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Cecilia Silva Pereira ORCID iD: 0000-0002-3036-6906

Karel A.C. De Schamphelaere ORCID iD: 0000-0002-5063-922X

Effects of Ni on a *D. magna* population

THE UNEXPECTED ABSENCE OF NICKEL EFFECTS ON A DAPHNIA POPULATION AT THREE TEMPERATURES IS CORRECTLY PREDICTED BY A DYNAMIC ENERGY BUDGET INDIVIDUAL-BASED MODEL (DEB-IBM)

Cecília M.S. Pereira^{1,a,b}

Karel Vlaeminck^{*1,a,c}

Karel Viaene^c

Karel A. C. De Schamphelaere^a

^a Ghent University (UGent), Laboratory of Environmental Toxicology and Aquatic Ecology, Environmental Toxicology Unit (GhEnToxLab), Campus Coupure – Building F – 2nd floor, Coupure Links 653, B-9000 Ghent, Belgium

^b Laboratory for Systemic Physiology and Ecotoxicological Research, University of Antwerp, Belgium

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^c ARCHE (Assessing Risks of Chemicals) Consulting, Liefkensstraat 35D, B-9023 Ghent (Wondelgem), Belgium

*Address correspondence to karel.vlaeminck@ugent.be

¹ Both authors contributed equally to this manuscript.

Abstract:

Recent studies have shown that temperature affects chronic Ni toxicity to *Daphnia magna* at the individual (apical) level. However, the effect of temperature on Ni toxicity to *D. magna* at population-level is unknown. The present study investigated whether the effect of temperature on chronic Ni toxicity to *D. magna* assessed on apical endpoints can be extrapolated to the population-level. The results of the performed population experiment showed no consistent Ni effects on total *D. magna* population abundance at 15, 20 and 25°C although the Ni concentrations tested were previously reported to significantly reduce reproduction in *D. magna* individuals. This result supports the idea that ecological risk assessment should not extrapolate as such from apical endpoints to the population-level. A Dynamic Energy Budget Individual-Based Model (DEB-IBM) was calibrated using apical Ni toxicity data at 15, 20 and 25°C. The goal was to investigate whether the calibrated DEB-IBM would be able to predict the unexpected absence of effects at population-level and to further investigate the effect of temperature on Ni toxicity to a *D. magna* population. At the population level, the calibrated DEB-IBM correctly predicted the unexpected absence of Ni effect to a *D. magna* population. Detailed analysis of simulation output suggests that the predicted lower Ni sensitivity at population level occurs because Ni-induced mortality is compensated by reduced

starvation (less intra-specific competition). Extrapolated EC50 values for population density predicted that the effect of temperature on Ni toxicity to *D. magna* populations was smaller (1.9-fold higher at 25°C than at 15°C) than on Ni toxicity to *D. magna* apical reproduction (the EC50 is 6.5-fold higher at 25°C than at 15°C, previous study). These results show that the DEB-IBM can help to replace population experiments by *in silico* simulations and to optimize the experimental design of population studies.

Keywords: population-level effects, population modeling, metal toxicity, temperature, Nickel, DEB-IBM.

INTRODUCTION

In aquatic ecosystems, where 95% of the species are ectothermic, temperature plays a prominent role (Willmer et al. 2004). The external temperature determines the body temperature of ectothermic organisms, which in turn determines all physiological rates (Paul et al. 2004; Walsh et al. 2014). Population dynamics are also determined by temperature (Jiang et al. 2014; Pratt 1943). For instance, Pratt et al. (1943) showed that maximum *Daphnia magna* population abundance was higher at 18°C than at 25°C and that population structure differed among temperatures.

An increase of temperature is commonly associated with an increase of metal toxicity (Heugens et al. 2001; Sokolova and Lannig 2008). However, the majority of the available studies have focused on acute toxicity. In addition, studies on the effect of temperature on chemical toxicity have, to our knowledge, only assessed apical endpoints (i.e. endpoints that are directly measurable from in-vivo toxicity tests, such as mortality, reproductive failure or reduced growth of single individuals).

An important goal of Ecological risk assessment (ERA) is to protect populations against chemical exposure but ERA is still most commonly based on apical endpoints (Galic et al. 2010; Jager et al. 2014; Preuss et al. 2009). However, previous studies have shown that effect concentrations estimated based on apical endpoints may not reflect the effects at population-level (Gagneten and Vila 2001; Viaene et al. 2015).

Recent studies have shown that temperature affects chronic Ni toxicity to *D. magna* on apical endpoints (Pereira et al. 2017; Pereira et al. 2018). The effect concentration that reduced reproduction by 50% (EC50) increased 2.0 to 6.5-fold with an increase of temperature from 15 to 25°C, indicating lower toxicity at higher temperatures (Pereira et al. 2017; Pereira et al. 2018). Furthermore, in Pereira et al. (2018), the multigenerational Ni effects on *D. magna* reproduction showed different patterns at different temperatures. In addition, in Pereira et al. (2018), the EC10 estimated in the first generation were considered to be protective for *D. magna* in a long-term context. However, the EC10, most commonly used for chronic ERA, were based on apical endpoints thus disregarding the population-level effects. Therefore, in the present study, we investigated whether the effect of temperature on chronic Ni toxicity to *D. magna* assessed on apical endpoints could be extrapolated to population-level. A population experiment was performed with *D. magna* exposed to three Ni concentrations, i.e. 0 µg L⁻¹ (control), and 12 and 100 µg L⁻¹, previously reported to significantly reduce the apical endpoint reproduction (Pereira et al. 2018).

In a population, organisms compete for food and can be limited by other factors (i.e. intraspecific interaction), such as crowding and light (Martin et al. 2013a; Preuss et al. 2009; Viaene 2016). Daphnids can detect the presence of other individuals through the

presence of metabolites or pheromones released into the environment (Gust et al. 2016), which can influence their growth and reproduction (Nishikawa and Ban 1998). Therefore, the fitness of an organisms and their response to chemical stressors not only depend on the temperature effect but may also depend on population density (Gust et al. 2016; Jiang et al. 2014).

Differences between the Ni effects on apical reproduction and the Ni effect at population-level were expected, but it was also expected that at $100 \mu\text{g Ni L}^{-1}$, a concentration that reduced apical reproduction by 70, 50 and 20% at 15, 20 and 25°C , respectively (Pereira et al. 2018), effects at population-level would be observed. However, the experiment in the present study showed an absence of any consistent Ni effects on total *D. magna* abundance and population structure at 15, 20 and 25°C (see Results section). To explore the possible reasons driving these different results, the effect of temperature on Ni toxicity to a *D. magna* population was studied with a population model: a Dynamic Energy Budget Individual-Based Model (DEB-IBM) (Martin et al. 2013a). DEB-IBM is a generic individual-based implementation of the Dynamic Energy Budget (DEB) theory (Martin et al. 2012). The IBM describes population dynamics through individual behavior, while DEB describes physiological process on an individual-level. In IBM, each organism is generated (at birth) and simulated as an individual entity within the population (Galic et al. 2010). DEB will translate environmental conditions to metabolic functions (i.e. energy assimilation, maintenance, growth, and reproduction) (Kooijman 2009). For each organism, these DEB processes are calculated based on a set of differential equations. DEB-IBM thus links individual metabolism to population

dynamics. The DEB-IBM for *D. magna* can extrapolate the effect of toxicants measured at apical endpoints to the effects on population dynamics (Martin et al. 2013b).

We calibrated the DEB-IBM using the apical toxicity data for *D. magna* and Ni at different temperatures that were reported in Pereira et al. (2018). We used the DEB-IBM to simulate *D. magna* populations and compared model predictions to observations from the population experiment made in the present study. Ni concentrations in the population experiment were based on the effect levels reported by Pereira et al. (2018) at the individual level. Considering that these concentrations showed significant effects on reproduction, the initial goal of the experiment was to examine if these effects would propagate to the population level and if the temperature-effect on *D. magna* Ni sensitivity would still be present at the population level. Additionally, we wanted to test whether the DEB-IBM, calibrated on apical toxicity data at different temperatures, would be able to predict and help explain the absence of effects of Ni at the population-level.

MATERIAL AND METHODS

Daphnia magna population experiment

Organism cultures and test media. The *D. magna* clone K6 was cultured in aquaria in a modified M4 medium (Pereira et al. 2017). This medium was also used for exposure. Prior to actual Ni exposure, organisms were acclimated for two generations to each temperature treatment (Mitchell and Lampert 2000). More detailed information about the acclimation process and test medium can be found in Pereira et al. (2017).

Experimental design. A *D. magna* population was exposed to three Ni concentrations at 15, 20 and 25°C. The three Ni concentrations tested were 0 µg L⁻¹ (control); 12 µg L⁻¹ (nominal concentration), which we expected would produce about a 30% effect on *D. magna* reproduction at 15°C and 0 % of effect at 20 and 25°C; and 100 µg L⁻¹ (nominal concentration) which we expected would result in about a 50% effect at 20°C, 20% at 25°C and 70% effect at 15°C). These conditions and expectations follow the results of previous conventional reproductive toxicity testing (Pereira et al. 2018).

The population experiment started with 6 egg-carrying females that had already released their 3rd or 4th brood and 10 neonates (<24h) per replicate. Four replicates were performed for each Ni treatment at each temperature treatment. Organisms were exposed in 2 L of medium in aquaria that were continuously aerated. Daphnids were fed daily with *Raphidocelis subcapitata* (formerly known as *Pseudokirchneriella subcapitata*) with a food density of 2.5 mg C L⁻¹ in the first week and 5 mg C L⁻¹ in the following weeks. To consider the different generation time (i.e. time to first brood) observed at the different temperature treatments (Bae et al. 2016; Paul et al. 2004; Walsh et al. 2014) the experiment was conducted during 9 weeks (63 days). Half of the medium was renewed three times a week (every Monday, Wednesday and Friday). The total abundance and the population structure (i.e. number of neonates, juveniles and adults) were recorded twice a week, every Monday and Thursday. When the number of organisms in the aquaria was above 300, it was necessary to take subsamples to facilitate counting (Wetzel and Likens 2000). Two subsamples of each aquarium were taken after gently spreading the organisms in order to have a homogeneous distribution of the organisms in the aquarium. The volume of the subsamples was measured and was between 125 and 200 mL. Three

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population size classes of *D. magna* were quantified by sieving the medium through sieves with different mesh sizes, i.e. adults ($> 800 \mu\text{m}$), juveniles (between 800 and 500 μm) and neonates ($< 500 \mu\text{m}$) (Preuss et al. 2009).

The mean of both subsamples was used to estimate the number of organisms in 2L aquaria. The coefficient of variation was calculated for all size classes and if the coefficient of variation was larger than 20% a 3rd or even a 4th subsample was taken. Every two weeks (14 days) 50% of each of the 3 size classes were removed at random (i.e. culling) to avoid population collapses due to overcrowding (Van Doorslaer et al. 2009). The exposure was performed under a controlled light cycle (16 h of light: 8 h of dark) (OECD 2012).

Chemical analysis. Dissolved metal concentrations, dissolved organic carbon (DOC), pH and O₂ were measured once a week, both in fresh and old medium. Water samples of fresh and old medium of all treatments were collected weekly for analysis of total (only of fresh medium) and dissolved concentrations of Ni, major cations (Na, K, Ca and Mg) and DOC. Dissolved concentrations refer to 0.45 μm membrane filtered concentrations (Acrodisc Filter, Supor Membrane, PALL, Newquay, Cornwall, UK). amples for metal analysis were acidified to a final concentration of 0.14 mol·L⁻¹ of HNO₃ prior to analysis. Metal analyses were performed using iCAP 7000 Series ICP-OES (Thermo Scientific). The samples for DOC analysis were measured with TOC-L CPH (Shimadzu, Duisburg, Germany). All data analyses were performed based on the mean dissolved concentrations of new and old medium.

The reference material used in all chemical analyses and the quantification limits can be consulted in Supplemental Information (Table S1). Temperature remained stable in the three treatments during the 9 weeks of the population experiment (15.2°C (± 0.6), 19.7°C (± 1.3) and 25.0°C (± 1.0); mean $\pm$ standard deviation). The dissolved organic carbon (3.5 ± 0.4 mg L⁻¹) and the pH (7.9 ± 0.1) also remained stable during the experiment (SI, Table S2). At 20°C, the measured concentration in the 12 μ g Ni L⁻¹ treatment was below the target concentration at four sampling time points, but the concentration in the 100 μ g Ni L⁻¹ treatment remained stable during the whole exposure (which was 9 weeks) (Supplemental Information: Table S2). At 15 and 25°C, the 12 and 100 μ g Ni L⁻¹ treatments remained stable during the whole exposure (Supplemental Information: Table S2).

Data analysis – Population experiment. For each endpoint at each counting day and at each temperature a Dunn's test was applied to test for statistical differences between control and Ni treatments. The Benjamini & Hochberg (Benjamini and Hochberg 1995) correction method was used to adjust the *p* values for multiple comparisons within each temperature. At 15°C, reliable data was only available until 21 days of exposure as a result of an experimental error (tap water was mistakenly used as dilution water for media preparation instead of deionized water). The statistical analysis was performed using the *dunn.test* package in R software (Team 2017). Only consistent effects of Ni were considered reliable and used for further data interpretation. A consistent effect of Ni was defined as an effect in the same direction that was found for at least 2 consecutive sampling dates (Van de Perre et al. 2016).

DEB-IBM

An IBM based on the DEB theory for *D. magna* was used to extrapolate effects of Ni to the population-level at 15, 20 and 25°C. An existing DEB-IBM for *D. magna* (Martin et al. 2013a) was calibrated using apical Ni toxicity data (endpoints: reproduction and survival) at 15, 20 and 25°C (Pereira et al. 2018). The calibrated model was used to predict (blindly) Ni effects at the population-level, i.e. it was independently validated against the dataset from the population experiment described above. Similar methods have been applied in the past to extrapolate effects of chemicals from the individual to the population-level (Hochmuth 2016; Martin et al. 2013a; Viaene 2016).

Model design and calibration of the DEB-IBM. A generic DEB-IBM implementation in NetLogo by Martin et al. (2013a) was developed to predict Ni effects for *D. magna* populations. To simulate the conditions of the population experiment, the original model needed to be adjusted for different temperatures (1), different food conditions (2) and the effects of Ni (3). A brief description of the adjusted DEB-IBM as well as a pseudo code is given in the Supplemental Information.

In the first adjustment the temperature effects were implemented through the Arrhenius equation. The Arrhenius relationship (Equation 1) is used to implement temperature-dependent metabolic rates in the DEB-IBM (Hochmuth 2016; Kooijman 2009). The Arrhenius temperature parameter T_A is species-specific and has been determined before for *D. magna* (Kooijman 1988).

$$k(T) = k_1 * \exp\left(\frac{T_A}{T_1} - \frac{T_A}{T}\right) \quad (1)$$

where k is the metabolic rate at temperature T (K), k_I is the metabolic rate at the reference temperature T_I (293 K) and T_A is the Arrhenius temperature (6400 K for *D. magna*) (Kooijman 2009).

The second adjustment considers the implementation of correction factors to adjust food-related parameters, in order to account for the differences in algal food and species-specific physiological characteristics (e.g. inter-clonal differences in *D. magna* in terms of food size preference, food handling time, digestion time, etc.). In the original model, food-related parameters were based on the algae *Desmodesmus subspicatus* as food source (Martin et al. 2013b; Preuss et al. 2009). However, in the present study the *R. subcapitata* was used as food. To account for such differences, the maximum surface-specific assimilation rate (p_{Am}) (J d⁻¹cm⁻²) and the food half-saturation constant (K) (number of cells L⁻¹) were corrected (Kooijman et al. 2008) (Equation 2 and 3).

$$K_{food} = K * corr_K \quad (2)$$

$$p_{Am,food} = p_{Am} * corr_{pAm} \quad (3)$$

By multiplying the p_{Am} , we thus implemented a correction factor to take into account differences in energy assimilation efficiency due to clone-specific physiological characteristics and food type compared to the original model. Additionally, knowing the food type is different and feeding characteristics may differ between clones (Repka 1997), the K was corrected as well. Correction factors were determined for each temperature treatment separately since assimilation rates depend on the temperature (Yurista 1999). Optimization of the food correction factors was based solely on the control data of the standard reproduction experiment described by Pereira et al. (2018).

The calibrated correction factors for the p_{Am} (corr_{pAm}) were 0.79, 0.84 and 0.76 respectively for 15, 20 and 25°C. For K , the correction factors (corr_K) were 0.82, 1.48 and 1.23 for 15, 20 and 25°C respectively.

The third adjustment was the estimation of the effect parameters for the different physiological modes of actions (PMoAs) to account for Ni toxicity (Martin et al. 2013a).

In DEB theory, stressors affect the metabolic rates and thus the energy flows, which is expressed by changing one or more parameters. Depending on the PMoAs, the life history of *D. magna* will be altered. To describe the relationship between the environmental stressor concentration (in this case Ni) and the level of stress on *D. magna*, two dose-response models were considered, the hockey-stick and the log-logistic relationship. We finally selected the hockey-stick relationship as this described the effects of Ni on size and reproduction better (Supplemental Information: Figures S1 and S2).

The PMoAs considered were inhibition of assimilation, general maintenance (with an increase on general maintenance costs i.e. both structural maintenance and maturity maintenance), structural maintenance (increase of structural maintenance costs), maturity maintenance (increase of maturity maintenance costs), growth costs (an increase in costs for growth) and on reproduction costs (Hochmuth 2016; Martin et al. 2013b). The models with the different PMoAs (as indicated by Martin et al. 2013a and Martin et al. 2013b) were calibrated with the apical Ni toxicity data of *D. magna* from Pereira et al. (2018). The best fitting model, i.e. the one that showed the lowest Euclidean distance (ρ), was further used in the DEB-IBM calibrated model.

For calibrating the food correction factors and the effect parameters for Ni toxicity, an Approximate Bayesian Computation (ABC) was used (van der Vaart et al. 2016). The model was run for 10 000 iterations, with randomized values of the parameters to be optimized in each iteration. The optimized parameter values were calculated from the 100 best fits based on the ρ between the model output (predictions) and apical Ni toxicity data of *D. magna* (endpoints: size and reproduction) from Pereira et al. (2018) (observations) (Equation 3; van der Vaart et al. 2016).

The ρ is determined as:

$$\rho(m_i, D) = \sqrt{\sum_j \left(\frac{m_{i,j} - D_j}{sd(m_j)} \right)^2} \quad (4)$$

where $m_{i,j}$ is the model output of endpoint j in iteration run i , D_j is the observed data of endpoint j , and $sd(m_j)$ the standard deviation of endpoint j of all simulation runs. Optimal parameter values for each PMoA can be found in the Supplemental Information (Supplemental Information: Table S4).

Lethal effects of Ni are modelled by introducing a stochastic chance of dying that increases with the Ni concentration. Based on Equation 5 from De Laender et al. (2008) the daily mortality rate of a given survival experiment can be calculated:

$$m(Ni) = \frac{1}{t_{exp}} * \ln \left(1 + \left(\frac{[Ni]}{LC_{50}} \right)^\beta \right) \quad (5)$$

where LC_{50} is the 50% lethal concentration, β is the slope of the dose response curve, and t_{exp} the duration of the experiment (in days). From the daily mortality rate, the stochastic chance of dying $p_m(Ni)$ can be calculated as:

$$p_m(Ni) = (1 - e^{-1 * m(Ni)}) (6)$$

Based on the survival data from Pereira et al. (2018), values for $LC50$ and β were determined for each temperature (Supplemental Information: Table S5).

Validation of the calibrated DEB-IBM. With the calibrated model (i.e. with the temperature adjustment, optimized food correction factors, and calibrated effect parameters for Ni toxicity), we mimicked the population experiment described above to know how well the model predicted the observation on population dynamics. The same conditions as the experiment were applied in the model simulation (i.e. daily food addition, medium replacement three times per week, and culling every 14 days). The measured dissolved Ni concentrations were imposed to the simulations. The PMoA that best predicted the data of the apical experiment (based on the Euclidean distance) was used for these simulations. The predictions of the calibrated DEB-IBM were validated against the (independent) dataset from the population experiment at 15, 20 and 25°C.

Extrapolation of the ECx for a Daphnia magna population. The calibrated model was used to further extrapolate the effects of temperature on Ni toxicity to *D. magna* population and to predict at which Ni concentrations effects would be observed. We again imposed the same conditions as in the population experiment, but now varying the Ni concentrations from 0 to 300 $\mu\text{g L}^{-1}$. Population-level effect concentrations were determined for equilibrium population abundance (i.e. the mean population density after 8 and 9 weeks of simulations – when the population density reaches a steady state). With the *drc* package in R, population-level ECx values were calculated based on regression of

a log-logistic dose-response curve (*llogistic* – 3 parameters log-logistic curve) (Ritz et al. 2016; RStudio Team 2017).

RESULTS AND DISCUSSION

Population experiment

The population experiment showed no consistent Ni effects on total *D. magna* population abundance at 15, 20 and 25°C (Figure 1), although the Ni concentrations tested (0, 12, 100 $\mu\text{g L}^{-1}$) were previously reported to significantly reduce the apical endpoint reproduction in a standard reproduction test (Pereira et al. 2018). The tested concentration 12 $\mu\text{g Ni L}^{-1}$ corresponds to ~30% of effect on *D. magna* apical reproduction at 15°C and to 0% of effect at 20 and 25°C (Pereira et al. 2018). 100 $\mu\text{g Ni L}^{-1}$ corresponds to 70, 50 and 20% of effect at 15, 20 and 25°C, respectively (Pereira et al. 2018). Furthermore, population structure was not affected by Ni: no consistent effect of Ni on the number of neonates, juveniles and adults were observed at any temperature. Also, no consistent effect of Ni on the ratio between the number of juveniles and the number of adults were observed at any temperature (Figure 2).

Previous studies have also shown that effect concentrations estimated based on apical endpoints may not reflect the effects at population-level (Gagneten and Vila 2001; Viaene et al. 2015). In Viaene et al. (2015), after 1 week of exposure to 62 $\mu\text{g L}^{-1}$ of pyrene, a concentration reported to have an effect on the apical endpoint survival (EC50 for immobilization = 68 $\mu\text{g L}^{-1}$), the total *D. magna* population abundance was reduced, after 2 weeks the *D. magna* population abundance had begun to recover but the adult abundance was longer affected. The results of Viaene et al. (2015) suggests that apical

effect concentrations do not (always) translate to population-level effects in a straightforward way. Another study showed a *Ceriodaphnia dubia* population became extinct when exposed to $20 \mu\text{g Cu L}^{-1}$, a concentration that reduced the apical endpoint reproduction by 50% (Gagneten and Vila 2001). To our knowledge, until now the effect of Ni to a *D. magna* population was only studied by Münzinger (1994) and only at 20°C in an 8-week study. In that study, the total abundance of the population held in flow through cultures was significantly reduced by 120, 160 and $200 \mu\text{g Ni L}^{-1}$ compared with the control treatment. The study did, however, not report effect concentrations on apical endpoints. Therefore, it is difficult to compare to the present study. The present study and the studies of Viaene et al. (2015) and Gagneten and Vila (2001) demonstrate that very different outcomes (quantitatively) can be obtained for population-level endpoints than for apical endpoints. Thus, conventional ecotoxicological results (i.e. based on apical endpoints) cannot be straight forward extrapolated to population-level effects.

Along the 9 weeks of the experiment the major cations concentrations remained stable at 15°C and 25°C but not at 20°C (SI, Table S3). One could argue that the different major ion concentrations at 20°C could compromise the comparison of the effect of Ni to the *D. magna* population with that at other temperatures. However, this is unlikely because the biotic ligand model for chronic Ni toxicity to *D. magna* (Deleebeeck et al. 2008) that we applied to media composition only estimated a 1.2-fold difference between the estimated EC10's between the media at 15, 20 and 25°C .

DEB-IBM predictions

In the population experiment of the present study the effect of Ni on a *D. magna* population was absent at 15, 20 and 25°C. Therefore, to further investigate the effect of temperature on Ni toxicity to a *D. magna* population a DEB-IBM (Martin et al. 2013a) calibrated using apical Ni toxicity data at 15, 20 and 25°C was used (Pereira et al. 2018).

Physiological modes of action. To predict the population response to Ni we firstly had to identify the PMoAs which are affected by Ni. The PMoA with an effect on growth costs (an increase in costs for growth) was able to predict the Ni effect on the apical endpoints size (length) and reproduction observed in Pereira et al. (2018). Furthermore, it presented the lowest ρ and the best fits to the observations (Figure 3 and 4; SI, Table S4). The PMoAs with an effect on general maintenance costs (i.e. both structural maintenance and maturity maintenance), on structural maintenance costs and on assimilation (decrease in feeding ability) also predicted the Ni effect on the apical endpoints size and reproduction relatively well, but the ρ were considerably higher (Figure 3 and 4; SI, Table S4). The PMoAs with an effect on reproduction costs and maturity maintenance costs did not predict an effect on growth. Overall, these results indicate that the PMoA with an effect on growth costs best predicted the Ni effects on size and reproduction.

The relation between the best-fit PMoA in this study (increased costs of growth) and the known mechanisms of Ni toxicity is not straightforward. Firstly, the mechanisms of Ni toxicity are not fully understood. Brix et al. (2017) identified as potential mechanisms of Ni toxicity in aquatic organisms the disruption of Ca, Mg and Fe homeostasis. Also, previous studies showed Ni exposure decreased respiration rates, glycogen and adenosine

triphosphate (ATP) concentrations in *Daphnia* (Pane et al. 2004; Pane et al. 2003). Pane et al. (2004) showed that the exposure of *D. magna* to Ni decreased Mg concentrations and reduced the ATP levels. That study suggested those two endpoints could be related since the Mg^{2+} is a cofactor for ATP (Stryer 1995). The ATP, the main source of energy in cells, must be bound to a Mg^{2+} ion in order to be biologically active (Stryer 1995). It would be worthwhile to investigate if the effect of Ni on Mg and ATP levels that are necessary for the growth of organisms (to build new units of structure, e.g. a new cell) could manifest into an increased cost for growth

The mechanisms of Ni toxicity reported in the paragraph above could equally well manifest into other PMoAs, since, ATP is necessary for all biological functions and therefore can have an effect on all PMoAs. Magnesium is essential for several biological functions including structural stabilization of nucleic acid (Wolf and Cittadini 2003), which could relate to the PMoAs of general maintenance costs and on structural maintenance costs. Also, Mg is essential for cell membranes and to promote specific structural organization of enzymes (Wolf and Cittadini 2003) which may translate into an effect on the PMoA assimilation. Ashauer and Jager (2018) already pointed to the importance to link the PMoA as defined in the DEB to physiological level endpoints (what happens at the cellular level) to enable the extrapolation from *in vitro* and *in vivo* toxicity studies to *in silico* studies (Ashauer and Jager 2018).

Predictions vs. observations. Overall, the DEB-IBM calibrated to our control condition and based on the apical Ni toxicity data (Pereira et al. 2018) predicted the absence of Ni effect to a *D. magna* population at 12 and 100 $\mu\text{g Ni L}^{-1}$ at 15, 20 and 25°C in accordance with the observations from the population experiment (Figures 5, 6 and 7). Only at 15°C

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and 100 $\mu\text{g Ni L}^{-1}$, there seemed to be an underprediction in juvenile, adult and total population density. This indicates that there might be a significant effect of Ni at 15°C at a concentration of 100 $\mu\text{g Ni L}^{-1}$. Unfortunately, due to the lack of reliable data beyond 21 days of exposure, it is not possible to conclude if this trend would have continued. The total abundance of the population over time and the size structure predictions were generally in accordance with the observations (Figures 5, 6 and 7). Only at 20°C, the predicted population density at the end of the simulation is higher than observed, for all Ni treatments. This is due to an overprediction of the juvenile density at 20°C (Figure 7).

Extrapolation of the Ni EC's for a Daphnia magna population at 15, 20 and 25°C. The extrapolated EC50 for a *D. magna* population of Ni (i.e. using the average of the total population abundance from week 8 to week 9) was lower at 15°C and it was 2.4-fold higher at 20°C than at 15°C and 1.3-fold higher at 20°C than at 25°C (Figure 8, Table 1). The extrapolated EC50 values indicated that the effect of temperature on Ni toxicity to a *D. magna* population was smaller than the effect of temperature on Ni toxicity to *D. magna* (apical) reproduction. Our previous experiment showed chronic Ni toxicity to *D. magna* increased with the increase of temperature with a 6.5-fold difference between the EC50 values for *D. magna* apical reproduction (i.e. 17.3 ± 2.2 , 75.1 ± 8.9 , $129.1 \pm 9.4 \mu\text{g L}^{-1}$ at 15, 20 and 25°C, respectively) (Pereira et al. 2018). The effect of temperature on Ni toxicity on *D. magna* population equilibrium density was thus smaller than on *D. magna* apical reproduction.

The predicted EC50 values for population equilibrium density are higher than those reported for the apical endpoint of individual reproduction (i.e. a 5.5-fold, 3.1-fold and 1.4-fold difference at 15, 20 and 25°C, respectively). This difference in Ni effects

between the apical and population level is most likely caused by compensating mechanisms that occur in population context, where individuals compete with each other, which is not the case in the standard *D. magna* reproduction test. If more individuals in the population die because of Ni exposure, more food will remain available for the surviving, less sensitive individuals. In line with this hypothesis, we performed a detailed analysis of the model to better understand the predicted (absence of) effects at the population level (Supplemental Information: Section 4). We investigated the different causes of mortality with increasing Ni concentration, as well the changes in DEB energy fluxes over time. We predicted with the DEB-IBM, by tracking in a simulation the cause of death of each individual, that when Ni is present most mortality is caused by Ni, whereas in the control treatment this is predicted to be starvation. Investigating the DEB energy fluxes in detail, the DEB-IBM indeed predicts that a compensation mechanism occurs between food limitation and Ni toxicity. Since more individuals die at intermediate Ni concentrations, more food will be available for the survivors, indicating that they are less limited by food. More energy can then be invested in reproduction, compensating - at the population level - for the Ni induced mortality. Barata et al. (2002) have experimentally confirmed the compensation of food limitation by toxic effects through a series of a population experiments with cladocerans. They observed that toxic stress compensates the stress of the lack of food. Reduction in population growth rate (by an increase in mortality due to toxic stress) caused by a toxicant is compensated since more food is available for survivors, increasing resource efficiency. Similar outcomes have been reported for fruit fly populations (Gui et al. 2008). In conclusion, because of this compensation mechanism, caused by the joint effect of Ni stress and resource

limitation, *D. magna* is less sensitive to Ni at the population level (regardless of the temperature).

The present study together with Viaene et al. (2015) and Gagneten and Vila (2001) show that care should be taken when extrapolating directly from apical to population-level endpoints. Population experiments are time consuming and expensive but the use of population models as DEB-IBM can help the scientific community in replacing or optimizing the population experiments. In the present study, the calibrated DEB-IBM predicted the unexpected absence of Ni effect to a *D. magna* population at the tested concentrations. Furthermore, we were able to use the calibrated DEB-IBM to extrapolate the ECx for a *D. magna* population of Ni. This type of information would have been very useful to optimize the experiment design e.g. choosing test concentrations. It has been shown that the DEB-IBM can be calibrated to different test species and different environmental conditions to extrapolate stressor effects to the population-level (Ashauer and Jager 2018; Hochmuth 2016; Jager et al. 2014; Martin et al. 2013b; Viaene 2016). For future population studies the prior use of the DEB-IBM to estimate population-level effects of chemical stressors would be helpful to improve the design of the experiment and therefore to optimize the time and the financial resources.

CONCLUSION

The present study showed the absence of consistent Ni effects on a *D. magna* population at 15, 20 and 25°C at Ni concentrations previously reported to significantly reduce the apical endpoint of reproduction. These results caution that extrapolating from standard toxicity test endpoints to population-level endpoints may be inaccurate. Interestingly, the

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simplified DEB-IBM calibrated using apical Ni toxicity data at 15, 20 and 25°C (Pereira et al. 2018) predicted this unexpected absence of Ni effect to a *D. magna* population. Detailed simulations suggest that the lower sensitivity at population level is most likely due to ecological compensation between Ni stress and resource limitation stress (related to intra-specific competition taking place in a population context). Furthermore, the extrapolated EC50 values (predicted with the DEB-IBM) suggest that the effect of temperature on Ni toxicity to *D. magna* population density is considerably smaller than the effect of temperature on Ni toxicity to *D. magna* reproduction. DEB-IBM can help to optimize the experimental design of population studies or even to replace population experiments by *in silico* simulations.

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Data availability

Data pertaining to this manuscript are deposited in figshare.

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Figures

Figure 1. Average total abundance of *Daphnia magna* population exposed during 9 weeks (63 d) to nickel (Ni) (0, 12 and 100 $\mu\text{g Ni L}^{-1}$) at 15, 20 and 25°C. Every 14 days half of the population was reduced by random culling. Vertical lines represent the days (every 14 days) that 50% of each of the 3 size classes (i.e. neonates, juveniles and adults) were removed at random to avoid population collapses due to overcrowding. At those time points the total abundance of *D. magna* population reported refers to the abundance just prior to the culling.

Figure 2. Ratio of the number (No) of juveniles and the number of adults of *Daphnia magna* population exposed during 9 weeks (63 d) to nickel (Ni) (0, 12 and 100 $\mu\text{g Ni L}^{-1}$) at 15, 20 and 25°C. Vertical lines represent the days (every 14 days) that 50% of each of the 3 size classes (i.e. neonates, juveniles and adults) were removed at random to avoid population collapses due to overcrowding.

Figure 3. Calibration of the DEB-IBM for *Daphnia magna* with different physiological modes of action, i.e. an effect on growth costs (an increase in costs for growth), on general maintenance costs (both structural maintenance and maturity maintenance), on structural maintenance costs and on assimilation (decrease in feeding ability). Predictions are plotted versus the observations from the apical nickel (Ni) toxicity dataset from Pereira et al. (2018). The hockey-stick relationship between the Ni concentration and the level stress on the PMoAs was used. Each graph shows *D. magna* cumulative reproduction as a function of the Ni concentration at 15, 20 and 25°C. Cumulative reproduction is expressed as the number of offspring per individual female produced until the organisms in the control treatment released the fifth brood as percentage of control treatment (cumulative reproduction (relative to control)). Lines represent predictions and dots observations.

Figure 4. Calibration of the DEB-IBM for *Daphnia magna* with different physiological modes of action, i.e. an effect on growth costs (an increase in costs for growth), on general maintenance costs (both structural maintenance and maturity maintenance), on structural maintenance costs and on assimilation (decrease in feeding ability). Predictions are plotted versus the observations from the apical nickel (Ni) toxicity dataset from Pereira et al. (2018). The hockey-stick relationship between the Ni concentration and the level stress on the PMoAs was used. Each graph shows *D. magna* size (as percentage of control treatment) as a function of the Ni concentration at 15, 20 and 25°C. Lines represent predictions and dots observations.

Figure 5. Observed and predicted population dynamics for *Daphnia magna* under 0, 12 and 100 $\mu\text{g Ni L}^{-1}$ during 9 weeks at 15 (data available only until 21 d), 20 and 25°C. Dots represent data points of the total population abundance and line the predictions of the model using the physiological mode with an effect on growth costs (an increase in costs for growth). Vertical lines represent the days (every 14 days) that 50% of each of the 3 size classes (i.e. neonates, juveniles and adults) were removed at random to avoid population collapses due to overcrowding.

Figure 6. Observed and predicted population dynamics for *Daphnia magna* under 0, 12 and 100 $\mu\text{g Ni L}^{-1}$ during 9 weeks at 15 (data available only until 21 d), 20 and 25°C.

Dots represent data points of the adult abundance and line the predictions of the model using the physiological modes of action mode with an effect on growth costs (an increase in costs for growth). Vertical lines represent the days (every 14 days) that 50% of each of the 3 size classes (i.e. neonates, juveniles and adults) were removed at random to avoid population collapses due to overcrowding.

Figure 7. Observed and predicted population dynamics for *Daphnia magna* under 0, 12 and 100 $\mu\text{g Ni L}^{-1}$ during 9 weeks at 15 (data available only until 21 d), 20 and 25°C. Dots represent data points of the juveniles (and neonates) abundance and line the predictions of the model using the physiological modes of action mode with an effect on growth costs (an increase in costs for growth). Vertical lines represent the days (every 14 days) that 50% of each of the 3 size classes (i.e. neonates, juveniles and adults) were removed at random to avoid population collapses due to overcrowding.

Figure 8. The extrapolated concentration response curves of nickel to a *Daphnia magna* population abundance (i.e. using the average of the total population abundance from week 8 to week 9) at 15, 20 and 25°C. Marker points represent average of the predictions and lines are the fitted concentration response curves. The concentration response curve were fitted through the results of the simulations with the *drc* package (LL.3, three parameter log-logistic curve) in R. Vertical lines indicate the extrapolated EC50 values at 15, 20 and 25°C.

Table 1. The extrapolated effect concentrations (ECx) nickel (Ni) to a *Daphnia magna* population (i.e. using the average of the total population abundance from week 8 to week 9) (+/- standard error) ($\mu\text{g L}^{-1}$) at 15, 20 and 25°C.

ECx	15°C	20°C	25°C
EC10	63.7 (± 0.2)	171.9 (± 0.8)	151.0 (± 0.3)
EC20	74.0 (± 0.2)	191.2 (± 0.6)	161.8 (± 0.2)
EC50	95.6 (± 0.2)	229.3 (± 0.4)	182.1 (± 0.2)