Interpreting toxicity data in a DEB framework; a case study for nonylphenol in the marine polychaete *Capitella teleta*

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Abstract
Dynamic Energy Budget (DEB) theory provides a powerful framework for interpreting toxicity data, but is not broadly applied in ecotoxicology yet. One of the reasons is the fact that estimation of DEB parameters from experimental data is certainly not a trivial affair. Almost every data analysis raises new questions, which require the formulation of specific mechanistic hypotheses. These hypotheses should be translated into model adaptations, which can subsequently be tested on the data set. In this paper, we demonstrate this procedure by analyzing a case study for ecotoxicity within the DEB framework. The case study we selected is a previously published data set for 4-n-nonylphenol in the polychaete worm *Capitella teleta* (formerly *Capitella* sp I). Particular aspects of interest in this study are an apparent slow initial growth, and low-dose stimulation (hormesis) of growth and reproduction. In this publication, we explicitly discuss our deviations from the standard DEB model in terms of the underlying hypotheses, the existence of alternative hypotheses to explain the data, and how limited additional experiments can be designed to decide between alternative explanations.

Keywords: Dynamic Energy Budgets, *Capitella teleta*, Nonylphenol, Life-cycle toxicity, Hormesis, Dose-response analysis

1. Introduction

Dynamic Energy Budget (DEB) theory (Kooijman, 2001) provides a powerful framework for interpreting toxicity data (Alda Álvarez et al., 2006a; Jager et al., 2006; Jager et al., 2010). A DEB analysis considers the toxic effects on all life-history traits over the entire life cycle in one consistent mechanistic framework. Such an analysis helps to understand how toxicants affect the acquisition of resources, and their use for growth, development, maintenance and reproduction. In this way, DEB theory provides a link to effects at higher levels of organization (e.g., populations, Jager and Klok, 2010), but also lower ones (e.g., gene expression, Swain et al., 2010). Understanding toxicant effects in a mechanistic sense is essential to make useful extrapolations beyond the laboratory test conditions, e.g., to the population response under various food situations (Jager and Klok, 2010). Despite the advantages of a DEB-based analysis, this approach is not broadly applied in ecotoxicology yet. One of the reasons is probably the perceived complexity of the theory. Furthermore, obtaining realistic values for all of the required parameters is certainly not a trivial affair (Kooijman et al., 2008; Van der Meer, 2006); most toxicity experiments are not designed with a DEB-based analysis in mind. Consequently, there will be data gaps to be filled before this theory can be applied. Furthermore, it is difficult to perform such an analysis of toxicity data on a routine basis, certainly without background knowledge of the theory.

One of the biggest challenges is that virtually every analysis deviates from the predictions of the standard DEB model in some way, and thereby raises questions regarding organism biology and/or experimental setup (see e.g., Alda Álvarez et al., 2005; Jager and Klok, 2010). When the model fit of the data is unsatisfactory, working in a DEB framework poses restrictions. If the model cannot describe the body size data, for example, we cannot simply select a different growth curve. Instead, we need to come up with a plausible mechanistic hypothesis, consistent within the theory, respecting mass and energy balance of the individual, and subsequently test this hypothesis on the experimental observations. The result of such an analysis is therefore not a simple summary statistic after a particular test duration, but rather an integral explanation of the observed effects data for all endpoints over time. This explanation follows in a formal manner from a set of assumptions: the standard assumptions in the DEB model (Sousa et al., 2008), appended with a set of general assumptions for toxic effects (Jager et al., 2010), and a set of additional hypotheses for this particular compound and experimental situation. In other words, the model equations follow uniquely from the assumptions; if the model fit is poor, one (or more) of the assumptions does not hold.

In this paper, we will demonstrate how this framework of fitting and hypothesis generation operates in practice, by employing a case study for a previously published ecotoxicological data set. In this publication, we will explicitly focus our discussion on deviations from the standard DEB model, in terms of relevance of the underlying assumptions. We will discuss the existence of alternative hypotheses to explain the data, and how limited additional experiments can be designed to decide between the alternative explanations. We selected a case study on the effects of 4-n-nonylphenol on life-history traits in the marine polychaete, *Capitella teleta* (formerly *Capitella* sp I) (Hansen et al., 1999). With this case study, we do not claim to provide a definitive answer to the toxicity mechanism of this compound in this species. Rather, we intend

to clarify the strategy for a full-blown DEB analysis of toxicity data, show how to deal with data gaps and deviations from model predictions, and finally illustrate the differences between a descriptive analysis and approaching a data set from a DEB-based perspective, and the added benefits of the latter.

2. Description of the experimental setup

2.1. Test organism

The deposit-feeding polychaete *C. teleta* is a well-studied opportunistic species, which dominates the benthos in organically enriched as well as polluted environments (Lopez and Levinton, 1987; Tsutsumi, 1990). This species demonstrates a high tolerance, both to organic and inorganic contaminants (Selck et al., 1998; Selck et al., 2003). *C. teleta* has lecithotrophic development (meaning that the mother provides yolk in the egg to sustain the embryo) and reproduces via dispersing larvae, which mature rapidly after settlement. The larvae have a high survival rate, and consequently, this species has rapid population growth rates and can reach densities of up to 400,000 individuals m^{-2} (Méndez et al., 1997). Age at maturity and age at first reproduction depends on the sediment's organic matter (OM) content, but is generally found to be around 30 and 40-50 days, respectively, in clean natural sediment with at least 1% OM (Hansen et al., 1999; Ramskov and Forbes, 2008). Settlement may be delayed in the absence of suitable substrate without suffering post-settling mortality, and is generally observed to happen shortly (hours to days) after hatching (e.g., Cohen and Pechenik, 1999), although settlement may be delayed in the presence of sediment-associated toxicants (A. Palmqvist, unpubl. obs). At least in some *Capitella* species, the larvae are able to maintain or even increase their carbon content in filtered sea water for up to two weeks; suggesting that they can either feed on suspended bacteria or take up dissolved organic molecules (Qian and Chia, 1993). On average, one brood per week is produced, with a total of about 5-7 broods during a lifetime. The number of eggs per brood ranges between some 100 to more than 500 eggs (Hansen et al., 1999), depending on the organic matter content in the sediment and female age (Ramskov and Forbes, 2008). The presence of toxicants has been demonstrated to adversely affect a number of the individual life-history traits (e.g., survival, growth, reproduction) in *C. teleta* (e.g., Hansen et al., 1999; Linke-Gamenick et al., 2000; Ramskov et al., 2009).

2.2. Test compound

NonylPhenol EthOxylates (NPEO) comprise more than 80% of the AlkylPhenol EthOxylates (APEO), which are one of the largest surfactants groups. Nonylphenol (NP) constitutes the main degradation product of APEO and is considered a fairly stable metabolite with endocrine disrupting properties (at least in vertebrates). NP is hydrophobic (log K_{ow} 4.48), persistent in the environment, bioaccumulates, and has shown adverse chronic effects on aquatic organisms (Staples et al., 2004). Due to its persistence and lack of degradation in anaerobic sediments, NP is found in the aquatic environment in high concentrations (Langston et al., 2005). For example, Navarro-Ortega et al. (2010) have recently reported between 69 and 6000 µg NP/kg sediment in the Ebro River Basin, and Langston et al. (2005) reviewed published concentrations in freshwater and marine sediment from different places worldwide, reporting values as high as 72 mg NP/kg sediment in the Great Lakes. This study applies the single

isomer 4n-NP, which is not necessarily representative for technical NP (see e.g., Preuss et al., 2010, and references therein).

2.3. Original data set

The experimental data set that we use is a partial life-cycle toxicity test for *C. teleta* exposed to 4n-NP in the sediment; full experimental details are provided in Hansen et al. (1999). The experiments start with the introduction of newly released larvae in beakers containing NP-contaminated sediment in concentrations 0 (control), 14, 52 and 174 mg/kg dry weight of sediment (measured in sediment after addition). Starting from day 14, body size and egg production were determined every week, and at the same time sediment and water were renewed, until the experiment was terminated at day 78. Survivorship was high and reported not to be influenced by the treatment. Therefore, effects on mortality were not considered.

The original data from this experiment were unfortunately lost, so we extracted the data for body volume and egg production per body volume from the graphs in the original publication. Data for reproduction were recalculated to cumulative number of eggs per reproductive animal, using the volume at each census day. Body volumes were translated to volumetric length (cubed root of volume) which allows a clearer view of the initial growth pattern in the figures.

2.4. Additional measurements

For a DEB-based analysis, it is important to know the size at birth. This is essential to allow for an estimation of the energetic costs for a single egg, which in turn is needed to correctly interpret the reproduction rate in energetic terms. Unfortunately, Hansen and co-workers first started their observations of body size after 14 days. Therefore, a new measurement of *C. teleta* egg size was made (n=8) using eggs from the same culture as used by Hansen and co-workers. This produced an average volume of 4.1 (range 2.9-4.8) $\cdot 10^{-3}$ mm^3, estimated from the longest and shortest axes of the egg, assuming the egg is shaped like a spheroid (Bridges, 1996; Qian and Chia, 1991). The measured egg diameter correspond well with earlier published data (Bridges, 1996; Eckelbarger and Grassle, 1983). This also corresponds well to the estimated body volume of $6.3 \cdot 10^{-3}$ mm^3 for 3-5 days old swimming larvae (S. Pedersen, unpubl. data). We assume that egg volume is roughly similar to body volume after hatching.

3. Description of the DEB model and adaptations

3.1. Model description

We will provide a short overview of DEB theory in the following; a detailed discussion of the underlying concepts can be found in Kooijman (2001) or Nisbet et al. (2000). DEB theory provides a set of simple rules that specify how all living organism acquire resources (energy and building blocks), and how they use these resources to maintain themselves, grow, develop and reproduce, over their entire life cycle. The term ‘budget’ in DEB refers to strict conservation of mass and energy; all ‘expenses’ need to be paid by acquiring resources from the environment. The basic structure of a DEB model for an animal is shown in Figure 1. There are four somewhat abstract state variables in the model (the large boxes in Fig. 1). None of

these states can be directly measured in the organism, but they have a logical connection to observable traits like body size and egg production.

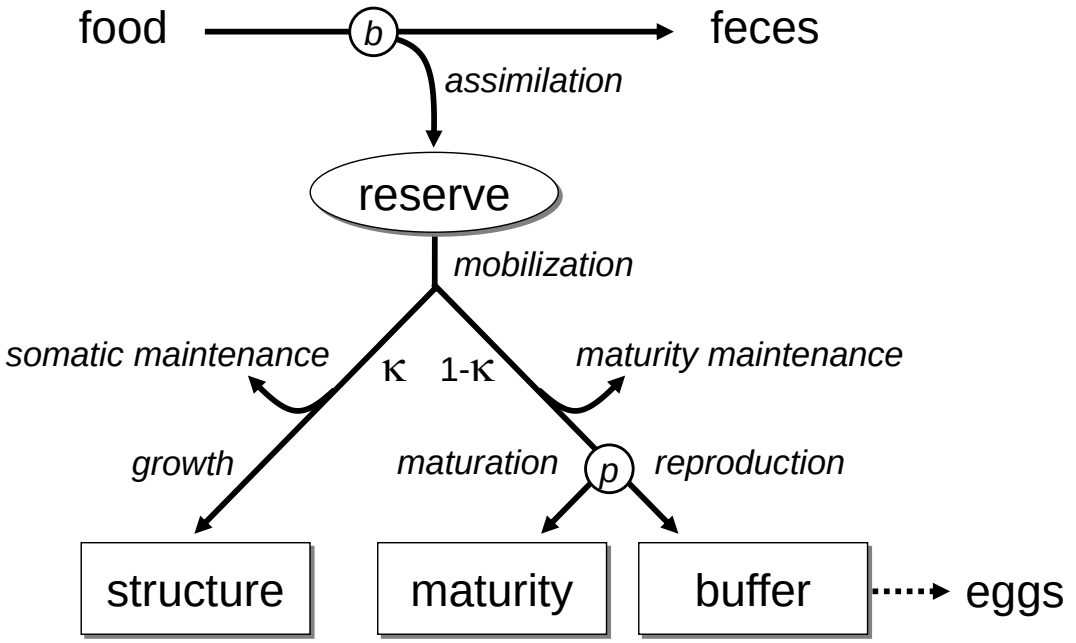

Fig. 1. Structure of the standard DEB model. The two nodes represent the switches in the life history at birth (*b*, start of feeding) and puberty (*p*, switch investment from maturation to production of gametes).

Biomass is partitioned into structure and reserve. Only structure requires maintenance, and only reserves are used to fuel metabolic processes such as growth, maintenance and reproduction. Reserves should not be thought of as inert materials waiting for further use; they include compounds that have an active metabolic function. The distinction between compounds classified as reserves and structure is in their dynamics, and in their response to nutritional status. The inclusion of reserves as a state variable is supported by the empirical observation that organisms do not die immediately when there is no food available, and even can continue to grow and reproduce for a while. The reserves buffer the organism against rapid changes in food availability.

The energy mobilized from the reserves is divided according to a fixed fraction κ over somatic maintenance and growth on the one hand, and maturity maintenance, maturation and reproduction on the other (Fig. 1). Maturity is though of as 'information' and therefore has no contribution to body mass; it is quantified by the amount of reserves that are allocated towards maturation. Maturity thresholds mark the initiation of feeding ('birth') and the start of investment in reproduction ('puberty'). Adults are assumed not to invest in further maturation but instead allocate resources into a reproduction buffer. This buffer is converted into eggs during spawning events.

When toxicants affect growth and reproduction, there are energetic consequences. If fewer resources are used for the processes of growth and egg production, where did the energy go to? Toxic effects on life-history traits are thus associated with a change in one or more DEB parameters (Jager et al., 2006; Jager et al., 2010). In principle,

every DEB parameter may be affected by a toxicant, but we depart from the assumption that a single parameter is affected, and consider additional target parameters until we obtain a satisfactory explanation of the toxicity. Even though we cannot measure DEB parameters directly, a change in each individual parameter has specific consequences for the life history traits; a 'physiological mechanism of action' (Alda Álvarez et al., 2006a). We can therefore use the toxicant's pattern of effects over the life to reconstruct the most likely affected parameter(s).

To link external toxicant levels to DEB parameters, we need to address toxicokinetics and the internal dose-response relationship. In toxicity experiments, body residues are not routinely determined. However, we can derive some information from the time course of the observed effects. When we assume one-compartment accumulation with simple first-order kinetics, we can estimate the time to equilibrium (and thus an 'elimination' rate constant) from the time pattern of effects. This rate constant does not necessarily reflect whole-body elimination; it represents the slowest removal or repair process in a chain of events that links the external concentration to the targeted DEB parameter. We do not have access to the absolute body residue, and thus cannot estimate bioconcentration factors or uptake rate constants. To estimate the dominant elimination rate constant from toxicity data requires a scaled version of the toxicokinetic model (see Jager et al., 2010).

We used a scaled version of the DEB equations as presented in Kooijman et al. (2008), in the appendix of that paper (the dimension of 'energy' is scaled out of the equations). We added the equations for scaled internal concentration and toxic stress as given in Jager et al. (2010). Note that this formulation is more extensive than the simplified version as used for most DEB-based treatments of toxic effects (see Jager and Klok, 2010, for discussion). The full set of equations is provided in the supporting information. The model was implemented in Matlab R2010a. Parameters were estimated on the basis of likelihood maximization (combining the fits on body size and reproduction), and confidence intervals were derived by profiling the likelihood function (see Jager et al., 2004).

3.2 Link between state variables and observations

In most DEB-based analyses, the observed body volume would be directly compared to the structural volume from the DEB model (see Fig. 1). However, the physical body volume, as measured in this experimental data set, also contains contributions from reserves and the reproduction buffer. As long as the reserve density (mass of reserves over mass of structure) remains constant in time and with toxicant exposure, we can safely ignore the contribution of reserves to volume (structural volume will then be proportional to the actual volume).

The contribution of the reproduction buffer to measured volume also requires consideration, especially in animals that produce large clutches of offspring (such as *C. teleta*). However, the current data set only provides mean body volume and mean reproductive output for a population of animals. It is unlikely that all individuals convert their reproduction buffer into eggs at the same moment, and so the body volume measurements of adults contain an unknown contribution of the reproduction buffer, at the time of measurement, and we therefore chose to ignore the reproduction buffer in the model. Lets consider, as a rough estimation, an adult of 15 mm^3 that

produces a clutch of 500 eggs of $4.1 \cdot 10^{-3}$ mm^3 each. In that case, the contribution of the eggs to the body volume is 14%, and on volumetric length 4.4% only. Therefore, we judge the error made by ignoring the reproduction buffer an acceptable one, and directly compare structural length from DEB to the cubic root of body volume.

Apart from its effect on body size, the existence of a reproduction buffer also implies that egg production is discontinuous in individuals. However, this behavior is not clearly seen in the data of Hansen and co-workers, probably because the individuals are not synchronized and there is only one observation per week. We will therefore compare the reported data for egg counts in a cumulative manner to the continuous reproduction rate from the DEB model. A more detailed representation of the reproduction behavior, and its effect on body volume, would have been preferable. This requires following body size and egg production of individual worms over time, preferably with body size observations just before and after spawning.

3.3. Default parameters

For several of the basic DEB parameters, we can depart from defaults if required (Kooijman, 2010, offers a complete set of defaults based on maximum body size only). In this analysis, we fixed a few parameters to a default value. The fraction of allocated reserves that is fixed in eggs (κ_R: reproduction efficiency) is probably close to one, and Kooijman et al. (2008) suggest a value of 0.95. At maximum food, the scaled functional response (f: scaled ingestion rate) is equal to 1. Many invertebrates seem to have a rather constant size at first reproduction, when faced with different levels of available food. In a DEB context, this translates to a similar value for the somatic and maturity maintenance coefficients, and thus to a ratio between somatic and maturity maintenance k of 1 (see Table 1). In *Capitella*, this value appears to be lower, as body size at first reproduction decreases with decreasing food availability (Ramskov and Forbes, 2008). However, in a DEB context, puberty marks the start of investment in reproduction, which precedes the appearance of the first clutch of eggs. If clutch size is affected by food limitation, it is possible that investment in the reproduction buffer still starts at a fixed size for different food levels, even though the release of the first clutch does not. Therefore, we will still depart from a value of k=1, but will also evaluate whether lower values fit better.

3.4. Initial growth in juveniles

Under constant conditions, DEB theory predicts that growth in the control situation should follow the Von Bertalanffy curve. However, the growth curve for *C. teleta* shows a deviation from this pattern. The initial growth in the larvae must be slower than expected from the standard DEB model. This implies that one or more of the underlying assumptions are violated. The standard model follows from the assumption that food availability is constant, and that the maximum ingestion rate is proportional to the surface area of the animal. *Capitella* spp. feed on selected components (sediment particle up to a certain size) of the sediment. For many species (including *C. teleta*), it is unclear whether the juveniles prefer the same food as the adults; their natural sustenance may differ from the food available in laboratory tests. It is thus possible that the larvae initially experience food limitation. This hypothesis was used before to explain deviating growth curves in other species (Jager et al., 2005; Jager and Klok, 2010). In this case, no additional food was added to the sediment, so it is

possible that initial feeding is restricted. Another likely explanation is that settling of the larvae in the sediment was not instantaneous (see discussion above). Hansen and co-workers assumed that settlement occurred within hours after their introduction to the experimental containers, but this was not confirmed by observations.

Unfortunately, we do not have knowledge about what happens to the larvae in their first few days after introduction to the experimental containers. In complete absence of data for this time period, we use an *ad hoc* solution: assume a lag time (the state of the organism does not change in the first days after introduction, and the length of this period is estimated from the data). Alternative explanations are that the feeding efficiency is a function of body size (Jager et al., 2005), or that the larvae go through a period of initial starvation (e.g., because they may initially require bacteria which may need some time to develop in the test system). A more detailed analysis of the behavior of the larvae in this initial time period is needed to select the most appropriate explanation for this apparent slow initial growth.

3.5. Hormesis in a DEB context

Comparing the response of the control to the lowest exposure concentration, it becomes clear that the lowest tested concentration of NP actually has a consistently positive effect on growth and reproduction (Hansen et al., 1999). At higher doses, the effect becomes negative. This type of response is often observed in toxicity data. When it cannot be explained as 'random' variation in the experimental data, this phenomenon of stimulation at lower doses is referred to as 'hormesis' (e.g., Forbes, 2000). The first thing to realize is that calling something hormesis does not explain this behavior; it is only a name for the phenomenon. Clearly, any organism has to obey the laws for conservation of mass and energy; an increase in performance therefore has to be paid in some way (Forbes, 2000). Working in a DEB context forces us to come up with a quantitative hypothesis. Following this logic, there are three basic paths to explain hormesis in this study: 1) the toxicant increases the assimilation of resources, 2) the toxicant affects the distribution of assimilated resources, which implies that any improved performance is associated with reduced performance on another trait (e.g., an increase in egg number linked to a decrease in egg size, Hammers-Wirtz and Ratte, 2000), or 3) the toxicant acts as a 'medicine' and relieves an unforeseen stress in the experimental system (e.g., parasites or secondary metabolites produced by algae or bacteria). Even combinations of these three options may occur.

For any hormetic effect on reproduction, it is essential to study the energy investment per egg (e.g., energy content, dry weight, hatchling size). Additionally, it is important to scrutinize the test setup to look for possible effects through food (density or quality) or other organisms (e.g., parasites). In this particular case, we lack the information to decide which of these options is most realistic. Because the effect occurs on both growth and reproduction we start from hypothesis 1 and treat hormesis as an increase in apparent food availability. From an evolutionary perspective, it seems unlikely that an animal will scale up its feeding rate in response to a toxicant (an individual that also increases feeding in absence of the toxicant would have an evolutionary advantage). Also, there is little evidence that feeding rate (measured as mass of sediment passing through the gut per worm body volume) in *C. teleta* shows any reaction after exposure to sediment-bound metals (Selck et al., 1998) or organic

compounds (Méndez et al., 2001; Selck et al., 2003). A more likely possibility is that NP increases the quantity or palatability of the available food in some way. NP might be used as an energy source for micro-organisms that in turn can serve as food for *Capitella*. Even though the organic matter content in the sediment was high (7.4%), and should not be limiting, bacteria may comprise a more easily digestible food source than the refractory humic substances that comprises the main part of the total organic carbon content in sediments (Lopez and Levinton, 1987). Alternatively, micro-organisms might be negatively affecting the food quality in the control, but are inhibited by the lowest tested dose of NP (see Jager and Klok, 2010, for a similar explanation). If food quality or quantity is an issue, the hormesis will decrease or even disappear under different (more optimal) test conditions.

4. Analysis of effects of nonylphenol in *Capitella teleta*

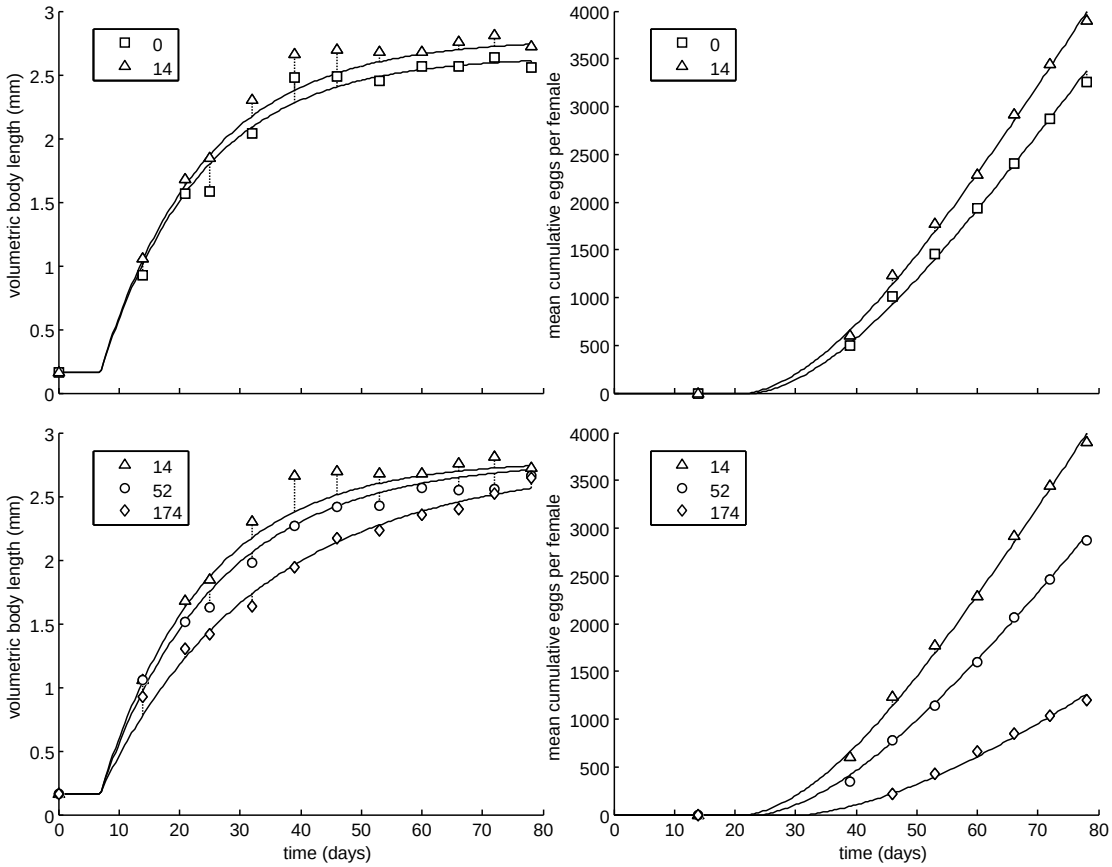

Fig. 2. Fits of the DEB model to data. NP is assumed to enhance food availability by the same amount (approx. 5%) in all treatments. At higher concentrations, NP is assumed to increase the costs for structure, and the costs for maturation/reproduction according to a linear relationship with threshold. Response in the control and lowest concentration (top row) is separated from the response at all NP concentrations (bottom row) to enhance readability. Symbols represent the different sediment concentrations of NP (given in the legends in mg/kg_{dwt}).

Table 1. Parameter estimates resulting from the model fit of Fig. 2. Estimates are given with 95% likelihood-based confidence intervals (n.e. is not estimated). Model equations are provided in the supporting information.

Symbol	Description	Value and unit
Physiological parameters		
g	energy investment ratio	2.42 (1.33-20[a]) [-]
v	energy conductance	1.68 (1.15-11.1) mm d^{-1}
k_M	somatic maintenance rate coefficient	0.250 (0.210-0.292) d^{-1}
k	ratio of maturity (k_J) and somatic maintenance (k_M)	1 (n.e.) [-]
κ	allocation fraction to soma	0.817 (0.738-0.883) [-]
κ_R	reproduction efficiency	0.95 (n.e.) [-]
U_H^p	scaled maturity at puberty	1.39 (0.706-2.02) mm^2 d
u_H^{bp}	maturity at birth, relative to puberty	0.865 (0.505-1.28) $\cdot 10^{-3}$ [-]
f	scaled ingestion rate at 0 mg/kg NP	0.951 (0.923-0.964) [-]
	scaled ingestion rate at >0 mg/kg NP	1 (n.e.) [-]
T_{lag}	initial lag time	6.97 (5.74-8.08) d
Toxicity parameters		
k_e	dominant 'elimination' rate constant	0.236 (0.0747-100[a]) d^{-1}
c_0	NEC for effect on assimilation	8.45 (0-14.9) mg kg_{dwt}^{-1}
c_T	tolerance for effect on assimilation	146 (106-176) mg kg_{dwt}^{-1}

[a] this is the highest reasonable value that is allowed in the estimation.

4.1. Evaluating the starting hypotheses

Assuming a lag time for the juveniles leads to a good fit of the growth curves for all treatments (Fig. 2). The duration of this phase is estimated from the data set as 7 days regardless of NP exposure (Table 1). Although it is clearly unrealistic to assume that there are no changes in the organism's state variable for the first week, a more convincing explanation would require further experimental evidence (especially observations on settlement and body size in the first two weeks after hatching).

A good fit of the growth and reproduction data was obtained by assuming that the presence of NP increases the assimilation of energy from food (which can represent higher availability or quality of food, or increased ingestion or assimilation efficiency). This enhancement was estimated as some 5% (see parameter f in Table 1, the confidence interval for f at 0 mg/kg NP does not include one), and assumed to be the same for all concentrations of NP (as the simplest hypothesis). This does not prove that NP increased feeding rates and/or assimilation efficiencies; it is a testable hypothesis which is consistent with the observed patterns in growth and reproduction simultaneously.

The assumption of equal values for the rate coefficients for somatic and maturity maintenance fits the data well (k = 1, Table 1). Allowing this parameter to be freely estimated from the data leads to a lower value (around 0.6), which provides a slightly better fit (not significant; likelihood ratio test, α=0.05). Given that we are not completely sure about the mode of action of NP, which can influence the size at puberty too (Jager et al., 2010), this data set does not offer strong support for a value of k smaller than one. As k=1 appears to be a good approximation for many invertebrates, we propose to stick to this default value.

4.2. Physiological mode of action for NP

The growth curve for the highest concentration of NP strongly suggests that costs for making new structure are increased. An increase in this DEB parameter leads to a decrease of the growth rate, but has no effect on the ultimate size (the growth costs do not determine ultimate size; ultimate size is determined by the ratio of the surface-specific assimilation rate to the volume specific maintenance costs). Thus, the toxicant effects are clearly visible half-way through the test, but not anymore at the end of the exposure period (see also Alda Álvarez et al., 2006a; Alda Álvarez et al., 2006b). This DEB mechanism provides a good fit on the data for body size. Other mechanisms that affect the growth patterns are a decrease in assimilation (e.g., decreased feeding rates), an increase in maintenance (e.g., for detoxification), or a decrease in the allocation fraction κ. However, these mechanisms all imply a dose-related decrease in the ultimate size of the individuals. This was not clearly observed in the current data set, and these mechanisms therefore resulted in poorer model fits than the assumption of a change in structural costs. A lack of effect on ultimate size might also result when body residues of NP decrease again over the course of the test (e.g., due to a decrease in bioavailability over the test duration). However, such a time-dependent decrease in exposure concentration is unlikely in view if the experimental setup (weekly renewal of sediment), and should also decrease the effects on the reproduction pattern over time, which was not observed in the data.

Increasing the costs for making new structure implies an indirect effect on reproduction. Body size determines feeding rates, and a slower growth therefore affects the reproductive output. However, the effect on the reproduction rate is larger than predicted by the increase in growth costs alone. A good fit is obtained by assuming that NP also increases the costs for maturation and reproduction by the same factor as the costs for structure. This makes some sort intuitive sense as both growth costs and maturation/reproduction costs are complementary processes in the DEB scheme (see Fig. 1). However, increasing maturity maintenance also fits reasonably well.

The no-effect concentration (c_0) is estimated at 8.45 mg kg_{dwt}^{-1} (Table 1). The confidence interval includes the first tested dose, which implies that it is unclear whether there is already a negative effect of NP at this exposure concentration, or only the positive effect via feeding. The dominant 'elimination' rate is not well identified by the data; the confidence interval extends all the way up to infinity (which would imply instantaneous steady state).

For this analysis, we assumed that NP did not affect the energy investment per egg. If the investment per egg changes, this would have had consequences for the interpretation of the effects on the reproduction rate. An excellent fit also results when we increase the investment per egg by the same factor as the costs for structure. For the highest concentration of NP, this would amount to an increase in the investment per egg of a factor of 2; a difference that should certainly be measurable. However, it is presently not so clear if and how animals change the investment per egg as a result of toxicant or food stress. Two closely-related *Capitella* species demonstrated different reproductive strategies: many small eggs vs. few large eggs (Qian and Chia, 1991). These authors suggest that fecundity, egg size and egg energy content are

mediated by food availability. Bridges (1996) on the other hand, found no clear relation between sediment treatment (mud alone or mud enriched with sewage, algae or oil) and offspring investment in *C. teleta*. Apparently, the response of investment per egg may differ substantially between members of the same genus.

5. General discussion

5.1 Strategy for using DEB to interpret toxicity data

As our case study demonstrates, the procedure for interpreting toxicity data within a DEB framework is rather different from fitting dose-response curves. We start from the standard DEB model for an animal, and attempt to fit it to the complete set of experimental data (all endpoints, all time points). In many cases, the resulting fit will not be satisfactory. One thing to do first is to scrutinize the experimental data; did we overlook something in the experimental setup that violates one (or more) of the assumptions in the standard model? The next step would be to generate mechanistic and testable hypotheses as to why the fit may deviate from the expectations. Subsequently, these hypotheses are translated into an adaptation of the standard model, which is again tested on the experimental data. This iterative process continuous until we are satisfied by the fit.

However, a fit can be good for the wrong reasons; how can we be sure that the additional hypotheses were realistic? There might be an alternative set of assumptions that yields a similar, or better, fit to the data. In this case, where we were working from a previously published data set, we cannot be sure. However, the generated hypotheses will have testable consequences for measurable model output. We can thus use the adapted DEB model to produce a set of predictions that can be tested in additional experiments. Such additional experiments might also involve other endpoints (e.g., respiration, hatching time), or the same endpoints under other test conditions (e.g., different temperature or food situation). Furthermore, such additional studies may also be shorter and focus on a specific part of the life cycle (e.g., the first weeks after hatching).

Of course, one should realize that DEB theory is an idealization of a complex biological system. Therefore, DEB like any theory is always ‘wrong’ in detail. However, for a theory to be useful it needs to capture the essence of the system, for the questions that need addressing. In this case, the question is whether DEB is a useful tool for interpreting toxicity data in *C. teleta*, and to extrapolate toxic effects to untested conditions.

5.2 Evaluating toxicity of NP

At the lowest tested dose, NP stimulated growth and reproduction. This stimulation was consistent with an effect on food availability or palatability. To test this hypothesis, it is possible to repeat (a part of) the test in a sediment system with varying food quantity and/or quality, although this might also affect NP bioavailability. The most likely mechanism of action of NP on *C. teleta* in this test was an increase in the costs for making new structure. An additional effect was required on reproduction (on top of the indirect effects through a decrease in growth rate), but the current data set did not allow distinguishing between three options with a

rather similar fit: an increase in costs for maturation/reproduction, increase in maturity maintenance, or an increase in the energy investment per egg. In the life-history data sets that have been analyzed with DEB so far, it appears to be a common situation that an increase in the costs for structure is associated with an additional effect on reproduction. This was the case for the nematode *Caenorhabditis elegans* exposed to pentachlorobenzene (Alda Álvarez et al., 2006b), the nematode *Acrobeloides nanus* exposed to cadmium (Alda Álvarez et al., 2006a), and to some extent the water flea *Daphnia magna* exposed to PAHs (Jager et al., 2010). At this point, we are not sure about the nature of this link. It could just be accidental or perhaps point at a physiological relationship between the processes of growth, maturation and reproduction that we have not considered before.

Possible confounding in the interpretation of the mechanism of action results from fact that we have no information about whether the investment per egg depends on NP. Relatively small-scale experiments could help to shed light on these issues.

5.3. Possibilities to use the results from a DEB analysis

One of the main strengths of a DEB-based analysis is that it offers a mechanistic explanation of the data set. Therefore, the theory allows for an educated extrapolation to untested conditions, such as the population response under limiting food availability (e.g., Jager et al., 2004), different temperature (Alda Álvarez et al., 2006a), or in the absence of 'experimental artifacts' such as hormesis and initial food limitation (Jager and Klok, 2010). However, the analysis with this extended DEB version forces us to think about a part of the life cycle that was not observed in the experiment: the embryonic stage. Before we can make an educated extrapolation, we need to assess hatching success, hatching time, and hatchling size (both under control and exposed conditions). The number of eggs alone will not be enough when the toxicant also affects the mother's investment per egg (see Hammers-Wirtz and Ratte, 2000), or when the chemical is transferred to the egg and affects embryonic development.

Extrapolation to the population level may also be served by a more detailed analysis of the rules for the reproduction buffer. In our analysis, we ignore this buffer, which means that we considered the egg production to be continuous after puberty. For the comparison to the experimental data this is defensible, but this simplification can have serious repercussions for population dynamics. To gain better understanding of the reproduction buffer requires following individuals more closely to allow better estimates for the actual time of egg production (and the variation between individuals). Furthermore, realistic assessment of population dynamics requires consideration of the energetic effects of 'density dependence' (Linke-Gamenick et al., 1999), and the response to starvation. Constant high food levels are unrealistic for most natural populations. However, when food availability decreases, an individual might face the situation that the maintenance needs cannot be paid anymore from the mobilized reserves allocated to growth and somatic maintenance. In that case, the individual will die, or (more likely) will deviate from the standard DEB rules. How they deviate seems to be highly species specific and requires experimental observations.

5.4. Data needs for a DEB-based analysis

Working in a DEB framework puts different demands on the experimental data than a descriptive analysis. In principle, this partial life-cycle data set is already a very good starting point. However, the questions we raised and the hypotheses we generated point at shortcomings in our understanding of the *C. teleta* life history. Clearly, the DEB analysis would have benefitted from having more information about the initial part of the life cycle (size at birth, initial feeding, settlement and growth). For adults, it would have been advantageous to have growth and reproduction data for the individuals to specify the rules for the conversion of the reproduction buffer into eggs, and to quantify the contribution of the buffer to the body volume. Furthermore, information about the investment per egg (in terms of e.g., caloric content, dry weight) would be helpful to interpret the reproduction rates.

To our knowledge, this is the first DEB-based analysis of a *Capitella* species. For any new species, it is most useful to start with the life-cycle analysis of growth and reproduction at different constant food levels (Kooijman et al., 2008). Such data provide the best opportunity to uniquely identify all parameter values (including the value of k). The current case study shows that it is possible to work with toxicity data alone, but this situation is certainly not optimal.

5.5. Outlook

With this case study, we have demonstrated how life-cycle toxicity data are used in a DEB framework. The time pattern of every life-history trait is considered quantitatively within a single model for the energy budget. We discussed which observations were missing, and how we could generate and test hypotheses to fill the data gaps. These hypotheses are testable, and should indeed be tested before the DEB model and its parameters are used to make strong statements about NP effects under untested conditions. With this DEB analysis, we have answered some questions but also generated several new ones. However, in our opinion, these new questions pave the way for follow-up investigations to increase our understanding beyond the original questions.

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