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Limitations of extrapolating toxic effects on reproduction to the population level

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Abstract. For the ecological risk assessment of toxic chemicals, standardized tests on individuals are often used as proxies for population-level effects. Here, we address the utility of one commonly used metric, reproductive output, as a proxy for population-level effects. Because reproduction integrates the outcome of many interacting processes (e.g., feeding, growth, allocation of energy to reproduction), the observed toxic effects in a reproduction test could be due to stress on one of many processes. Although this makes reproduction a robust endpoint for detecting stress, it may mask important population-level consequences if the different physiological processes stress affects are associated with different feedback mechanisms at the population level. We therefore evaluated how an observed reduction in reproduction found in a standard reproduction test translates to effects at the population level if it is caused by hypothetical toxicants affecting different physiological processes (physiological modes of action; PMoA). For this we used two consumer–resource models: the Yodzis-Innes (YI) model, which is mathematically tractable, but requires strong assumptions of energetic equivalence among individuals as they progress through ontogeny, and an individual-based implementation of dynamic energy budget theory (DEB-IBM), which relaxes these assumptions at the expense of tractability. We identified two important feedback mechanisms controlling the link between individual- and population-level stress in the YI model. These mechanisms turned out to also be important for interpreting some of the individual-based model results; for two PMoAs, they determined the population response to stress in both models. In contrast, others stress types involved more complex feedbacks, because they asymmetrically stressed the production efficiency of reproduction and somatic growth. The feedbacks associated with different PMoAs drastically altered the link between individual- and population-level effects. For example, hypothetical stressors with different PMoAs that had equal effects on reproduction had effects ranging from a negligible decline in biomass to population extinction. Thus, reproduction tests alone are of little use for extrapolating toxicity to the population level, but we showed that the ecological relevance of standard tests could easily be improved if growth is measured along with reproduction.

Key words: Daphnia; *dynamic energy budget; ecological risk assessment; ecotoxicology; ontogenetic symmetry; physiological mode of action, PMoA; population dynamics; reproduction test; Yodzis-Innes.*

Introduction

Current ecological risk assessment of chemicals is largely based on standard tests on individuals in the laboratory. However, the main goal of risk assessment is to avoid effects on populations, communities, and ecosystems that would impair their persistence and functioning (EC 2009, EU 2012, Galic et al. 2012). Therefore, effect assessments on individuals need to be extrapolated to higher levels of biological organization. This is often done by applying safety factors to account for ecological and environmental effects that cannot be assessed in the laboratory.

Whether these safety factors are safe remains unknown, in particular when considering sublethal effects, which do not lead to mortality, but still might accumulate to unacceptable effects on populations. Thus, it is critical to know how well the sublethal effects, or "endpoints," measured in current standard tests can be extrapolated to the population level (Hammers-Wirtz and Ratte 2000, Forbes and Calow 2002, Grimm and Martin 2013, Gabsi et al. 2014). Is the uncertainty in this extrapolation only of a

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quantitative nature, or might current endpoints fail, even in qualitatively capturing population-level effects?

The most commonly used sublethal endpoint in chemical risk assessment is the reproductive output of individuals (Ashauer et al. 2011). The experimental protocols for reproduction tests vary for different species, but in general, involve recording the number of offspring produced by individuals exposed to different concentrations of a toxicant. For example, for *Daphnia*, newborn individuals are placed in isolation in a small volume of water with a high algae concentration, which is frequently renewed. The *Daphnia* are checked daily for reproductive events, and the number of newborns are counted and then removed. After 21 days, the test is ended, and the cumulative reproductive output per individual is reported as a function of the toxicant dose to which they were exposed. Apart from its direct relevance for population dynamics (every reproductive event alters population abundance), a compelling argument for the use of reproduction in chemical risk assessment is that it integrates the outcomes of many interacting processes: feeding, growth and development, allocation of energy to embryos, and survival of embryos. Therefore, if a toxicant affects any one of these processes, the effects will be detected by a change in reproductive output (Alda Alvarez et al. 2006, Agatz et al. 2013). Conversely, if, for example, only feeding or growth were measured and the toxicant acts via embryonic mortality, the assay would give a false negative (Jager et al. 2007). One final justification for the use of reproductive output is that when measured along with survival, at multiple points in time, the effects of toxicity on the intrinsic population growth rate, r, can be calculated with the Lotka equation. Thus, effects on reproduction can be used to extrapolate to at least one measure of population performance (Forbes and Calow 2002).

Identifying the physiological processes affected by stress (physiological mode of action, PMoA; Alda Alvarez et al. 2006) is unnecessary for extrapolating to the population level in hypothetical, constant environments where populations grow exponentially. However, conditions under which populations grow exponentially lack the feedback processes that are ubiquitous in real systems, for example, between a population and its resource. Feedback on population dynamics mediated by a resource involves several steps. First, the population consumes the resource; as the population increases, so does the pressure on the resource. Individual organisms in the population initially respond to changed resource densities through changes in feeding rate. A changed feeding rate then affects other physiological rates, notably growth, development, reproduction, and mortality rates. The population change is the aggregate effect of these changes in individual physiological responses. Toxicants and their different PMoAs may impact some or perhaps all of the physiological rates. In short, there are many feedback routes through which exposure to a toxicant may impact the dynamics of a resource-limited population.

We here explore the consequences of some of these feedback mechanisms through a practical example based on current practices in risk assessment of chemicals. We start with a situation typically faced in risk assessment: we know how much a given concentration of a chemical reduces reproductive output over some time interval (OECD 2008), but we do not know which physiological process the toxicant affects. We explore the consequences of this uncertainty by evaluating effects at the population level of hypothetical toxicants that disrupt different physiological processes, i.e., have different PMoAs. To link the individual and population levels, we use two different models: the Yodzis-Innes (YI) model (Yodzis and Innes 1992), and an individual-based implementation of dynamic energy budget theory (DEB-IBM; Martin et al. 2012).

The YI model uses two differential equations to describe biomass dynamics in interacting consumer and resource populations, yet is grounded in an explicit representation of individual dynamics (de Roos and Persson 2013) that requires three assumptions: (1) all consumer energetic fluxes (feeding, assimilation, maintenance costs) are proportional to mass, (2) production of new biomass via reproduction and somatic growth have the same production efficiency, and (3) the mortality rate of consumers is independent of size. Collectively, these three assumptions represent a special case referred to as ontogenetic symmetry (de Roos et al. 2013, de Roos and Persson 2013, Persson and de Roos 2013).

Models that assume ontogenetic symmetry are known for one organism (the zooplankter *Daphnia*) to give good fits to laboratory population data (Nisbet et al. 1997*a*), to capture some important characteristics of natural populations (Murdoch et al. 1998), and are used in many food-web (e.g., Brose et al. 2006) and ecosystem models (e.g., NPZ models reviewed by Franks [2002]). Yet violations of the symmetry assumptions are the rule rather than the exception (de Roos and Persson 2013), and the nature and magnitude of asymmetry within a population can fundamentally alter its qualitative dynamical behavior (e.g., stage-specific biomass overcompensation; Schröder et al. 2009, Ohlberger et al. 2011). It is of considerable relevance for ecological risk assessment that some types of stress are symmetry breaking, and hence, cannot be considered by obligately symmetric models. For example, some toxicants reduce the reproductive output of adults, without altering their growth (Jager et al. 2007, Preuss et al. 2010). The introduction of a stressor that affects only reproduction violates the symmetry assumption that the efficiency of biomass production via somatic growth and reproduction is equal. Thus, for asymmetrically acting stressors, we cannot extrapolate

TABLE 1. Dynamical equations and model characteristics of the Yodzis-Innes model with and without stress for the populations and individual levels.

Equations and characteristics	Formula
A) Dynamical equations	
Population	
Resource biomass dynamics	$\frac{dR}{dt} = \rho(R_{\max} - R) - (f \times M \times C)$ where $f = R/(R + H)$
Consumer biomass dynamics	$\frac{dC}{dt} = (f \times M \times \sigma \times C) - [T \times C] - (\mu \times C)$
Individual	
Individual growth, W (mass), and reproduction, F (number of individuals), dynamics	if $W(t) < \omega_A$ $\frac{dW}{dt} = ([M \times \sigma] - T)W(t), \frac{dF}{dt} = 0$ if $W(t) \geq \omega_A$ $\frac{dW}{dt} = 0, \frac{dF}{dt} = ([M \times \sigma] - T)\frac{\omega_A}{\omega_B}$
B) Model characteristics	
Population	
Condition for equilibrium	$f^* M \times \sigma - T = P^* = \mu$
Equilibrium for resource	$R^* = \frac{H(T + \mu)}{(M \times \sigma) - T + \mu}$
Equilibrium for consumer	$C^* = \frac{\sigma \times \rho(R_{\max} - R^*)}{T + \mu}$
Individual	
Cumulative reproduction after t_{obs}	$F(t_{obs}) = \frac{\omega_A}{\omega_B}\left[t_{obs}(M \times \sigma - T) - \ln\frac{\omega_A}{\omega_B}\right]$
C) Stressed population and individual traits as a fraction of their unstressed values	
Population	
M-stressed C^* : C^*	$\frac{C_M^*}{C^*} = \frac{R_{\max} - R_M^*}{R_{\max} - R^*}$
T-stressed C^* : control C^*	$\frac{C_T^*}{C^*} = \frac{(T + \mu)(R_{\max} - R_T^*)}{(T_s + \mu)(R_{\max} - R^*)}$
Individual	
M-stressed F : control F	$\frac{F_M(t_{obs})}{F(t_{obs})} = \frac{t_{obs}(M_s \times \sigma - T) - \ln(\omega_A/\omega_B)}{t_{obs}(M \times \sigma - T) - \ln(\omega_A/\omega_B)}$
T-stressed F : control F	$\frac{F_T(t_{obs})}{F(t_{obs})} = \frac{t_{obs}(M \times \sigma - T_s) - \ln(\omega_A/\omega_B)}{t_{obs}(M \times \sigma - T) - \ln(\omega_A/\omega_B)}$

Notes: Following de Roos and Persson (2013), we consider a resource, R, following semi-chemostat growth dynamics with a dilution rate ρ and a maximum resource biomass, R_{max}. A consumer population, C (biomass), feeds on the resource with a type II functional response, where M is the maximum mass-specific ingestion rate, and H is the half-saturation coefficient. Ingested energy is assimilated with an efficiency of σ (the remainder excreted). The consumer population loses biomass through maintenance costs (respiration) and mortality at mass-specific rates, T and μ, respectively. Individual growth and reproduction dynamics assuming ad libitum food, $f = 1$, are given. Individuals are born with a mass ω_B, and grow until they reach their adult mass ω_A, after which point they allocate all net biomass production to reproduction. Formulae are shown for the reproductive output (number of individuals) at the end of a toxicity test $F(t_{obs})$, where t_{obs} is the length of the experiment, and for stressed population and individual traits as a fraction of their unstressed values. For both population and individual traits, the subscript s indicates the altered parameter value under stress, and subscripts M and T indicate the stressed trait value for feeding and maintenance stress respectively.

toxicity to the population level with the YI model. Because of this limitation, and because we want to know if realistic deviations from ontogenetic symmetry matter, we analyze the DEB-IBM model, where these assumptions are not made.

DEB-IBM removes the constraint of ontogenetic symmetry by modeling the growth, development, and reproduction of each individual explicitly with a dynamic energy budget, DEB; Kooijman 2010). DEB theory provides a generic model of individual energy acquisition and use. Unlike the YI model, DEB populations are ontogenetically asymmetric. The asymmetry is due in part to mass-specific maintenance costs of individuals increasing more rapidly with size than their surface-area-specific feeding rates. DEB theory has been used extensively to model how stress affects growth and reproduction by altering energetic fluxes regulated by different physiological processes. The development of DEB theory within an individual-based modeling framework has been used to link individual energetics

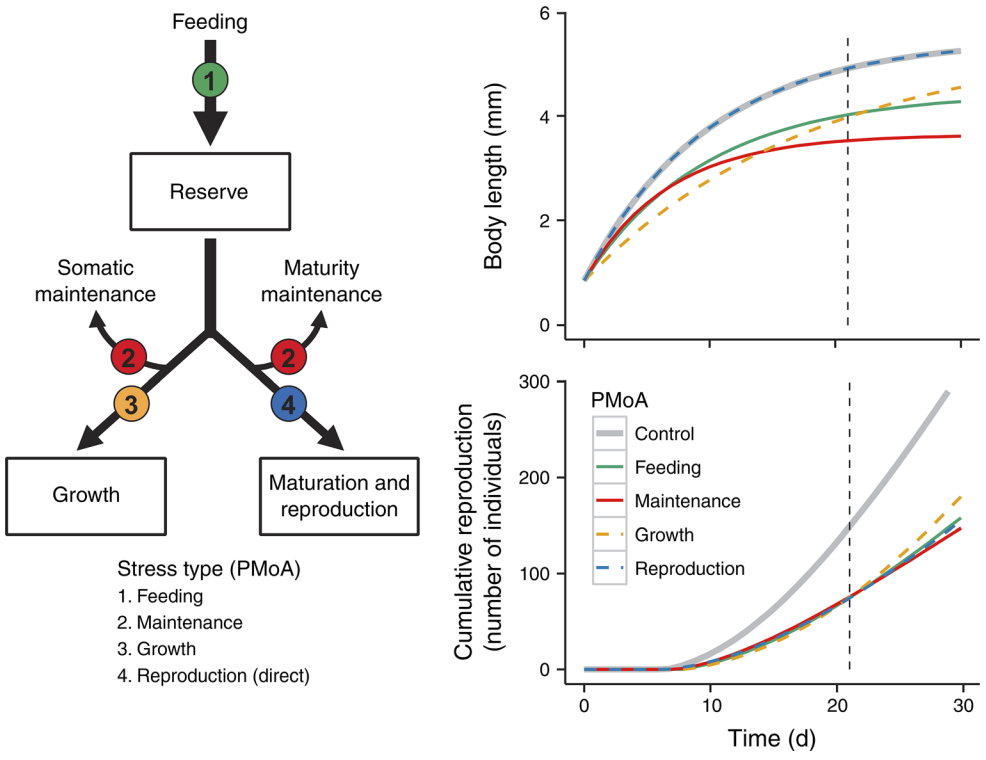

FIG. 1. Schematic diagram showing the patterns of effect on growth and reproduction for various physiological modes of action (PMoA) in the individual-based implementation of dynamic energy budget theory (DEB-IBM) model. Each number in the left panel represents the point where toxicity via a specific PMoA disrupts energy flow. In the right panel, the growth trajectory and reproductive output associated with stress via each PMoA are shown. The growth and reproductive pattern for each PMoA corresponds to the stress level required to result in a reduction in reproduction of 50% relative to control after 21 days (indicated by the vertical dashed line) in a standardized *Daphnia* reproduction test.

with their population-level consequences. More recently, the DEB-IBM framework was adapted for *Daphnia magna*, and the model predictions were tested against data from population experiments in non-stressed environments (Martin et al. 2013*a*). DEB-IBM has also been used to predict the population-level response to a chemical stressor from individual-level toxicity data (Martin et al. 2013*b*).

We first give a brief overview of both models. We then use the YI model to identify relevant feedback mechanisms associated with stress caused by certain PMoAs. Then we use DEB-IBM to identify the circumstances under which these mechanisms can account for the population-level response to stress when more realistic and ontogenetically asymmetric representations of individual ontogeny are used (DEB-IBM). Although ecotoxicology provided the original motivation for this study, the results are applicable to a wide range of natural and anthropogenic stressors.

Description of Yodzis-Innes Model

The YI model is summarized in Table 1 and gives the dynamical equations for the resource, R, and consumer biomass, C. Although not immediately apparent from the equations, YI explicitly represents individual growth (dW/dt) and reproduction dynamics (dF/dt; see Appendix A for a detailed derivation of the individual- and population-level models). Thus, we can evaluate how stress on different physiological processes, or parameters, affects both individual- and population-level traits. We take advantage of this feature to explore the sensitivity of a population to stress relative to its effect on individual reproduction. We consider stress with two different PMoAs (Fig. 1). The first PMoA, feeding stress, acts by decreasing the mass-specific maximum ingestion rate (M), while the second, maintenance stress, acts by increasing the mass-specific maintenance parameter (T). We then plot the stressed equilibrium consumer biomass (C^*_M and C^*_T, subscripts denote which parameter the stress affects) as a fraction of the unstressed consumer biomass C^*, against the fractional reduction in cumulative reproduction due to stress observed in a 21-d reproduction test, $F_M(t_{obs})$ and $F_T(t_{obs})$, compared to the control $F(t_{obs})$.

Description of DEB-IBM

We used a nonspatial, DEB-based model of *Daphnia magna* (Martin et al. 2013*a*) based on the generic DEB-IBM framework (Martin et al. 2012). The individual model consists of a set of coupled differential equations, based on standard DEB theory, describing the dynamics of state variables that characterize the energetic and

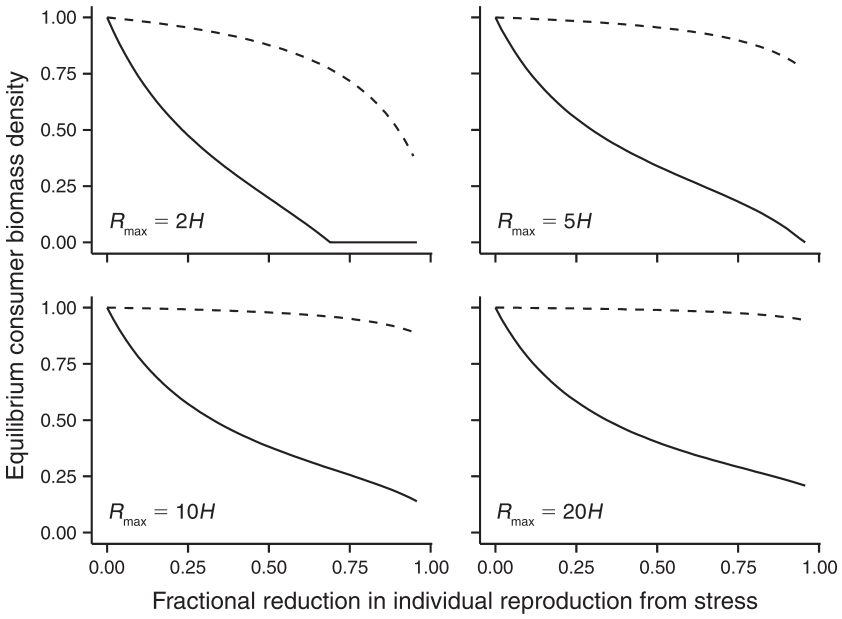

FIG. 2. The relationship between stress measured at the individual level (cumulative reproduction after 21 days) and effects at the population level (equilibrium consumer biomass, as a fraction of control population value), in the Yodzis-Innes model. The two PMoAs evaluated were feeding stress (dashed black line), which reduces individual reproduction via a reduction in maximum ingestion rate, and maintenance stress (solid black line), which does so by increasing metabolic losses through respiration. Results are shown for four levels of maximum resource density (R_{max} set at 2, 5, 10, and 20 times the half-saturation coefficient, H). Parameters used for these figures were taken from the default mass-specific values given in de Roos and Persson (2013), and are given in Appendix A.

developmental state of each individual. A full model description following the ODD protocol (a standard format for describing IBMs; Grimm et al. 2006, 2010), is included in Appendix B.

As with the YI model, we modeled hypothetical stressors that affect specific individual-level processes (parameters), which in turn alter the magnitude of a specific energy flux, and ultimately life-history output, over time. In addition to feeding and maintenance stress, we evaluated two other physiological processes not accessible in the YI analysis due to symmetry constraints: stress that decreases the proportion of surviving embryos (reproduction stress), and stress that increases overhead costs for growth (growth stress). Typical patterns of effect at the individual level are shown in Fig. 1, assuming a constant stress exposure over time. Reproduction stress reduces reproductive output directly, without altering growth of the mother. The remaining three PMoAs result in simultaneous reductions in growth and reproduction; however, the manner in which growth and reproduction are affected over time differs. Growth stress acts by increasing the costs of synthesizing new biomass, which results in reduced conversion efficiency in energy allocated to growth. As a result, growth stress does not affect the asymptotic size of individuals, but it reduces the rate at which they approach their asymptotic size (Jager and Zimmer 2012). Feeding and maintenance stress reduce the growth rate and ultimate size reached by an individual. However, relative to feeding stress, maintenance stress reduces growth rates of larger *Daphnia* more severely (Fig. 1). This is because in DEB theory, the maintenance flux is assumed to be proportional to structural volume, and thus increases with size at a faster rate than other fluxes that scale with surface area.

For each PMoA, we conducted individual-level simulations to calculate the required change in the stressed parameter to result in a reduction of 25%, 50%, 75%, 90%, and 95% in the OECD *Daphnia* reproduction test (assuming ad libitum food at 20°C). The resulting values of stressed parameters were then used as inputs for population-level simulations (values given in Appendix B).

Population simulations

The DEB-IBM population, like the YI consumers, fed on a dynamic resource with semi-chemostat population growth

$$\frac{dR}{dt} = \rho(R_{max} - R) - P_R \quad (1)$$

where ρ is the dilution rate, R_{max} is the equilibrium density of resource in the absence of predation (maximum resource density), and P_R is the resource depletion flux from the feeding *Daphnia* population. Feeding rates of individuals scale with surface area (L^2), and follow a Holling type II functional response. Thus the depletion flux, summed over all individuals, i, is

$$P_R = \sum_i f \times L^2 \times J_R \quad (2)$$

where J_R is the maximum surface-area-specific ingestion rate and f, as in the YI model, is the scaled functional response (type II).

Simulation experiments were initiated with 10 newborn *Daphnia*. We allowed a period of 150 d for *Daphnia* populations to reach their equilibrium dynamics. After 150 d, the parameters of *Daphnia* were adjusted according to the PMoA and the effect level of the treatment, i.e., chronic exposure to the hypothetical chemical for 21 d (Appendix B). To avoid including transient dynamics in our estimation of equilibrium population effects, we waited 150 d after exposure to begin measuring population characteristics. After this transitory phase, we recorded the average of various population-level characteristics over a 300-d period. For each simulation, we recorded the percentage of populations surviving, mean population biomass and abundance, mean resource density, and as a basic metric of size structure, the mean size of individuals in the population over the 300-d observation period.

Simulations were run with the same R_{max} values (as a multiple of the half-saturation coefficient, H) as in YI model (2, 5, 10, and 20 times H). The density of *Daphnia* in the DEB-IBM simulations increased with R_{max}. Because we were interested in differences relative to control populations, we standardized the average number of *Daphnia* simulated by adjusting the volume of water being simulated so that control populations for all values of R_{max} consisted of, on average, 150 individuals. The algae dilution rate, ρ, also strongly affected total *Daphnia* abundance, but did not affect the proportional response to toxicity for any of the metrics measured. Therefore we used a ρ of 0.05, but our results apply for any value of ρ, provided R_{max} is large enough to sustain a population.

Analysis of the Yodzis-Innes Model

In the Yodzis-Innes model, the relationship between stress observed at the individual level (cumulative reproduction) and effects at the population level (equilibrium population biomass) was different for stressors with different PMoAs (Fig. 2). The difference in magnitude of population-level effects for stress can be accounted for by considering the different feedbacks of the two PMoAs. For a population at equilibrium, net specific biomass production, P, must balance biomass losses through mortality. When feeding stress is induced, the reduction in maximum mass-specific ingestion rate, M, temporarily reduces the net biomass production of the consumer, unbalancing biomass production and mortality ($P \neq \mu$). At the same time, the rate of depletion of the resource by consumers is reduced, and thus resource density increases, subsequently increasing P. The new equilibrium is reached when resource density, R, has increased enough for the new scaled functional response, f, to compensate for the initial stress-induced decline in P, so that production once again balances mortality. As a consequence, feeding stress reduces the equilibrium consumer biomass, C^*, indirectly via its effects on R^* (Table 1). The magnitude of this reduction is determined by the difference in resource growth rate in the stressed population relative to the unstressed population. In a semi-chemostat system, resource growth rate decreases as R^* approaches R_{max}, and the magnitude of the reduction in resource growth rate for a given increase in R^* is negatively related to R_{max}.

For maintenance-stressed populations, the reduction in equilibrium biomass shown in Fig. 2 is due to a combination of two mechanisms. First, as with feeding stress, a reduction in consumer productivity due to increased maintenance costs requires an increased R^* for net biomass production to balance biomass loss via mortality, thereby indirectly reducing C^* by decreasing the resource growth rate. However, unlike feeding stress, maintenance stress contributes a second term in the equilibrium consumer biomass equation that depends on the stressed parameter (Table 1). The key difference between the two PMoAs is that resources not consumed due to feeding stress remain available to the consumer population, while with maintenance stress, the resource is consumed but then lost to the system through respiration. This additional, direct mechanism is independent of the resource; hence, maintenance stress resulted in significant reductions in equilibrium biomass even for high values of R_{max}.

In summary, analysis of the YI model highlights the importance of knowing which physiological process is responsible for an observed reduction in individual reproduction. The PMoAs of stress mattered, because the physiological processes they affected were associated with different feedback mechanisms. It can also be shown that for these two PMoAs, stress will not alter the size structure, and therefore effects on population abundance will be proportional to the effects on biomass (see Appendix A).

Analysis of DEB-IBM

Population biomass

For stress that induced a given reduction in reproduction, the reduction in population biomass depended on the maximum resource level and on PMoA (Fig. 3). For all PMoAs, stress reduced population biomass proportionally more when the maximum resource density, R_{max}, was low. However the extent to which R_{max} controlled biomass loss varied considerably among PMoAs. Maintenance stress had the most severe effect on population biomass, with substantial losses even at high R_{max} (Fig. 3). In low R_{max} environments, populations exposed to high levels of maintenance stress were driven to extinction. Feeding and growth stress resulted in the second and third largest proportional reductions in population biomass, with their order depending on

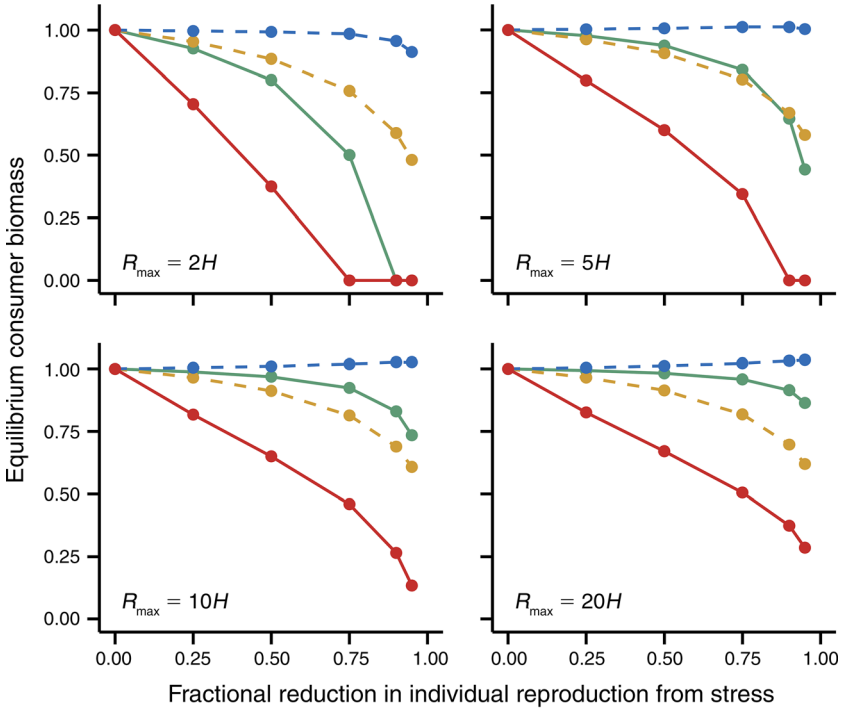

FIG. 3. The relationship between stress measured at the individual level (cumulative reproduction after 21 days) and effects at the population level (equilibrium population biomass, as a fraction of control population value) in the DEB-IBM model. The different lines represent hypothetical chemicals with different PMoAs (line styles as in Fig. 1). Results are shown for four levels of maximum resource density (R_{max}, as in Fig. 2). Results are presented as a fraction of unstressed populations. Each point represents the mean of 100 simulations.

R_{max}. Population biomass response to feeding stress depended more strongly on R_{max} than growth stress. Reproduction stress led to negligible reductions in biomass at all effect levels and R_{max} values tested.

We evaluated the extent to which the direct and indirect feedback mechanisms identified in the YI model applied to the DEB-IBM results, where more realistic and asymmetric representations of individual ontogeny were assumed. By plotting the ratio of stressed population biomass to control population biomass against the ratio of the stressed resource growth rate to the resource growth rate in the control, we could partition out indirect resource-mediated mechanisms (Fig. 4). This revealed that population biomass response to feeding stress was entirely indirect, and explained by a reduced resource growth rate (Fig. 4A). Maintenance- and growth-stressed populations were also affected by this indirect mechanism, but were also affected by an additional direct reduction in biomass production efficiency. Also like the YI model, the resource dependence of the indirect mechanism explained the increased severity of biomass loss when R_{max} was low (Fig. 4).

Population abundance and size structure

Feeding and maintenance stress in DEB-IBM led to negligible changes in average body size, with all combinations of effect level and R_{max} falling within 5% of control populations (Fig. 5A). Because these stress types had negligible effects on size structure, the observed effects on population abundance were roughly equivalent to those observed on biomass (Fig. 5B vs. Fig. 4). Conversely, growth and reproduction stress significantly altered the average size of individuals in populations. Growth stress resulted in a reduction in the mean length of individuals in populations, while reproduction stress increased it (Fig. 5A). Furthermore, the effects on population abundance were no longer identical to the effects on biomass for these PMoAs (Fig. 5B). For example, growth stress reduced population biomass while actually increasing population abundance. Similarly, reproduction stress reduced population abundance, while scarcely impacting biomass. Because the mass of an individual is proportional to its length cubed, relatively moderate changes in mean length led to large differences between effects on biomass and abundance.

The symmetry of a population's response to stress, in terms of abundance and biomass, depended on the symmetry of the toxic effect on individual energetics (Fig. 6). PMoAs (feeding and maintenance stress) that impaired biomass production symmetrically through the somatic and reproductive pathways also resulted in symmetrical changes in equilibrium biomass and abundance. Conversely, asymmetric individual-level stress

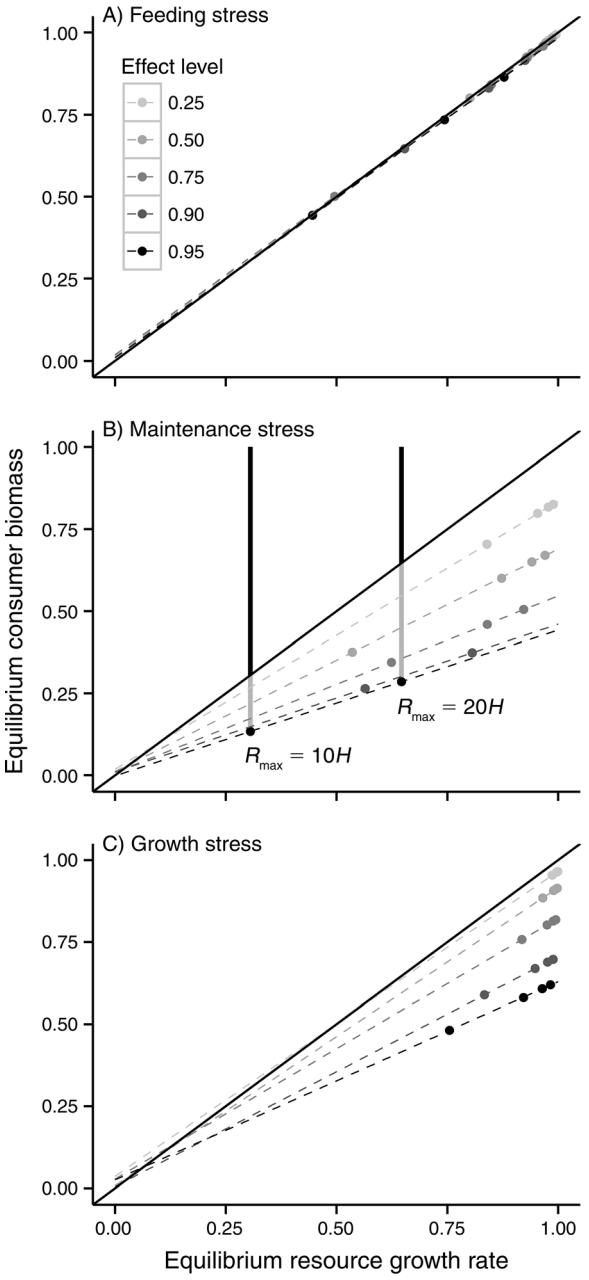

FIG. 4. Partitioning of stress-mediated reductions in population biomass due to direct and indirect mechanisms. For (A) the feeding PMoA, nearly all the variation in the reduction in *Daphnia* population biomass can be explained indirectly by a reduction in resource growth rate. For the two other PMoAs that led to a significant reduction in *Daphnia* population biomass, (B) maintenance and (C) growth stress, the reduction in population biomass was due to the combined effects of decreased resource productivity (indirect) and reduced efficiency of *Daphnia* populations in converting a given amount of resource into population biomass (direct). The regression lines (thin dashed lines) fitted through all R_{max} values for a given effect level form a straight line with an intercept near zero. This indicates that the direct effect is proportionally constant for all resource levels. Panel (B) demonstrates the relative partitioning between the indirect (thick black vertical line), and direct (thick gray vertical line) mechanisms. Within each effect level, the points represent, from right to left, the R_{max} values of 20, 10, 5, and 2 times the half-saturation coefficient of the *Daphnia*. Effect levels with less than four data points indicates that all populations for the lower R_{max} went extinct. Consumer biomass and resource growth rate are both presented as a fraction of control population value.

caused an asymmetric population response in response to growth or reproduction stress. Stress that asymmetrically affected the reproductive and somatic pathways led to changes in the size structure of the population, and asymmetric changes in population biomass and abundance.

DISCUSSION

Many regulatory decisions use reproduction or its derived consequences for population growth rate as a proxy for population-level effects. Implicit in the use of these metrics is the assumption that they convey relevant information about the population-level response. Our analysis of two models that link these levels of organization challenges this assumption by showing that the mapping of toxic effects from the individual to population level is contingent on the physiological process affected by a stressor. Even though we standardized effects of the different PMoAs by comparing them at levels where they produce the same observed effects at the individual level, effects at the population level were drastically different. For example, toxicants with different PMoAs considered identical based on their effects on individual reproduction could have population effects ranging from a negligible decline in biomass to extinction. Because reproduction data alone is insufficient to identify the specific physiological process interrupted by a toxicant, current widely used practices in ecological risk assessment will have limited ability to extrapolate to the population level. As a broad principle, this has long been appreciated by ecologists, but our analyses elucidate the direct and indirect feedbacks associated with the physiological process affected by stress.

Analysis of the YI model revealed that for feeding-stressed populations, effects on equilibrium population biomass are due entirely to changes in resource growth rate associated with an increase in equilibrium resource density. This indirect mechanism is commonly found in consumer–resource systems, where an initial reduction in consumer productivity is compensated for by an eventual increase in resource density (Nisbet et al. 1997*b*, Abrams 2002). The compensatory capacity of the indirect mechanism depends on both the dynamics (e.g., semi-chemostat, logistic) and characteristics (parameters, e.g., carrying capacity) of the resource. Here, we considered a resource whose population growth rate decreased monotonically with density (semi-chemostat growth). Thus, a stress-mediated increase in resource biomass always reduces consumer biomass, and less

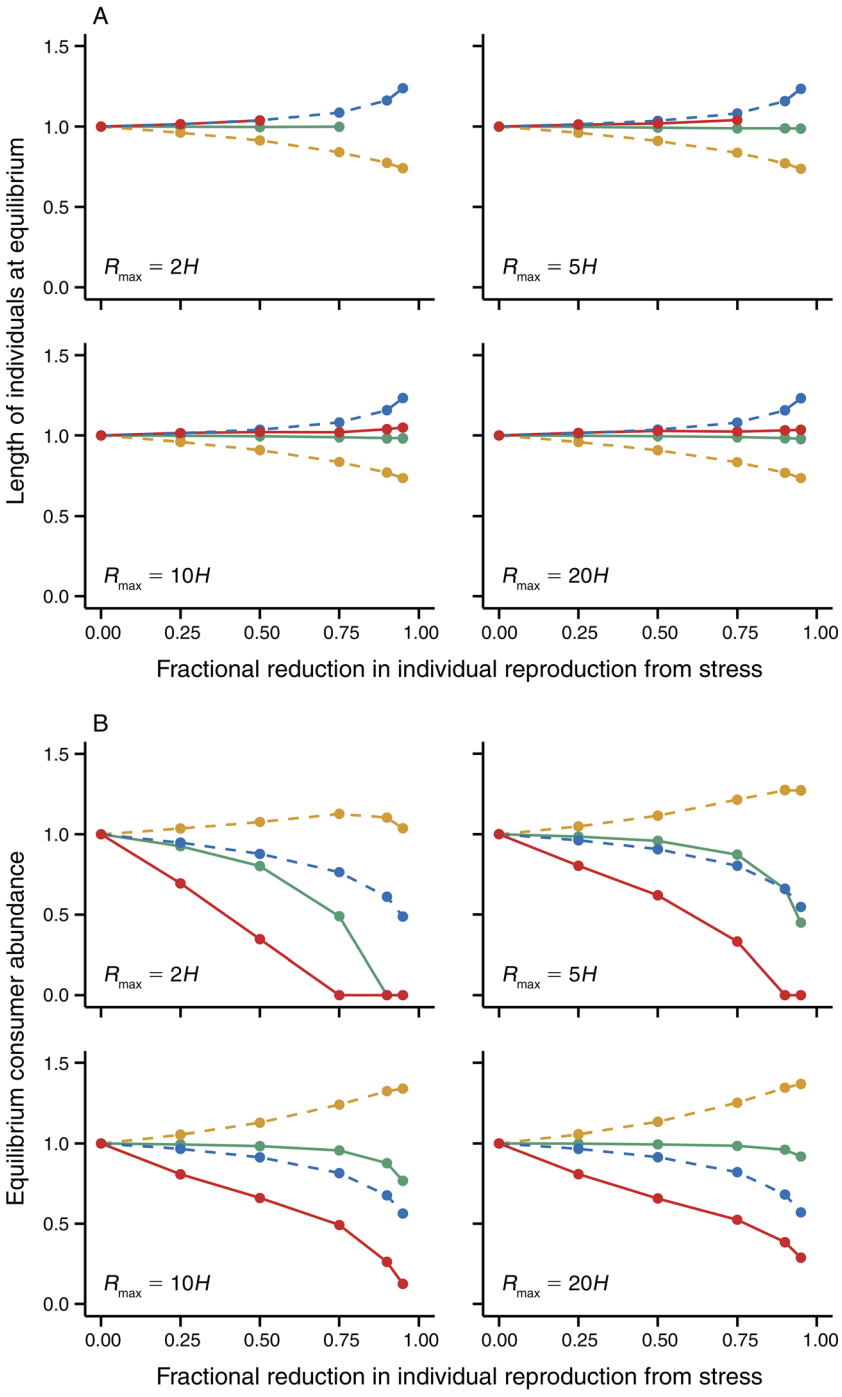

FIG. 5. The relationship between stress measured at the individual level (cumulative reproduction after 21 days) and effects at the population level; (A) mean length of individuals at equilibrium and (B) equilibrium population abundance in the DEB-IBM model. Both length and abundance are presented as a fraction of control population value. The different lines represent hypothetical chemicals with different PMoAs (line styles as in Fig. 1). Results are shown for four levels of maximum resource density (R_{max}, as in Fig. 2). Each point represents the mean of 100 simulations.

productive environments (low R_{max}) had lower capacity to buffer stress. Other types of resource dynamics, e.g., a logistically growing resource, have greater buffering potential; indeed, when resource dynamics are logistic, the indirect mechanism can actually cause an increase in consumer biomass in productive environments where in the absence of stress, consumers keep resource biomass well below their carrying capacity (Abrams 2002).

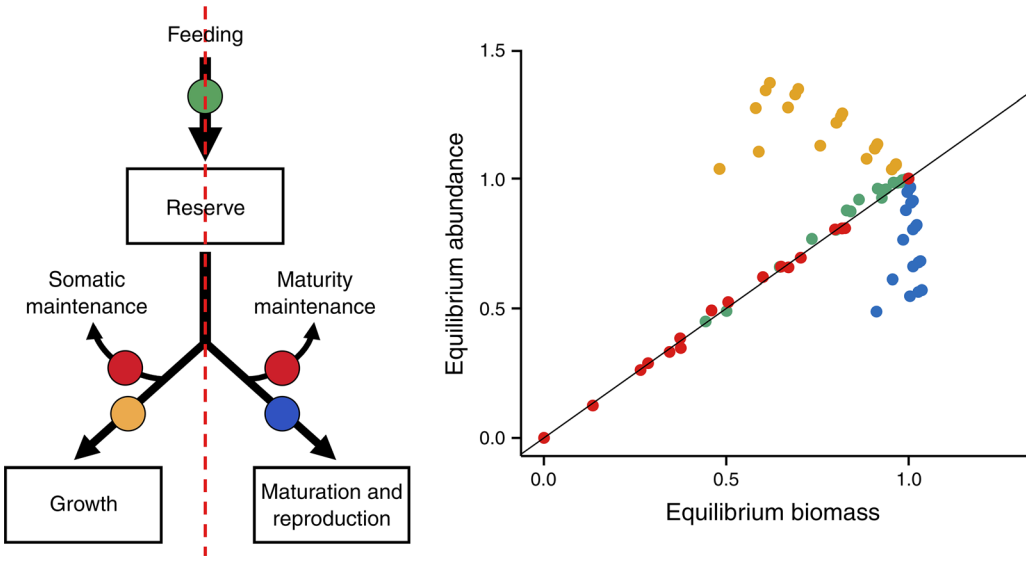

FIG. 6. The relationship between symmetry of stress at the individual level (left panel), and the symmetry of population response (right panel). The red dashed line differentiates stress types that alter energy allocation to growth and reproduction symmetrically (feeding stress, green; maintenance stress, red), and those that act asymmetrically (growth stress, orange; reproduction stress, blue). In the right panel, the symmetry of population response in terms of abundance and biomass is shown. Each point corresponds to the mean value of abundance and biomass for each combination of R_{max} and effect level as a fraction of control population value. The colors correspond with the PMoAs indicated in the left panel.

However, in both logistic and semi-chemostat systems, the buffering capacity of the indirect mechanisms is reduced in environments with low productivity (low resource carrying capacity), because resource growth rate decreases rapidly in response to its increase in biomass. In addition to this indirect resource-mediated mechanism, maintenance stress reduces consumer biomass directly, through a decrease in the efficiency of converting food to new biomass. Unlike the indirect mechanism, this direct mechanism is not resource dependent. Thus populations exposed to a stressor that increases maintenance costs will show reductions in population biomass and abundance even in productive environments.

Our results suggest that the two feedback mechanisms found in classic consumer–resource models are important, even in the absence of ontogenetic symmetry. The population response to feeding stress in DEB-IBM, like YI, was due exclusively to the indirect resource-mediated feedback. Maintenance-stressed populations in DEB-IBM were also affected by this indirect mechanism, but were subject to an additional, direct, resource-independent mechanism. As a result, DEB-IBM populations, like YI populations, showed significant biomass loss in response to maintenance stress even in environments with high resource productivity. Because both models are generic representations of consumer–resource systems, and because DEB is a generic representation of organismal physiology, we expect these direct and indirect effects to be important for a wide range of consumer–resource systems.

Analysis of the DEB-IBM model revealed an additional feedback mechanism not present in the YI model. Stress that alters the ontogenetic symmetry of energy allocation shifts the size structure of populations, so that changes in population abundance are no longer directly proportional to changes in biomass, and in some cases can even lead to abundance and biomass moving in different directions under stress. This provides a useful heuristic for predicting the qualitative response of a population to toxicity, by knowing which direction a stressor shifts individual energy allocation (Fig. 6). For example, in the case of a stressor that acts via embryonic mortality, we can predict a decline in abundance to biomass ratio, mediated by an increase in relative growth efficiency, and average individual size. This prediction is consistent with results from a population experiment using 3,4-dichloroaniline (DCA), a chemical that resulted in reduction in reproduction without effects on growth at the individual level. Exposure to 3,4-DCA reduced population density while increasing the proportion of the population consisting of large (adult) individuals (Preuss et al. 2010). Although in this case, the model and data suggest negligible effects on population biomass, changes in population structure and individual performance can have severe effects under several environmental scenarios, e.g., fluctuating conditions, predation (Gergs et al. 2013), or exposure to mixtures (Agatz et al. 2012).

Unfortunately, there are very few cases, like for 3,4-DCA on *Daphnia*, where toxic effects at the individual and population level are known. However, the comparison of toxic effects on populations at low and high

densities provides evidence for differing compensatory mechanisms in populations exposed to stress. In some cases, feedbacks can compensate, nearly completely, for the effects of stress observed in experimental conditions without feedbacks. For example, Sibly et al. (2000) found that copepod populations in high-food environments exposed to pentachlorophenol approached their equilibrium densities at a reduced rate, but at equilibrium, their biomass did not significantly differ from control populations. Other studies provide counterexamples, where feedbacks did not completely compensate for the effects of stress, and populations declined in response to chemical stress (Marshall 1978, Noël et al. 2006). We have documented several feedback mechanisms that may explain these differential outcomes in natural and experimental systems. However, experimental work measuring toxic effects on individual- (growth and reproduction) and population-level traits (abundance, biomass, and size structure), is needed to elucidate the extent to which the feedbacks identified, and possibly others, can explain and predict a population's response to stress.

The fundamental challenge for ecological risk assessment is that most measures of stress are made in a controlled, constant environment that lacks the important feedback processes present in the environments the risk assessment is charged with protecting. One solution to this problem would be for risk assessments to require experiments that include the relevant feedback processes in natural environments (e.g., population experiments). While more experiments with population-level feedbacks are badly needed, the extended spatial and temporal scales needed to run them make their use in standard risk assessment impractical. One way to circumvent the extrapolation problem is to use lower-level tests to identify the physiological process a given stressor affects and then use ecological theory or models to identify the relevant feedbacks associated with that process, and ultimately how they will affect the population response. As we have shown, a reduction in reproduction can be due to toxic impairment of many physiological pathways, and thus alone is insufficient to identify the PMoA. However, in many cases, the differing growth trajectories associated with various PMoAs (Fig. 1) can be used to identify the affected process. In some of the few cases where growth has been measured in addition to reproduction, patterns of effect consistent with those analyzed in the DEB-IBM model have been observed, for example, reduced growth rate and asymptotic size (Jager et al. 2004, Jager and Klok 2010, Agatz et al. 2012), reduced growth rate but unaffected asymptotic size (Alda Alvarez et al. 2006), and reductions in reproduction with no effects on growth (Jager et al. 2007, Preuss et al. 2010). When growth and reproduction are measured in combination over time, mechanistic-effect models can be used to fit growth and reproduction data in combination to identify the PMoA most consistent with the pattern of effect (Ashauer et al. 2011).

In conclusion, our results highlight the limitations of using reproductive output of individuals as an indicator of population-level effects. We propose a way forward, involving measuring growth in addition to reproduction, and using process-based models to interpret both data types simultaneously. If measuring growth in addition to reproduction is in some cases determined to be unfeasible, then a conservative approach could be used, by testing the population outcome under different PMoAs, and assessing risk assuming that the most harmful PMoA is responsible for the observed reduction in reproduction. However, when growth is measured, the approach outlined here and elsewhere (Jager et al. 2004, Martin et al. 2013*b*) provides a framework for classifying stressors (by PMoA). These types of classifications are badly needed in ecological risk assessment of chemicals, due to the near infinite number of chemical–species combinations.

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Supplemental Material

Ecological Archives

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