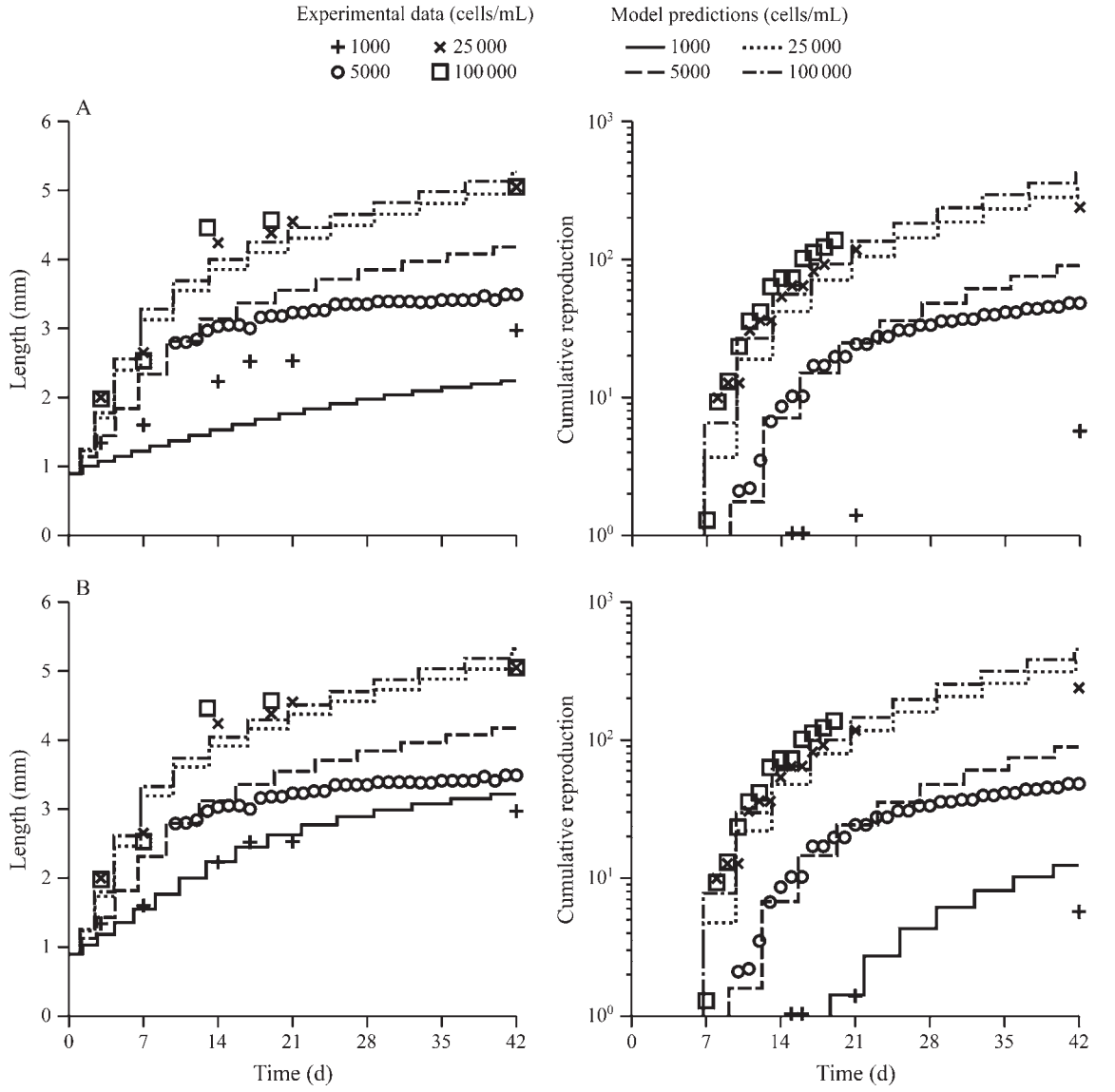

FIG. 3. Comparison of growth and reproduction trajectories under semichemostat conditions predicted by our model against 42-d experimental data from Sokull-Klüttgen (1998). Panel (A) fits to the data using method 1, where feeding level was calibrated using cumulative reproduction at 5000 cells/mL after 19 d. Panel (B) fits using method 2, where each food level was fitted individually (see *Methods* for details).

ness of fit: poor) for all data surveyed except for Glazier (1992). The model captures temperature dependence of endpoints well. The plots cover data across the range 20–25°C.

Most outliers in Fig. 2 derive from one study, Glazier (1992). Glazier reported that for both clones in his work, molt duration is higher at high food as compared to low food, which is contrary to almost all other data available on molt duration that we reviewed. Our molting model based on energy budgets constrains the molting to take longer when the energy assimilation is lower, so it is not surprising that our model matches Glazier's data very poorly. The length dependence of the minimum molt duration does make longer individuals (such as those fed high food) molt less frequently, but this effect is weak and is not the source of the mismatch between our model and Glazier's data.

In summary, the model describes the carbon budget of *D. magna* with considerable accuracy over a broad range of experimental clones and conditions. Age at first brood and number of molts or broods vary considerably across the data considered, but model predictions are in the observed range.

Time course of growth and reproduction

We next compared model predictions of the time courses of growth and reproduction against the 42-d experimental data under semichemostat conditions

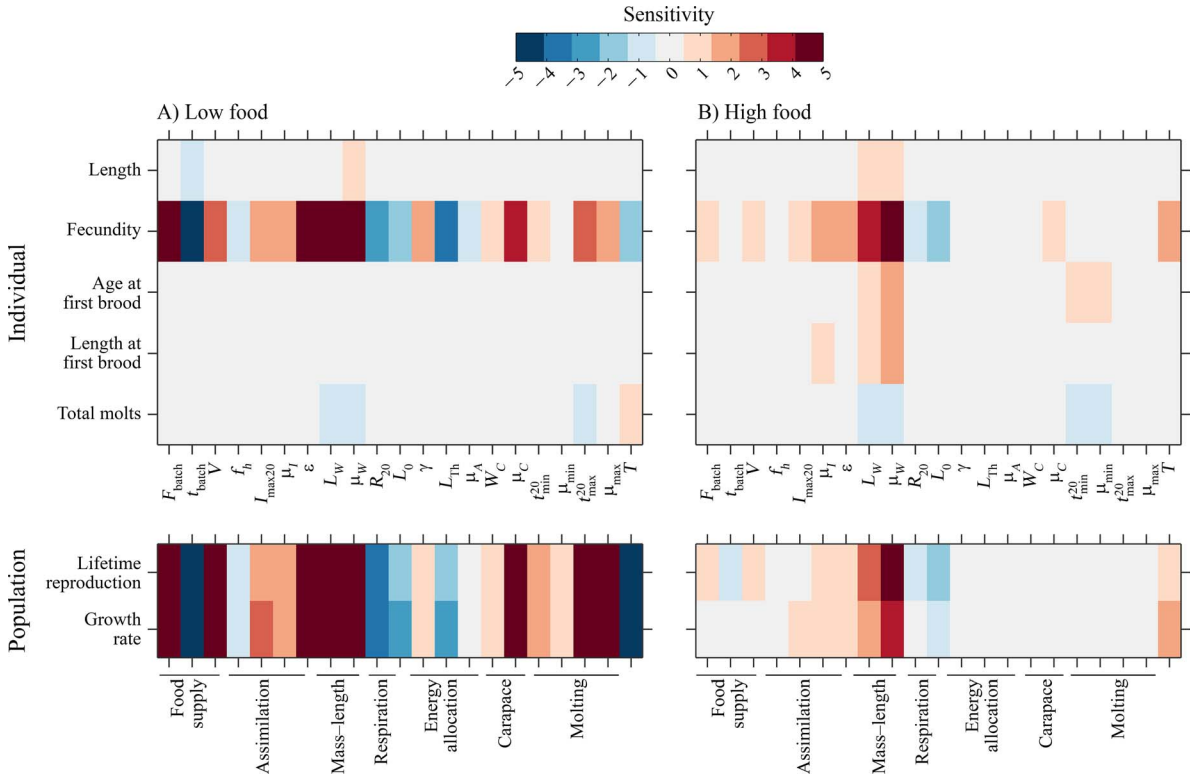

FIG. 4. Sensitivity heat map of the bioenergetic model endpoints to changes in parameter values under batch cultures measured by normalized sensitivity scores (limited to the range ±5). The *x*-axis lists the parameter and experimental conditions from Table 2, and the *y*-axis lists the experimental endpoints, including growth and reproduction. The *Daphnia* are fed daily at (A) low- and (B) high-feeding levels corresponding to normalized feeding levels of 0.11 and 1.1, respectively, under a simulated 21-d standard OECD (Organisation for Economic Co-operation and Development) batch experimental protocol (see *Methods*). The parameters are further grouped according to the physiological process they represent on the last row. In the lower two panels, the sensitivity of lifetime reproduction and long-run growth rate of the population are provided at low and high food.

(Sokull-Klüttgen 1998) and the results are shown in Fig. 3. With method 1 (single tunable parameter; Fig. 3A), the fits to the higher food levels have much smaller systematic bias than at low food, while cumulative reproduction is estimated better than the length of the individual at each time point. The predictions are even better, if experimental data are truncated after the standard duration of 21 d (see Appendix B: Fig. B2). However, a major failing, evident in Fig. 3, is the apparent poor performance of the model at the lowest feeding level of 1000 cells/mL, where the model predicts zero reproduction and much lower growth than is observed. With method 2 (functional responses estimated for each treatment; Fig. 3B), the fits at medium and higher feeding levels remain good, but the low-food performance is now consistent with observation (see Appendix B: Fig. B2).

Sensitivity of endpoints commonly used in toxicological assessments

The sensitivity heat maps of five endpoints characterizing individual performance to 21 d under standardized toxicity tests for low (population growth rate of 0.027 d^{-1}) and high food (population growth rate of 0.37 d^{-1}) supply are shown in Fig. 4, in a manner analogous to the large-scale microarray toxicogenomic assays on *D. magna*, e.g., Table 1 in Poynton et al. (2007). Cumulative reproduction (fecundity) is the most sensitive endpoint at both feeding levels, while the other four endpoints including final length are only sensitive to a few model parameters. At low food, fecundity is susceptible to changes in all processes and almost all model parameters.

The effect of food-supply parameters on all endpoints other than fecundity is minimal at high food when supply is sufficient and feeding rate largely saturates due to the type 2 functional response. At low food, food supply and the functional response both have strong effects; providing more food more regularly improves fecundity and to a lesser extent increases the final length of individuals. Assimilation efficiency affects mainly fecundity, but does so at both food levels and leads to increased reproduction even at high food, since, although *Daphnia* are feeding at their maximum rate, increased assimilation still leads to increased net production. According to the model, reproducing adult *Daphnia* allocate proportionally less energy to growth than juveniles and as a result, final length is not

markedly affected by assimilation parameters at either food level.

The mass–length relationship determines the conversion of carbon between mass and length at the molt. Moreover, increasing the mass–length relationship parameters increases the length equivalent of a given mass, which subsequently improves maximum feeding rate leading to increased mass gain. Therefore, the entire growth and reproduction dynamics of *Daphnia* is strongly influenced by the mass–length relationship.

The first brood of *Daphnia* at low food occurs very close to the 21-d experimental run and hence appears insensitive to all processes in 21-d sensitivity scores. The age at first brood at high food increases with the minimum molt duration, since *Daphnia* with enough food molt at the maximum rate. Juveniles allocate almost all their energy to growth and hence, length at first brood increases with assimilation.

Daphnia with higher respiration rates have less net production available for growth and reproduction. As mentioned earlier, *Daphnia* development is composed of purely growth in the pre-reproductive phase and much less growth in the reproductive phase after maturity. At high food, the former phase is relatively short, and 21-d experiments are mostly dominated by the latter phase. However, at low food, a significant duration of the experiments are spent in the former phase. The energy allocation parameters determine both the duration of the two phases and the transition time between the two. Thus at low food, increasing the length threshold at maturity reduces the time after maturity in which to produce eggs during a 21-d experiment. In addition, these parameters also determine the balance of energy allocation between growth and reproduction.

A larger carapace-mass-scaling exponent leads to a larger carapace for a given mass/length of individual, and the consequences of this are not intuitively obvious. A larger carapace mass for a given mass has two opposing effects on the maintenance cost. A larger carapace leads to a smaller somatic mass that respires, but requires a higher contribution to construct a larger new carapace at the molt. With our model parameterization, it appears that a larger carapace leads to a smaller maintenance cost and hence a higher net production (see Table 1, maintenance rate equation). Since these mature individuals allocate most of their net production to reproduction, any changes to the net production effected by the carapace is expressed predominantly on reproduction.

Sensitivity of reproduction to the maximum molt duration at low food reflects the functioning of *Daphnia* close to the maximum molt duration. The resulting longer molts allow individuals to distribute their carapace production costs and assimilate over a longer period of time, thus averaging periods of food and famine at low-food conditions and effectively increasing their net production. Coupled with the energy allocation of adults, this effect is most apparent on reproduction.

The initial length of the *Daphnia* also determines (in this study) the minimum energetic requirement for producing an egg (see Table 1). Thus, a larger initial length results in a smaller number of eggs produced from a given reproductive biomass and thereby a smaller cumulative reproduction. The total number of molts in 21 d is dependent on the molt duration parameters and conversion of energy (mass) to length as anticipated.

Fecundity is strongly sensitive to temperature at both food levels. Temperature increases shorten molt durations and increase respiration rates. At high food, while increased respiration reduces net production, feeding rates can be boosted by faster growth with shorter molt durations. At low food, individuals have to survive on the fixed feeding ration until the next feeding. The increased cost of respiration with temperature results in lower net production, which is manifested as lower fecundity. When total food is not limited (as with constant feeding even at low food, which is discussed in Fig. 5), this temperature effect on respiration disappears.

Sensitivity analysis for population level endpoints

The sensitivities of lifetime reproduction and of long-run population growth rate at low and high food supply rates are shown in Fig. 4, assuming a uniform background mortality rate of 0.03 d^{-1}. At high food, these largely track the sensitivity of fecundity in individuals. Thus, there is very weak sensitivity to food supply and assimilation, strong sensitivity to the parameters of the mass–length relationship, and intermediate sensitivity to the maturation threshold and temperature.

At low food, lifetime reproduction and long-run population growth rate are sensitive to the values of *all* parameters and the sensitivity is very similar to the cumulative reproduction to 21 d. The population endpoints have stronger sensitivity to the molting-related parameters and temperature than fecundity. The sensitivity of cumulative reproduction at 21 d is a reasonably good indicator of sensitivity of either population endpoint at both low and high food.

Sensitivity under constant food conditions

Daphnia populations in batch cultures experience extreme fluctuations in food availability, while populations in nature typically experience less extreme fluctuations. We therefore repeated our sensitivity analyses for the same ecotoxicological endpoints, but assumed constant low-food (long-run population growth rate 0.0281 d^{-1}) and high-food (long-run population growth rate 0.285 d^{-1}) conditions (Fig. 5).

The sensitivity heat map at high food in constant food experiments is very similar to that of batch cultures at high food (Fig. 4). The signs of the sensitivities are the same, although the relative strengths are slightly different. The only significant exception is the weak negative sensitivity to the half-saturation constant under constant high food. Under constant food, higher half-saturation constants in the type 2 functional response result in smaller overall feeding rates, and in well-fed

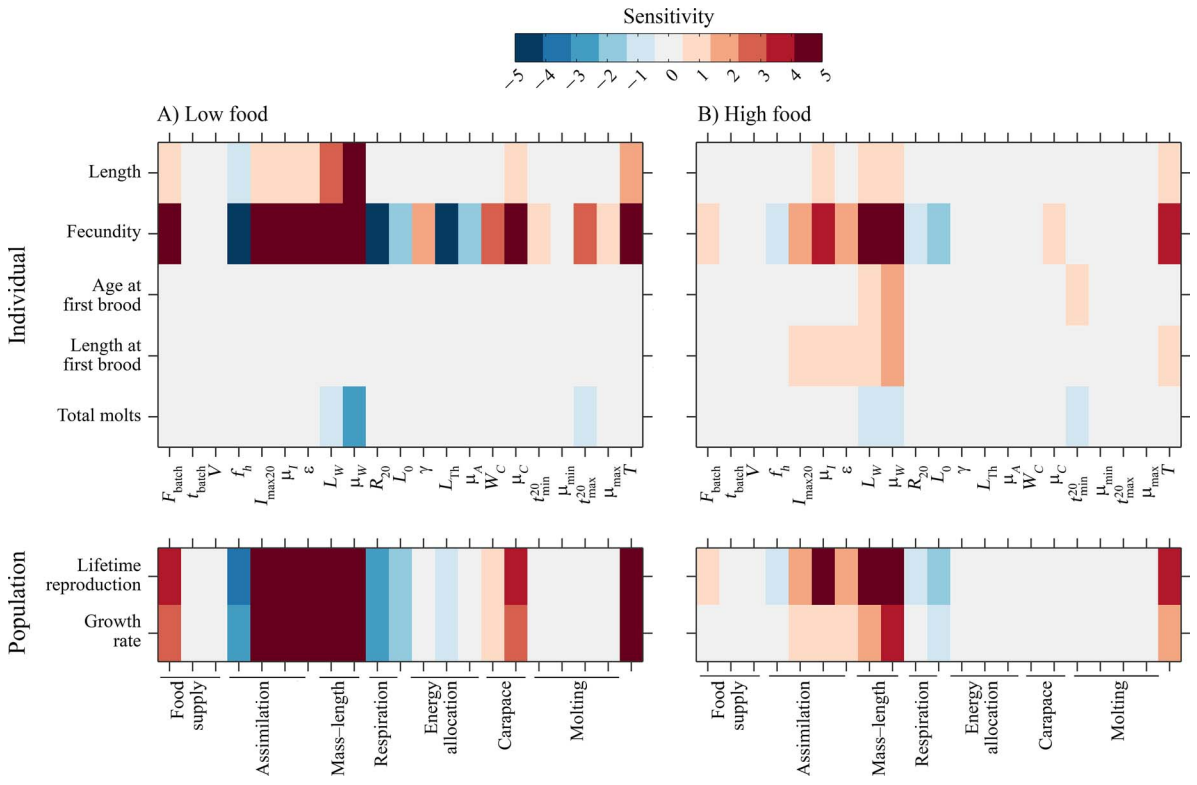

FIG. 5. Sensitivity heat map of simulated 21-d constant food cultures under (A) low food and (B) high food (normalized feeding levels of 0.03 and 0.3, respectively) for comparison with batch cultures in Fig. 4. We use the nominal experimental setup values in Table 2, but set the food dynamics to zero. As before, the sensitivity of population measures at the assumed mortality rate and grouping of parameters according to the relevant physiological processes are also shown.

adults, the decrease in assimilation reduces the overall reproduction. By contrast, in batch cultures the functional response has little influence on the performance of the largest animals, which consume almost all the food supplied.

At low food, the sensitivities with constant food are qualitatively similar to the sensitivities under batch cultures. The sensitivity of the half-saturation constant is negative, as observed at high food, since the sensitivity at constant food is driven by the derivative of the type 2 functional response, which is always negative. The two qualitative differences are negative sensitivity to the allocation function exponent, which controls the transition time spent in allocating resources to growth vs. reproduction, and the positive temperature sensitivity. As the allocation exponent increases, the transition time decreases, producing smaller adults that have lower maximum feeding rates, and hence, smaller reproduction. Unlike batch cultures, the feeding levels here are held constant, but assimilation (and net production) can still be increased by faster growth as a result of shorter molts at higher temperatures, irrespective of food conditions. Moreover, the loss of net production due to increased respiration is immaterial with constant food as there is no constraint on the total available food.

The final length at low food also shows significantly stronger sensitivity to parameters characterizing the mass–length relationship, assimilation, feeding, and carapace properties than its counterpart (Fig. 4). This can be attributed to the longer transition between prioritizing growth (for juveniles) and reproduction (for large adults) at low food, during which the changes in net production may have a large effect on length after 21 d, as the transition period extends beyond 21 d. As before, the sensitivity of the population parameters at both feeding levels closely tracks the sensitivities of fecundity.

Links to genomic data

Transcriptomic information on energy utilization processes.—In the studies examined, expression changes in genes involved in the metabolism of three key biomolecules (carbohydrates, lipids, and proteins) are pervasive, occurring across nearly all stressor conditions, including nutrient restriction and exposures to contaminants including metals, pharmaceuticals, and industrial chemicals (Appendix C: Table C1). With the exception of genes involved in specifically lipid transport dedicated to egg production (i.e., vitellogenin), the majority of observations where stressors negatively affected growth and/or reproduction showed increased expression of genes involved in the metabolism of energy substrates (13 of 21 observations and an additional six at specific

treatment levels in mixed responses; Fig. 6; Appendix C: Table C1). For example, Soetaert et al. (2007*a*) demonstrated that Cd exposure at levels that impacted growth, as well as carbohydrate, lipid, and protein content in *D. magna*, caused increased expression of genes involved in carbohydrate, lipid, and protein metabolic pathways. We hypothesize that this increased expression of genes involved in carbohydrate, lipid, and protein metabolic pathways reflects an increased total rate of energy utilization due to the increased energetic cost of maintenance processes needed to ameliorate pollutant effects.

Oxygen transport throughout the organism via heme groups represents an important process for sustaining aerobic energy metabolism. Therefore, expression of genes involved in oxygen transport may represent a plausible indicator of changes in respiration rates. Expression of genes involved in oxygen transport was indeed sensitive to a variety of chemical stressors including Cd (Shaw et al. 2007, Soetaert et al. 2007*a*, Connon et al. 2008, Poynton et al. 2011*a*, *b*), Ni (Vandenbrouck et al. 2009), Zn (Vandegehuchte et al. 2010), and fenarimol (Soetaert et al. 2007*b*). However, no general trend was observed across studies as observations were divided relatively evenly among genes having increased vs. decreased expression (Fig. 6). Further, expression was variable even for individual stressors, as was the case for studies on Cd where expression varied across studies and across concentration ranges (Appendix C: Table C2). Similar variability is, however, also observed in direct measurements of changes in respiration rates in response to Cd (Kettle et al. 1980, Barber et al. 1994, Knops et al. 2001).

In our model, an increased respiration rate would be expressed as an increase in the respiration rate constant R_{20} and/or a decrease in the nominal assimilation efficiency ε, since the latter takes account of costs of growth and specific dynamic action. According to Figs. 4 and 5, both would lead invariably to reduced reproduction and possibly to reduced growth at low food, consistent with findings on apical effects in the studies described (Appendix C: Tables C1 and C2).

Transcriptomic information on digestive and assimilation processes.—The rate of assimilation depends on the feeding rate and the assimilation (or digestion) efficiency. No transcriptional responses reported in Appendix C: Table C3 relate directly to feeding rates, but a number of studies reported effects related to digestion. Chemical-stressor exposures affected expression of genes involved in digestion and nutrient absorption that potentially impact assimilation (Poynton et al. 2007, 2008*a*, Soetaert et al. 2007*a*, *b*). Cu, Cd, and Zn exposures at trace concentrations below that needed to cause observable growth and reproductive effects caused decreased expression of transcripts involved in digestion and nutrient absorption (Poynton et al. 2007, 2008*b*). Conversely, higher Cd concentrations that negatively affected *Daphnia* growth, as well as organism level energy substrates, including reduced lipid, carbohydrate, and protein content predominantly caused increased expression of digestive genes (Soetaert et al. 2007*a*; Appendix C: Table C3). Similarly, the anti-ecdysteroidal fungicide fenarimol caused increased expression of genes involved in digestion at concentrations that negatively affected *Daphnia* growth and embryonic development (Soetaert et al. 2007*b*).

In view of the absence of transcriptomic data relating to the part-behavioral process of feeding, the above information must be complemented by data from direct-feeding measurements. Studies on organismal response of *D. magna* demonstrate that exposure to Zn reduces feeding rate and also decreases mass, energy reserves, and neonate production in chronic exposures (Muyssen et al. 2006, Poynton et al. 2007). Exposure to Cd also reduces feeding rate (e.g., Bodar et al. 1988*a*, Allen et al. 1995, Knops et al. 2001) and leads to reduced reproduction (e.g., Bodar et al. 1988*b*, Geffard et al. 2008). Figs. 4 and 5 show that fecundity largely tracks changes in assimilation rate with a weaker effect on growth that is more pronounced at low food. (Note that feeding rate decreases with increasing the value of the parameter f_h in the model). Thus, the growth/reproduction response is consistent with direct measurements of feeding and with the observed transcriptomic response related to digestion at low exposures. The increased expression of digestion-related processes at higher concentrations is possibly a compensatory response that is too weak to outweigh the other processes involved. Thus, the assimilation parameter of the DEB model is not only mechanistically informed by the results of transcriptomics data on digestive processes, but these data point to a possible organism level compensatory process for which we are aware of no data.

Transcriptomic information on carapace and molting processes.—*Daphnia* exposed to various pollutants displayed differential expression of genes involved in chitin metabolism, cuticle metabolism, and/or cuticle protein metabolism (Soetaert et al. 2006, 2007*a*, *b*, Poynton et al. 2007, 2008*a*, *b*, 2011*a*, Shaw et al. 2007, Connon et al. 2008, De Schamphelaere et al. 2008, Heckmann et al. 2008, Vandenbrouck et al. 2009, Vandegehuchte et al. 2010, Dom et al. 2012). However, there were no general trends in expression across chemical exposures where observations of mixed expression of genes within pathways (i.e., consisted of genes with increased as well as decreased expression) were predominant (Fig. 6; Appendix C: Table C4). The complexity of the responses likely reflects differences in chemical type, dose, and exposure duration. However, even within the chitin and cuticle metabolism pathways, there was diversity in responses.

The complexity of the observed genomic response underlying cuticle metabolism does not provide easily generalizable trends for use in the model. This is well illustrated by a result from De Schamphelaere et al. (2008), who found that zinc exposure shortened the second instar of *Daphnia*, but prolonged the two subsequent instars. If the latter patterns were to persist, the heat maps in Figs. 4 and 5 predict unambiguous reduction in fecundity, but that sensitiv-

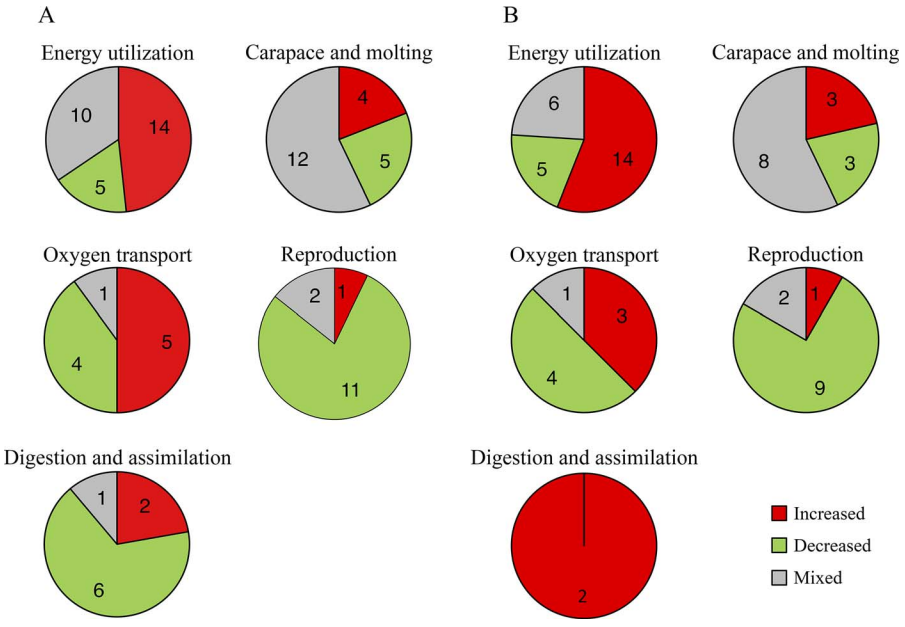

FIG. 6. Summary of functional- and pathway-level gene expression observations that mapped to key parameters within the DEB model. The observations were collected from a literature review of studies investigating global gene expression in *Daphnia* species summarized in Appendix C. The numbers in each pie piece are the number of observations. Panel (A) represents the sum of all functional-/pathway-level observations taken across all studies in the literature review (including treatments causing no apical impacts). Panel (B) represents the functional-/pathway-level observations that had parallel experimental connections to apical level impacts on organismal growth and/or reproduction.

ity of growth would depend on whether the primary effect was on the minimum or the maximum intermolt duration. The former is more important at high food and the latter at low food. Disentangling these options would require better mechanistic understanding of molting.

Transcriptomic information on processes related to reproduction.—The interpretation of changes in expression of genes related to reproduction requires considerable care, since reproduction is one of the apical endpoints. An observed organism-level change in reproduction may be due to either a direct impact on the physiological processes linked to reproduction or may be caused indirectly through the bioenergetic processes described above that are featured in our DEB model. Fig. 6 shows that in response to pollutant exposure, there is a general trend of decreased expression of genes directly involved in reproductive physiology, but this trend was representative both of studies where negative effects on reproduction were observed (Heckmann et al. 2008, Vandegehuchte et al. 2010) and of assays where only growth was observably affected by stressor exposure (Soetaert et al. 2006, 2007*a*, *b*, De Schamphelaere et al. 2008, Vandenbrouck et al. 2009, Jeyasingh et al. 2011), an outcome not possible in our model, except with juvenile animals. The trend persists even at low-exposure levels below the threshold for observed whole organism responses (Poynton et al. 2008*a*). With the caveat that two contaminants (in Appendix C: Table C5) are potential endocrine disruptors (fenarimol and propiconazole), the overall results are consistent with Figs. 4 and 5, which indicate reproduction is highly sensitive to changes in many model parameters, especially at low-food levels, but for precisely this reason they are somewhat less instructive without further mechanistic modeling, e.g., through variants of a model by Murphy et al. (2005). However, applying the transcriptomic results for reproductive physiology, especially the allocation of energy to egg production via vitellogenin within the DEB model provides a robust indication of stressor impacts on both growth and reproduction.

DISCUSSION

This work was motivated by the need to develop models that relate suborganismal effects of toxicants to physiological performance of individual organisms and subsequently to population dynamics. For many invertebrates, fecundity and mortality depend strongly on size and food supply; thus demography demands a model of the individual that relates growth and reproduction to the food environment. Ideally, the model will open the way to exploiting the rapidly growing body of suborganismal information on sublethal responses to toxicants, a particularly important source of information, since such studies typically demand much less time than either chronic toxicity tests or population studies. We formulated and parameterized a model of *Daphnia*, and then tested its ability to predict a number of endpoints commonly used in chronic toxicity studies. With just one adjustable parameter, the model successfully predicts

growth and reproduction from a wide array of experiments performed in multiple laboratories using different clones of *D. magna* raised on different food sources. Age at first brood and number of molts or broods vary considerably across the data considered, but model predictions are within the observed range. These endpoints are not seriously constrained energetically, so the higher variability in measured values is not surprising.

The most important challenge in relating model to data occurs in the few studies with low food, since *Daphnia* in natural environments commonly experience low food availability (McCauley and Murdoch 1987, McCauley et al. 1988). The model, with its default parameter values and a single tunable parameter, underestimates growth at low food after 21 days (top left panel of Fig. 2). The same limitation using the same approach was revealed in fits of complete time courses of growth and reproduction in experiments by Sokull-Klüttgen (1998) at three food levels (Fig. 3A). The fit to the data is much improved when the form of the functional response is allowed to change from its default form, thereby better calibrating the energy inputs in the model (Fig. 3B). We cannot exclude the possibility that the low-food performance reflects physiological changes, such as have been observed for *D. magna* during bouts of starvation/recovery (Bradley et al. 1991) or with *D. pulex* in fluctuating environments of batch cultures (McCauley et al. 1990*b*). However, the semichemostat design used by Sokull-Klüttgen (1998) does not generate large fluctuations in the food environment, but does generate results that are sensitive to the parameters in the functional response, well illustrated by the increased sensitivity of fecundity and length to the half-saturation constant, f_h, in the low- vs. high-food panels of Fig. 5.

One previous study on *Daphnia*, and several previous studies on other organisms have similarly required calibration of the functional response. Martin et al. (2013) fitted a simpler bioenergetic model, Kooijman's (2010) DEB model, to the Sokull-Klüttgen data and found it necessary to estimate the functional response separately for the different treatments. Two studies that used Kooijman's (2010) DEB model to describe the growth of the blue mussel *Mytilus edulis* (Rosland et al. 2009, Thomas et al. 2011) calibrated the functional response in a similar way. A study of the bioenergetics of the oyster *Crassostrea gigas* across six Atlantic ecosystems used the same calibration, but found systematic variation of half-saturation constant with food (phytoplankton) density (Alunno-Bruscia et al. 2011). An alternative, mechanistic approach, also from literature on bivalves, is to use a more detailed and parameter-rich representation of feeding and assimilation (Saraiva et al. 2011).

The overall conclusion from our data synthesis on properties of individuals is that the model describes the carbon budget of *D. magna* with considerable accuracy over a broad range of experimental clones. For any application requiring precise fitting to data for one particular clone, our model and the parameters in Table 2 provide a starting point. Parameter estimates could be refined using, for example, Bayesian methods (Johnson et al. 2013) or variants of the approach proposed for Kooijman's DEB model by Lika et al. (2011). The parameters in Table 2 from our data synthesis (Appendix A) are analogous to the pseudodata of Lika et al. and could be used to obtain priors for Bayesian estimation.

We made a connection to the population level by computing the long-run growth rate for a population experiencing the same food and temperature environments as individuals in the modeled experimental regimes. In common with earlier studies (e.g., Kooijman and Metz 1984, Muller et al. 2009*b*), we assumed a constant background mortality rate, independent of the state of the animal and of its food and temperature environments. This simplification permits qualitative discussion of sensitivity to stressors, but there is increasing evidence that the population dynamics of *Daphnia* is influenced by food-dependent mortality, in addition to the effects of growth and reproduction studied here (McCauley et al. 2008, Ananthasubramaniam et al. 2011, Martin et al. 2013). We are aware of no data on individuals that would allow parameterization of such a mortality term for any *Daphnia* species other than the very limited information from one single study reported by Nisbet et al. (2010). For this reason, we did not include analysis of mortality in this paper, but, although challenging, we recommend investigation of mortality in low food as a priority for future empirical studies.

The heat maps displaying the results of sensitivity analysis for individual and population endpoints are a powerful tool for relating effects at these two different levels of organization. With almost all model parameters, fecundity is the most sensitive endpoint for individual organisms, and there is broad correlation between the sensitivity of fecundity and of the long-run growth rate, as is desirable for the default metric used in 21-day toxicity tests. We noted earlier the strong sensitivity of long-run growth rates to many model parameters under low-food conditions; this has been long appreciated for parameters related to energy input (e.g., Kooijman and Metz 1984), but our findings generalize this result.

Some of the sensitivities in Fig. 4 and Fig. 5 relate to model assumptions with the weakest empirical support, for example, the constancy across environments of the mass–length relation and the formula for carapace mass. This opens the question of whether our results are particular to debatable, species-specific model details. As noted in the *Introduction*, theorists recognize important conceptual differences between empirically based bioenergetic models (such as the model in this paper) and models drawing on the more abstract DEB formalism of Kooijman (2010), an issue explored in detail by Nisbet et al. (2012). Yet two recent overviews of *Daphnia* dynamics (Nisbet et al. 2010, Peeters et al. 2010) suggest that the differences among the models may be less critical in practice than was previously thought, a view supported by the similarity of population dynamics in the very different bioenergetic models of McCauley et al. (2008) and Martin et al. (2013). There are, therefore, grounds for optimism that conclusions on individual to population connections are robust against small changes in the model.

The benefit of including some species-specific detail derives primarily from the possibility of relating *suborganismal* responses to changes in whole-organism performance. Cross-referencing the results of the sensitivity analysis against the suborganismal observations compiled across a review of *Daphnia* gene-expression experiments (Appendix C) revealed a number of connections. The overwhelming majority of expression studies represented ecotoxicological assessments where *Daphnia* were exposed to chemical stressors. A general trend of increased transcript expression for genes involved in both energy assimilation and utilization was observed for a variety of stressor exposures. The increased expression is hypothesized to reflect the costs of combatting pollutant effects, reflected as an increased respiratory rate in our sensitivity analyses. Another pervasive effect observed in the literature review was the differential expression of genes involved in chitin metabolism, cuticle metabolism, and/or cuticle protein metabolism in response to contaminant exposure. However, the complexity of responses precluded generalized associations between stressor exposure and effects on growth and/or reproduction. Further research into the mechanistic processes involved in molt physiology would be of great benefit for validating assumptions of the discrete molt component of the model. Finally, the observation that reproduction is highly sensitive to perturbation of the majority of the parameters in the model is consistent with the widespread observation that transcription of genes involved in reproductive physiology (especially allocation of energy to egg production via vitellogenin) were decreased with many chemical stressor exposures (Appendix C), but, as noted earlier, the generality of the observation of decreased reproduction limits the insight obtainable from these observations without further modeling.

We end with comments on the generality of our approach. One reason why ecological risk assessment is particularly challenging is the diversity of stressors, organisms, and ecosystems. This paper focused on a detailed, empirically based model of the physiology and population dynamics of just one model organism, *Daphnia*. *Daphnia* represents an important genus in many temperate freshwater bodies, so our work has immediate applicability, exemplified by recent empirical work, where multigenerational life-table experiments on *Daphnia* exposed to engineered nanoparticles (TiO_2) were used to calculate changes in long-run population growth rate using the Euler-Lotka equation (Jacobasch et al. 2014). The data demands for such studies are huge, and fitting a validated bioenergetic model like ours will reduce the effort and cost required for similar future investigations. However, our overarching objective is to advance towards general principles that will elucidate how effects of stressors propagate up through levels of biological organization, from gene expression to population dynamics. The challenge is achieving generality without sacrificing the security that comes from working with species-specific and thus rigorously testable models, exemplified by the model in this paper. DEB theory offers a route towards such generality, as the primary focus is on fundamental physiological processes that are common to many groups of organisms, supplemented by one more specific feature: explicit representation of molting. This study has demonstrated that the organism-level predictions from such models can be rigorously tested and that there is encouraging potential for connections to suborganismal processes.

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Supplemental Material

Ecological Archives

Appendices A–C are available online: http://dx.doi.org/10.1890/114-0498.1.sm