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Impact of engineered zinc oxide nanoparticles on the energy budgets of *Mytilus galloprovincialis*

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ABSTRACT

This paper characterizes the sublethal impact of engineered ZnO nanoparticles on the individual performance of the marine mussel *Mytilus galloprovincialis* within the context of Dynamic Energy Budget theory, thereby allowing an integrated evaluation of the impact of multiple stressors on various endpoints. Data include measurements of the impact of ZnO nanoparticles on body burden, feeding, respiration, shell length, biomass, and mortality of mussels kept in laboratory tanks for over 100 days. ZnO nanoparticles in the environment impair the mussels' feeding rate (EC_{50} for the maximum feeding rate is 1.5 mg ZnO nanoparticles L^{-1}). Zn accumulated in tissue increases respiration (EC_{50} for the respiration rate is 0.9 mg environmental ZnO nanoparticles L^{-1} with the body burden having reached its ultimate level), indicating that maintenance processes are more affected by ZnO nanoparticles than feeding. The feeding regime constrained growth and biomass production to the extent that the impact of ZnO nanoparticles on these processes was undetectable, yet the remaining measurements allowed the estimation of the toxicity parameters. The toxicity representation, combined with the DEB model, allowed the calculation of the effect of the nanoparticles on the expected lifetime production of reproductive matter. EC_{50} for the expected lifetime production of reproductive matter is less than 0.25 mg ZnO nanoparticles L^{-1}, indicating that that the ecological impact of ZnO nanoparticle exposure is stronger than its impact on individual physiological rates.

1. Introduction

Nanomaterials are now recognized as environmental pollutants with toxic potential. Hence there is an urgent need to characterize their toxicity in a variety of environmental contexts, as the production of nanomaterials is rapidly expanding and their use is increasingly widespread (Thomas et al., 2011a). Although many types of nanomaterials contain common and well-studied pollutants, notably those containing metal ions, the physical properties of nanomaterials, such as shape and size, may determine their environmental fate and bioaccumulation characteristics, thus influencing their toxic potential (Baun et al., 2008; Klaine et al., 2008). Consequently, relatively little is yet known about the potential toxic impact of nanomaterials on biological systems. In this study, we use Dynamic Energy Budget (DEB) theory to analyze the impact of ZnO nanoparticles, whose use is particularly widespread in pigments, cosmetics, sunscreens, and coatings (Klaine et al., 2008; Pitkethly, 2004), on the marine mussel *Mytilus galloprovincialis*.

Dynamic Energy Budget (DEB) theory is a process-based modeling framework with several successful applications in ecotoxicology (Billoir et al., 2007; Ducrot et al., 2007; Jager et al., 2010; Klanjscek et al., 2012; Kooijman and Bedaux, 1996a; Miller et al., 2010; Muller et al., 2010b). DEB theory uses three modeling modules to describe the toxic effects of pollutants on individual organisms (Billoir et al., 2008; Jager and Zimmer, 2012; Kooijman and Bedaux, 1996a,b; Kooijman et al., 2008). The first is a Dynamic Energy Budget (DEB) model that describes the rates at which organisms acquire resources from the environment and use the energy and nutrients therein for growth, maintenance and reproduction (Kooijman, 2010; Nisbet et al., 2000; Sousa et al., 2008). The second module is a toxicokinetic model describing the exchange of toxic compounds between the organism and the environment. The last module is a toxic effect model describing the impact of accumulated toxicants on processes as defined in DEB theory, which essentially amounts to changes in parameter values.

DEB theory has several attractive features making it particularly suitable for investigating the toxic effects of nanoparticles. It provides an integrative framework with which the combined effects of an arbitrary number of environmental factors, such as food availability and temperature, can be described. Thus, the severity of a toxic impact can be studied as a function of these factors. Furthermore, the theory can be used to infer the population consequences of toxic pollution (Alda-Alvarez et al., 2005; Jager and Klok, 2010; Muller et al., 2010b). A feature that is especially attractive for the assessment of toxic effects is that the toxic effect parameters determined from empirical studies are

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independent of experimental duration and the choice of endpoint. This means that toxic effects of some compound as determined with one endpoint (e.g. reproduction) can in principle be used to infer the impact of that compound on another endpoint (e.g. growth).

This paper aims at characterizing the impact of ZnO nanoparticles on the individual performance of the marine mussel *M. galloprovincialis*, and using this characterization for making projections of an ecologically relevant measure: the expected lifetime production of reproductive matter. We use data from Hanna et al. (Hanna et al., 2013) to estimate toxicity parameters in the framework of DEB theory. This extensive data set, one of the most elaborate data sets about the impact of any toxicant on marine mussels known to us, includes measurements on the impact of ZnO nanoparticles on body burden, feeding, respiration, shell length, biomass and mortality. Furthermore, there have been many previous studies using DEB models of *Mytilus* spp. (e.g. Filgueira et al., 2011; Handa et al., 2011; Rosland et al., 2009; Ross and Nisbet, 1990; Sara et al., 2012; Saraiva et al., 2012; Thomas et al., 2011b; Troost et al., 2010; van der Veer et al., 2006), so we have access to well-validated DEB parameters. Our integrated experimental-modeling approach testing the impacts of ZnO nanoparticles on an ecologically important marine suspension feeder advances our understanding of the ecological impacts of nanomaterials in marine coastal ecosystems that have been identified as potentially important environmental sinks for these emerging environmental pollutants (Canesi et al., 2012; Keller et al., 2010; Klaine et al., 2008; Montes et al., 2012; Scown et al., 2010).

2. Model

We use our previously tested modeling framework (Muller et al., 2010b) to investigate the toxic effects of ZnO nanoparticles on the energy budgets of marine mussels. The foundation of this framework is Kooijman's Dynamic Energy Budget (DEB) model. Since a detailed discussion of model assumptions and derivation can be found elsewhere (Kooijman, 2010; Nisbet et al., 2000; Sousa et al., 2008), we simply mention here that this model uses three state variables, i.e. structural biomass, reserve density and maturity, to describe the rates of resource acquisition, growth, reproduction, maturation, and maintenance. We have amended the standard DEB model with a rule for reserve mobilization under severe starvation conditions: in case of a deficit, the reserve mobilization rate increases to match maintenance demands. Because the theory strictly obeys mass and energy balances, it implies the dynamics of derived quantities, such as the respiration rate, without additional assumptions. Fig. 1 outlines the energy and material flows and state equations, Table 1 lists the equations used in this paper, and Table 2 explains symbols and conventions.

To account for toxicant exchange, we assume a toxicokinetic model based on a single animal compartment (see Table 1 for the dynamic equation for toxicant exchange). This model is frequently used in the framework of DEB theory (Jager and Zimmer, 2012; Kooijman, 2010 and references therein; Kooijman et al., 2008; Kooijman and Bedaux, 1996a; Muller et al., 2010a) and differs from the standard toxicokinetic model found in many textbooks by assuming that the rates of uptake and elimination are proportional to the surface area of an animal, not its biomass.

With regard to toxic effects, we recognize two potential targets of ZnO nanoparticles: maintenance and feeding/assimilation (Muller et al., 2010a). We assume that the maintenance rate potentially increases linearly with the body burden of ZnO nanoparticles beyond a threshold value, the no effect concentration (NEC). Furthermore, we assume that the rates of feeding potentially decline hyperbolically with the environmental concentration of ZnO nanoparticles (see Results & discussion section for motivation of actual toxic effect models used here). We used these toxic effect models previously to describe the impact of produced water in oil production on marine mussels (Muller et al., 2010b).

To investigate the longer-term ecological implications of sub-lethal effects of ZnO nanoparticle exposure, we calculate the expected lifetime production of reproductive matter. This extrapolation to longer timescales requires assumptions about mortality rates (see Eq. (10) in Table 2). We assume a constant post-settlement mortality rate based on the background mortality observed in the experiment. Although a constant mortality rate, independent of age and/or size, is unrealistic in nature, the use of this mathematically convenient assumption illustrates the impact of sublethal effects of ZnO nanoparticles on the expected lifetime reproductive effort of mussels.

3. Material and methods

The data analyzed here are from Hanna et al. (Hanna et al., 2013), who describe in detail the experimental configuration and analytical procedures used. In short, *M. galloprovincialis* with shell length of 2–6 cm were obtained from Taylor Shellfish Farms (Shelton, WA, USA) and grown in 40 7-liter tanks that initially contained 120 (small; average length 34.5 mm) or 100 (large; average length 47.5 mm) individuals at a mean temperature of 14 °C (sd = 1.2 °C). They were exposed to 0, 0.1, 0.5, 1, or 2 mg L^{-1} ZnO nanoparticles (except between days 84 and 98), which were obtained from Meliorum Technologies (Rochester, NY, USA) and characterized by the University of California Center for the Environmental Implications of Nanotechnology (Godwin et al., 2009; Keller et al., 2010). Except during the weekends the contents of the tanks were refreshed daily, after which approximately 14.5 mmol C per tank from a phytoplankton diet (Shellfish diet 1800, Reed Mariculture, Campbell, CA, USA) was added. Twenty mussels per tank were labeled to use for growth studies. Mussel length was measured prior to exposure, after 6 weeks, and after 12 weeks of exposure. At these times mussels were counted in each tank and survival was determined. Five mussels were removed from each tank every

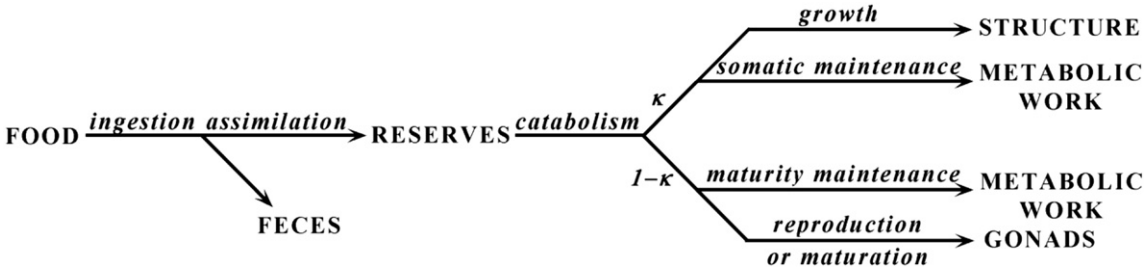

Fig. 1. Material and energy flows and primary state variables in mature *Mytilus galloprovincialis* according to DEB theory. The compositions of reserves and structure are constant, implying that conversion efficiencies for assimilation and growth, and the maintenance rate for a unit of structure are constant. Food is ingested at a rate proportional to the surface area of the mussels (i.e. the square of the shell length) and the scaled food density (type II functional response). Under non-starvation conditions, a constant fraction of mobilized reserves is used for somatic maintenance and growth, with maintenance having priority over growth; the remaining fraction is used for maturity maintenance and reproduction, with maturity maintenance having priority over maturation and reproduction. Under mild starvation conditions, somatic and maturity maintenance take priority over reproduction (for a more comprehensive description, see Kooijman, 2010; Sousa et al., 2008). Under more severe starvation conditions, the reserve mobilization rate is modulated to meet somatic and maturity maintenance requirements.

two weeks throughout the study and frozen for Zn analysis. Oxygen consumption rates of individual mussels were determined with a respirometer (duration 1 h) after 12 weeks for a subset of mussels from each concentration and size class. During the first three months the feeding potential of mussels was determined on a biweekly basis. Upon feeding, the clearance of algae in the tanks was followed for a period of 24 h (depletion occurred within a half day in the vast majority of cases). Mussel tissue was analyzed for elemental composition using a CE-440 CHN/O/S Elemental Analyzer (Exeter Analytical Inc., North Chelmsford, MA, USA).

Table 1
Equations (see Table 2 for an explanation of symbols).

State equations for shell length and reserve density

$$\frac{dL_w}{dt} = \frac{(\kappa v_c \delta_M m_E - j_{EM,c} L_w)_+}{3(\kappa m_E + y_{EV})}$$

provided this reserve mobilization rate is sufficient to meet maintenance demands:

$$\frac{dm_E}{dt} = \frac{y_{EX} J_X}{[M_V]\delta_M^3 L_w^3} - \frac{v_c m_E}{\delta_M L_w}$$

otherwise, i.e. when $[M_V]\delta_M^3 L_w^3 v_c m_E < j_{EM,c} + \frac{k_{J,c} E_H^p}{\mu_E}$:

$$\frac{dm_E}{dt} = \frac{y_{EX} J_X}{[M_V]\delta_M^3 L_w^3} - j_{EM,c} - \frac{k_{J,c} E_H^p}{\mu_E [M_V]\delta_M^3 L_w^3}$$

Feeding rate:

$$J_X = \min\{\overline{J_X}, J_{Xm,c}\}$$

i.e. if food is depleted before the next feeding (nearly always), the feeding rate is the mean feeding rate calculated from the amount of food added, number of mussels present and the interval duration between feedings; else food is abundant (a few cases) and feeding proceeded at its maximum rate.

Tank clearance rate:

$$\frac{dX}{dt} = -N\{J_{Xm,c}\} L_w^2 \frac{X}{X + X_K}$$

in which $\frac{X}{X+X_K}$ is the scaled functional response and N is obtained from census data

Rate at which reserves are allocated to reproduction:
if reserve mobilization rate is sufficient to meet maintenance demands:

$$J_{ER} = \frac{(1-\kappa) m_E [M_V]\delta_M^3 \left(v_c L_w^2 + y_{VE} j_{EM,c} L_w^3\right)}{\kappa y_{VE} m_E + 1} - \frac{k_{J,c} E_H^p}{\mu_E}$$

if reserve mobilization rate is insufficient to meet maintenance demands:

$$J_{ER} = \left([M_V]\delta_M^3 \left(v_c m_E L_w^2 - j_{EM,c} L_w^3\right) - \frac{k_{J,c} E_H^p}{\mu_E}\right)_+$$

Volume specific respiration rate (assuming negligible contributions of assimilation and growth):

$$[j_O] = \alpha j_{EM,c}$$

Total biomass:

$$M = M_V + M_E + M_R = (1 + m_E)[M_V]\delta_M^3 L_w^3 + M_R$$

in which at time τ, $M_R = M_{R,0} + \int \tilde{\eta} J_{ER} dt$

Dynamics of body burden of zinc:

$$\frac{dm_{Q+}}{dt} = \frac{k_{dw} C}{L_w} - \frac{k_{ew} m_{Q+}}{L_w} - \frac{3 m_{Q+}}{L_w}\frac{dL_w}{dt}$$

Table 1 (*continued*)

Toxicant effect functions:

$$\{J_{Xm,c}\} = \{J_{Xm}\}\left(1 + \frac{(C - C_{NEC})_+}{C_K}\right)^{-1}$$

$$v_c = v\left(1 + \frac{(m_{Q+} - m_{NEC})_+}{m_K}\right)^{-1}$$

$$j_{EM,c} = j_{EM}\left(1 + \frac{(m_{Q+} - m_{NEC})_+}{m_K}\right)$$

$$k_{J,c} = k_J\left(1 + \frac{(m_{Q+} - m_{NEC})_+}{m_K}\right)$$

Expected lifetime gonad production:

$$\overline{M}_R = \int_0^\infty \kappa_R J_{ER} e^{-ht} dt$$

4. Data manipulation and parameters

The number of mussels per tank declined during the course of the experiment because of sampling and background mortality, but the feeding routine remained unaltered. Accordingly, the per capita food ration varied during the week and gradually increased during the course of the experiment. We calculated the average daily feeding rate while taking into account the sampling schedule and correcting for background mortality from the census data collected in weeks 0, 6, and 12 (assuming a constant survival probability between censuses). The average per capita maximum surface area specific feeding rate was estimated from the Holling type II functional response (see Eq. (4) in Table 1) using algal tank clearance measurements and the estimated number of live mussels in each tank.

We converted mass measurements to C-moles, a standard measure used in DEB theory. The elemental composition neither differed between small and large mussels, nor changed during the experiment (results not shown). Accordingly, we used the average C content of biomass, 39.15%, to convert dry weight measures into C moles.

In order to parameterize part of the DEB model we used values and temperature correction factors for the closely related *Mytilus edulis* from Saraiva et al. (2011), which are part of the DEB parameter database (Kooijman, 2011). The values we used are listed in Table 2. Parameter estimation was done in Matlab with simple nonlinear least squares (tank clearance rates) or with weighted least squares with weights equal to the inverse of the variance of all measurements (respiration rates) or with weights equal to the inverse of the variance of measurements at each time point (all other data).

5. Results & discussion

We evaluate the impact of ZnO nanoparticles on five processes: Zn accumulation, feeding, respiration, production (growth) and expected

Table 2
Symbols (see Table 1 for equations). Estimated values ± SD; other values from the DEB parameter database (Kooijman, 2011) for 14 °C, unless indicated otherwise.

Symbol	Default value	Units	Interpretation
C	Variable	μmol/L	Environmental zinc concentration
C_K	1.45 ± 0.47	μmol/L	Toxicant scaling parameter for feeding
C_{NEC}	0	μmol/L	Environmental zinc concentration below which feeding is not impaired
E_H^p	52.82	J	Maturation threshold for reproduction (puberty)
h	$1.66\ 10^{-3}$ [a]	1/d	Specific mortality rate
j_{EM}	$3.10\ 10^{-3}$	mol reserve C/mol structural C d	Structure-specific maintenance rate without toxicants
$j_{EM,c}$	Variable	mol reserve C/mol structural C d	Structure-specific maintenance rate with toxicants
$[j_O]$	Variable	mol O_2/cm^3 d	Volume-specific respiration rate
J_{ER}	Variable	mol reserve C/d	Rate at which reserves are committed to reproduction
J_X	Variable	mol food C/d	Feeding rate
$\overline{J}_X$	Variable	mol food C/d	Average feeding rate
$J_{Xm,c}$	Variable	mol food C/d	Maximum feeding rate with toxicants, $\{J_{Xm}\}L_w^2$
$\{J_{Xm}\}$	2.50 ± 0.16	mol food C/cm^2 d[b]	Maximum surface area-specific feeding rate without toxicants
$\{J_{Xm,c}\}$	Variable	mol food C/cm^2 d[b]	Maximum surface area-specific feeding rate with toxicants
k_{dw}	0.788 ± 0.073	cm L/mol C d[b]	Zinc uptake rate parameter
k_{ew}	0.022 ± 0.003	cm/d[b]	Zinc elimination rate parameter
k_J	$1.57\ 10^{-3}$	1/d	Maturity maintenance rate
L_w	Variable	cm[b]	Shell length
$L_{w,0}$	Variable	cm[b]	Initial shell length
m_E	Variable	mol reserve C/mol structural C	Reserve density
$m_{E,0}$	0.25[c]	mol reserve C/mol structural C	Initial reserve density
m_K	235.2 ± 0.2	μmol Zn/mol total biomass C	Toxicant scaling parameter for maintenance and energy conductance
m_{NEC}	50[d]	μmol Zn/mol total biomass C	No-effect body burden of zinc
m_{Q+}	Variable	μmol Zn/mol total biomass C	Body burden of zinc
M	Variable	mol C	Total biomass
M_E	Variable	mol C	Reserve biomass
M_R	Variable	mol C	Biomass reserved for reproduction
$\overline{M}_R$	Variable	mol C	Mean lifetime production of reproductive matter
M_V	Variable	mol C	Structural biomass
$[M_V]$	$5.02\ 10^{-3}$	mol C/cm^3	Structural biomass density
N	Variable	#	Number of mussels in a tank
t	Variable	d	Time
X	Variable	mol C/L	Food density
X_K	0.01[c]	mol C/L	Half saturation food density
y_{EX}	0.13[c]	mol reserve C/mol food C	Yield of reserves from food
y_{VE}	0.76	mol structural C/mol reserve C	Yield of structure from reserves ($y_{VE} = y_{EV}^{-1}$)
α	6.4	mol O_2 mol structural C/mol reserve C cm^3	Respiration conversion factor
δ_M	0.2942	–	Shape coefficient
κ	0.7983	–	Fraction of catabolic power energy spent on maintenance and growth
κ_R	0.95	–	Fraction of reproduction energy fixed in gonads
μ_E	$697\ 10^3$	J/mol C	Chemical potential of reserves
v	$59.7\ 10^{-3}$	cm/d	Energy conductance without toxicants
v_c	Variable	cm/d	Energy conductance with toxicants

[a] Calculated from census data; see text.
[b] Units in shell length rather than volumetric length.
[c] Fixed.
[d] See text.

lifetime gonad production. We need to know the dynamics of zinc accumulation in order to quantify the toxic impact of ZnO nanoparticles on the energy budgets of mussels. The impact of ZnO nanoparticles on the rates of feeding is relatively direct, whereas the impact on growth represents an integrated response. Using the toxicity parameters estimated from the data on the first 4 processes, we calculate the expected lifetime gonad production.

Because zinc naturally present in seawater and feed may be a substantial source of this metal accumulated in mussel tissue, we relate the bioaccumulation of zinc by mussels to the total ambient zinc concentration. Accordingly, we do not distinguish among the different sources of zinc, which would complicate matters substantially. However, previous work indicates that approximately 70% of ZnO nanoparticles dissolve in seawater within 12 h at the concentrations used in our study (Miller et al., 2010). The environmental zinc concentration increases linearly with the nominal concentration of ZnO nanoparticles (see Fig. 2a). With the regression parameters estimated accordingly, we calculate the dissolved zinc levels to which mussels were exposed and use these concentrations for the estimation of the toxicant exchange parameters in Eq. (8) in Table 1 from bioaccumulation data. We use a constant length measure for each of the two size classes, as growth was insignificant during the duration of the experiment (see below; the mean shell length was 34.5 and 47.5 mm for small and large mussels, respectively). Despite the fact that the bioaccumulation data contain considerable scatter (see Fig. 2B and C), it is clear that zinc accumulates in mussels at a rate increasing with the nominal concentration of ZnO nanoparticles and that zinc accumulates relatively faster in small mussels than in large ones. The toxicant exchange rate parameters (see legend in Fig. 2) imply a bioconcentration factor of 37 mol Zn/mol C biomass per mol Zn/L or 1.2 mol Zn/gDW per mol Zn/L, which is well in the range of published values in the EPA ECOTOX database (EPA, 2011), thus indicating that although the data are variable, they yield realistic estimates for the exchange rate parameters.

We have not found evidence that feeding rates decline with increasing body burdens of zinc (results not shown). In contrast, the maximum feeding rate as determined with Eq. (4) in Table 1 does decrease with increasing levels of ZnO nanoparticles in the environment (see Fig. 3A). This impact is independent of exposure duration. Therefore, we lump the maximum surface area specific feeding rates determined at different time points (Table 1). With Eq. (9a) in Table 1 we estimate that the feeding potential is reduced by 50% with approximately 1.5 mg ZnO nanoparticles L^{-1} added to the environment (which corresponds to 13 μM Zn in the environment); the data indicate that there is no level of ZnO nanoparticles below which feeding is not impaired.

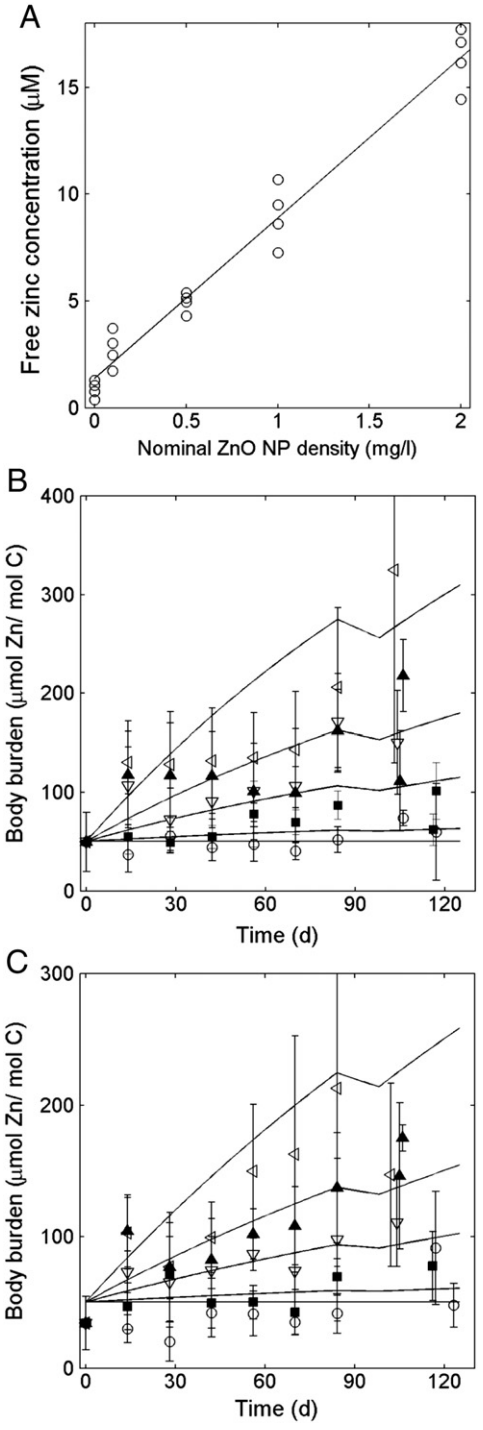

Fig. 2. Fate of zinc in the tanks with mussels. (A) ZnO nanoparticles partially dissolve, yielding a dissolved Zn concentration that increases linearly with the nominal ZnO nanoparticle density; the slope is 7.52 μmol Zn/mg ZnO nanoparticles and the intercept with the y-axis 1.37 μM Zn (represents dissolved zinc from seawater and feed). Accumulation of zinc in (B) small (average 34.5 mm) and (C) large (average 47.5 mm) mussels in tanks with 0 (open circles), 0.1 (closed squares), 0.5 (open downward-facing triangles), 1 (closed triangles) and 2 (open left-pointing triangles) mg ZnO nanoparticles/L; the error bars show the spread in standard deviations. The curves represent model fits of the solution of Eq. (8) (see Table 1) to the data. The bottom curve represents the fit at the lowest exposure level and the top curve the one at the highest exposure level; parameter estimates are listed in Table 2. The decline visible at the higher exposure levels between days 84 and 98 is the result of depuration, as in this time interval the mussels were not exposed to ZnO nanoparticles.

Possibly, given that the feeding potential does not depend on the body burden, nanoparticles impair feeding through a physical rather than a chemical mechanism, e.g. by damaging or clogging of the feeding apparatus.

In contrast, zinc accumulated in tissues rather than zinc in the environment affects the respiration rate (see Fig. 3B) and, to a lesser extent, growth (see Fig. 3C–D). Although the data contain a lot of scatter, there is a trend towards an increase in the respiration rate with the body burden of zinc. The increase in shell length was minimal in all treatments; yet there is a statistically significant decline of shell growth with increasing nominal ZnO nanoparticle densities (Hanna et al., 2013). In all treatments, the biomass content of small mussels remained approximately constant, whereas that of large mussels declined somewhat. These patterns show that the feeding regime was insufficient to support substantial growth; yet, we routinely observed a substantial amount of pseudofeces in the tanks before cleaning, implying that the yield of reserves from food must have been relatively low. Those patterns also suggest that large mussels lost reserves in order to remain viable. Furthermore, since daily feeding rates varied little (because the supply of food was limited) and growth was (biologically) insignificant, the increase in respiration rates with increasing body burdens of zinc indicates that maintenance was a primary target of toxicant action.

Using Eqs. 1, 2a, 2b, 4, 5a, 5b, 6, 7, 8, 9a, 9b, 9c and 9d, and the toxicodynamic parameters estimated from the bioaccumulation data (see Table 2) and the DEB core parameters for *M. edulis* from the DEB database (Kooijman, 2011), we estimate the toxic effect parameters from the combined data on respiration, shell length and total biomass. Because of the substantial scatter, we are not able to estimate the initial conditions of the state variables (besides the initial shell length of the small mussels), the no-effect body burden of zinc (see Eqs. (9b)–(9d)), the yield of reserves from food and the conversion factor α (see Eq. (6)). We set the no-effect body burden at a fixed value of 50 μmol Zn mmol C^{-1}, which is slightly above the mean body burden of zinc in the controls, and the initial conditions and α at arbitrary values, but within the range of possible values in the DEB database. Motivated by the considerable amount of pseudofeces production, we set the yield of reserves from food at a relatively low value of 0.13. The exact values chosen have relatively little impact on the estimation of the toxic effect parameter. The set of parameter values imply that mussels of both size classes and at all treatment levels maintain reserves throughout the experiment. Accordingly, mussels were not forced to resorb gonads or break down structural biomass in order to generate energy to remain viable, although this may have occurred to some extent.

The value for the toxic effect parameter, m_K, is estimated at 235 μmol Zn (mol C in biomass)$^{-1}$ with a modestly asymmetric 95% confidence interval as calculated from the log likelihood profile: 171–378 μmol Zn (mol C biomass)$^{-1}$. The relative width of the confidence interval reflects the fair amount of scatter in the data and indicates the great difficulty posed in estimating additional parameters. The sum of the toxic effect parameter and no-effect body burden represents the body burden at which the maintenance rate is double and the energy conductance half of those in uncontaminated environments. In steady state, with the bioconcentration factor given above, this sum corresponds to 7.8 μM Zn in the environment, which would be achieved with 0.9 mg ZnO nanoparticles L^{-1} in the environment. This is below the 1.5 mg ZnO nanoparticles L^{-1} at which feeding is reduced by 50%, indicating that the internal toxic effects of zinc are stronger than the external ones. However, both internal and external effects are biologically significant at relevant exposure levels.

By characterizing the impact of ZnO nanoparticles on physiological processes, we are able to make a projection of the expected lifetime performance of the mussels at different contaminant and food levels. Mortality rates are necessary for this analysis, information which is available as census data collected at 0, 6, and 12 weeks of the experiment. These data show that there is no significant difference among the survival probabilities of animals exposed up to 1 mg ZnO nanoparticles L^{-1}.

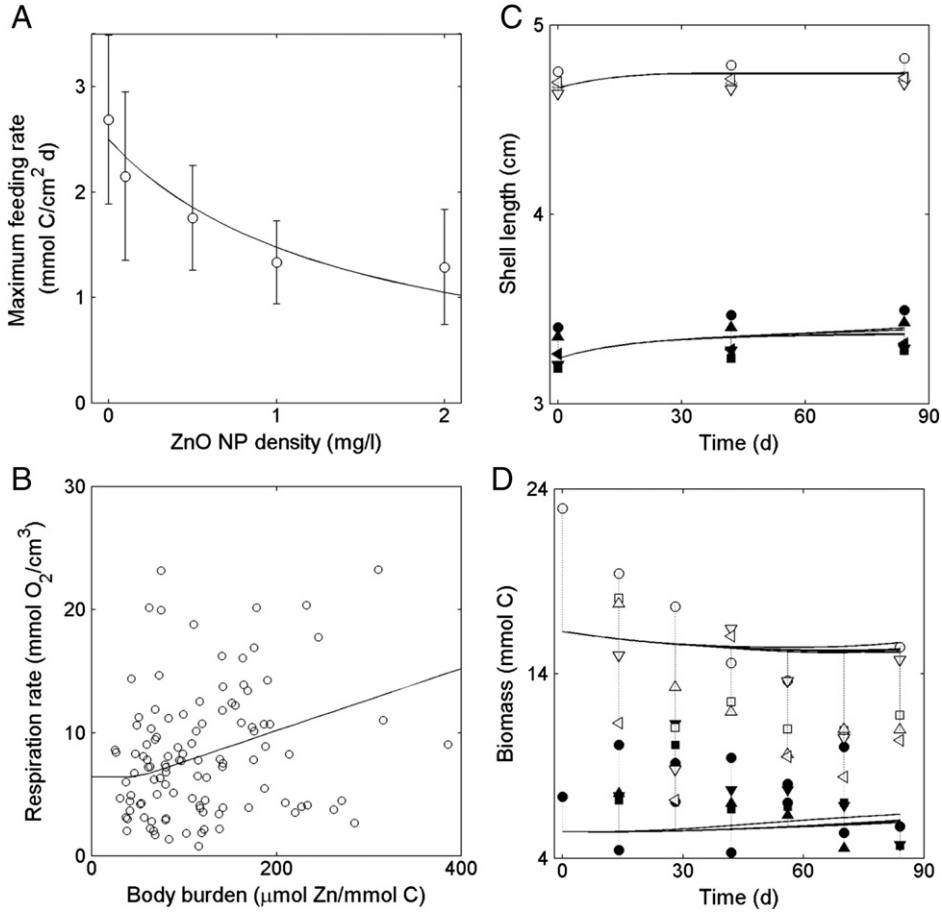

Fig. 3. Impact of ZnO nanoparticles on mussels. (A) ZnO nanoparticles in the environment directly impact the feeding capacity of mussels. The curve represents the fit of Eq. (9a) in Table 1; error bars show the spread of the data in standard deviations. (B) Zn accumulated in tissue tends to enhance the volume-specific respiration rate. The curve represents the fit of Eq. (6) in Table 1 in conjunction of those used to fit shell length and biomass data. (C) Shell length and (D) biomass content change relatively little during the experiment. Closed symbols represent small mussels (average 34.5 mm), whereas open symbols represent large mussels (average 47.5 mm) exposed to 0 (circles), 0.1, (squares), 0.5 (downward-facing triangles), 1 (upward-facing triangles) and 2 (left-pointing triangles) mg ZnO nanoparticles/L. Model fits are based on Eqs. 1–3 and 5–9. For parameter estimates see Table 1.

Therefore, we calculate the mean specific mortality rate, h, using all available census data except those obtained at the highest exposure level of 2 mg ZnO nanoparticles L^{-1}, and use h to make projections for the expected lifetime production of reproductive matter with Eq. (10) (see Table 1).

We present the results of this projection in two ways: the expected lifetime production of reproductive matter, $\overline{M}_R$, at various levels of food and ZnO nanoparticles scaled as a fraction of this production with abundant food in a clean environment (see Fig. 4a), and the expected lifetime production of reproductive matter at various levels of food and ZnO nanoparticles scaled as a fraction of this production at the respective food level in a clean environment (see Fig. 4B). Thus, the first scaling shows the impact of food availability and contaminant level on $\overline{M}_R$ relative to that of a mussel in a clean environment with abundant food, whereas the second scaling shows the impact of contaminant level on $\overline{M}_R$ relative to that of a mussel living in a clean environment but at the same food level as the impacted ones. The first scaling, displayed in Fig. 4a, shows that an increase in ZnO nanoparticle levels in the environment has a qualitatively similar effect on the expected lifetime production of reproductive matter as a decrease in food level. It also shows that the expected lifetime production of reproductive matter is substantially reduced even at the lowest experimental nominal exposure levels (0.1 and 0.5 mg ZnO nanoparticles L^{-1}), although the impact of ZnO nanoparticles on individual physiological processes is relatively modest at these contaminant levels (see Fig. 3).

This finding is corroborated by the projection results of the second scaling (see Fig. 4B), which illustrates the reduction of the lifetime production of reproductive matter as a function of ZnO nanoparticle exposure for each food level. This figure emphasizes the concentration at which the expected lifetime production of reproductive matter at a given food level is reduced by 50% (i.e. an EC_{50} measure). At all food levels, this EC_{50} value is less than 0.25 mg ZnO nanoparticles L^{-1}, which is about three and five times less than the EC_{50} for respiration and feeding at abundant food, respectively. This highlights an important shortcoming in conventional toxicity analyses, in which sublethal toxicity measures depend on the choice of endpoint. This problem is circumvented in the framework of DEB theory, as one toxicity measure can be 'translated' into another one, as the above analysis demonstrates.

In summary, DEB theory offers an integrated framework for the evaluation of the impact of toxicants and other environmental stressors or conditions on the performance of organisms. Provided that sufficient data are available, toxicity is characterized by parameters that are independent of the choice of endpoint and experimental duration. Once those parameters have been estimated, the impact of a toxicant on physiologically based endpoints not experimentally characterized can be inferred. Furthermore, this framework transcends the individual level, as it can be used to infer integrated responses to toxicant presence at a higher level of biological organization. We have used this method to show that ZnO nanoparticles affect the energy budgets of mussels through a reduction in feeding capacity and an increase in maintenance requirements, and that these two effects lead to a reduction in the expected lifetime production of reproductive matter. The latter reduction is relatively stronger than the impacts of zinc on feeding and maintenance, which stresses the importance of a methodology in which the

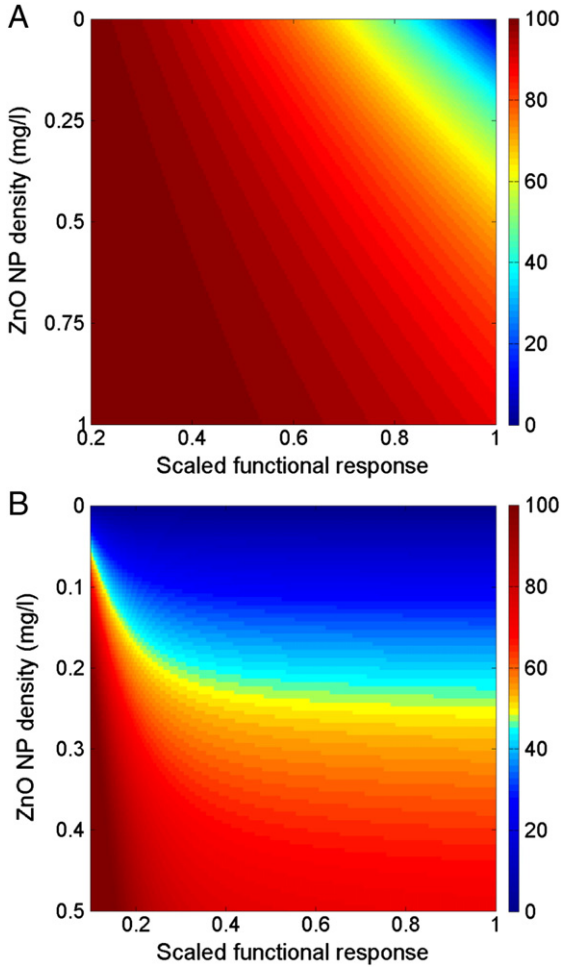

Fig. 4. Integrated impact of food availability and ZnO nanoparticle density on the expected lifetime production of reproductive matter of mussels. (A) The percentage decline in expected lifetime production of reproductive matter relative to that of a mussel in a clean environment with abundant food increases rapidly with decreasing food availability and/or increasing ZnO nanoparticle levels. ZnO nanoparticles have a stronger impact on the ecologically relevant measure of the expected lifetime production of reproductive matter than on physiological measures, such as feeding, respiration and growth (cf. Fig. 3). (B) The percentage decline in expected lifetime production of reproductive matter in mussels is expressed relative to that of a mussel living at a similar food level in a clean environment. At all food levels, the EC_{50} for the expected lifetime production of reproductive matter is <0.25 mg ZnO nanoparticles/L. This is about three and five times less than the EC_{50} for respiration and feeding at abundant food, respectively, indicating that the ecological impact of ZnO nanoparticle exposure is stronger than its physiological impact.

impact of engineered nanomaterials on multiple endpoints can be evaluated concomitantly.

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