Extrapolating contaminant effects from individuals to populations: a case study on nanoparticle toxicity to *Daphnia*

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January 30, 2022

Dear Drs. Desforges, Hickie, Gergs, and Weijs,

Please find the attached manuscript titled “Extrapolating contaminant effects from individuals to populations: a case study on nanoparticle toxicity to *Daphnia*” for consideration of publication in the special issue in Archives of Environmental Contamination and Toxicology titled “Development and Application of Bioaccumulation and Effect Models to Address Current and Emerging Issues in Ecotoxicology”. This is an original manuscript that has not been previously submitted to this or any other journal.

The effective management of ecological risk relies on our ability to predict the effect of stressors on the sustainability of ecosystems. However, Ecological Risk Assessments (ERA) most commonly use data on *individual* organisms due to the infeasibility of large, ecosystem or population-level experiments. Therefore, the extrapolation of stressor effects from the individual-level to higher levels of biological organization is of great importance when considering the potential effect of a stressor, specifically a contaminant in ecotoxicological applications. In this manuscript, we build on previous work (Stevenson et al. 2017¹) in which we predicted from data on *individual* life histories a threshold daily food requirement such that populations of *Daphnia pulicaria* exposed to silver nanoparticles (AgNPs) at one concentration will persist. We conducted an experiment exposing daphnid populations to the same concentration of AgNPs at which the individual-level data would predict persistence at all but the lowest food input rate which was close to the extinction threshold. We fit a bioenergetic model to the individual-level data published in Stevenson et al. 2017 and used these parameters in an Individual Based Model (IBM) which predicted population outcomes broadly matching those observed. This work highlights the importance of studies on individuals at ecologically plausible food levels. It also points to the importance of considering ecological feedbacks, specifically via resource dynamics, when making predictions across levels of biological organization.

We suggest the following reviewers for this manuscript: Drs. Christopher Salice (csalice@towson.edu), Steven Railsback (steven.railsback@humboldt.edu), Nika Galic (nika.galic@syngenta.com), Nina Marn (Nina.Marn@irb.hr), and Andre Gergs (andre.gergs@bayer.com).

Thank you very much for your consideration of our manuscript. We are excited about the special issue of this journal and think our work is a good fit for its aims, specifically the topics “Bioenergetic or other process-based models that improve characterization of contaminant accumulation and effects” and “Models capable of extrapolating effects across biological scales (e.g., molecular to whole organism to population)”.

Sincerely,

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¹ Stevenson, L. M., Krattenmaker, K. E., Johnson, E., Bowers, A. J., Adeleye, A. S., McCauley, E., & Nisbet, R. M. (2017). Standardized toxicity testing may underestimate ecotoxicity: Environmentally relevant food rations increase the toxicity of silver nanoparticles to *Daphnia*. *Environmental toxicology and chemistry*, 36(11), 3008-3018.

1 **Extrapolating contaminant effects from individuals to populations: a case**
2 **study on nanoparticle toxicity to *Daphnia***

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31 **Abstract:**

32 Ecological risk assessment (ERA) is charged with assessing the likelihood a chemical
33 will have adverse environmental or ecological effects. When assessing the risk of a potential
34 contaminant to biological organisms, ecologists are most concerned with the sustainability of
35 *populations* of organisms, rather than protecting every *individual*. However, ERA most
36 commonly relies on data on the effect of a potential contaminant on individuals because these
37 experiments are more feasible than costly population-level exposures. In this work, we address
38 the challenge of extrapolating these individual-level results to predict population-level effects.
39 Previous per-capita population growth rate estimates calculated from individual-level exposures
40 of *Daphnia pulicaria* to silver nanoparticles (AgNPs) at different food rations predict a critical
41 daily food requirement for daphnid populations exposed to 200 µg/L AgNPs to avoid extinction.
42 To test this, we exposed daphnid populations to the same AgNP concentration at three different
43 food inputs, with the lowest ration close to the extinction threshold predicted from data on
44 individuals. The two populations with the higher food inputs persisted, and the population with
45 the lowest food input went extinct after 50 days but did persist through two generations. We
46 demonstrate that we can extrapolate between these levels of biological organization by
47 parameterizing an individual-level biomass model with data on individuals' response to AgNPs
48 and using these parameters to predict the outcome for control and AgNP-exposed populations.
49 Key to successful extrapolation is careful modeling of temporal changes in resource density,
50 driven by both the experimental protocols and feedback from the consumer. The implication for
51 ecotoxicology is that estimates of extinction thresholds based on studies of *individuals* may be
52 reliable predictors of population outcomes, but only with careful treatment of resource dynamics.

53
54 **Introduction:**

One important goal of ecological risk assessment (ERA) of chemicals is to estimate the effects of a potential contaminant on *populations*, however population experiments are costly in terms of labor, materials, and organisms. In practice, ERAs often end up relying on data from experiments on individuals, and, in the case of ERAs for freshwater systems, they commonly use data from chronic toxicity tests (OECD 2012) in which *Daphnia* are exposed to increasing concentrations of a potential toxicant and their survival and reproduction are measured. From these individual-level data, it is possible to estimate metrics related to population-level responses, such as the long-run population growth rate in a constant environment (per-capita population growth rate, r), an approach that has been employed to predict the effect of toxicants on populations from individual level data for many years (Kooijman and Metz 1984). However, such metrics do not directly permit prediction of population-level impacts, unless they take account of potentially important feedbacks between the individuals that comprise the population and their environment. Recognition of this limitation has inspired calls for the use of ecological modeling to incorporate ecological complexity into ERA (Forbes et al. 2011; Forbes et al. 2009). The feasibility, and challenges, of the use of models to extrapolate ecotoxicological impacts across levels of biological organization was demonstrated for *Daphnia* by (Martin et al. 2013a; Martin et al. 2013b).

Our past work (Stevenson et al. 2017) studied the effect of silver nanoparticles (AgNPs) on individual *Daphnia* fed a range of algal food rations (mgC algal food per daphnid per day). We found that low food increased AgNP toxicity, and that two AgNP exposures (75 and 200 $\mu\text{g/L}$) had direct effects on daphnid survival (Stevenson et al. 2017). Specifically, 200 $\mu\text{g/L}$ AgNPs was overwhelmingly toxic at all but the highest food ration with no individuals exposed to 200 $\mu\text{g/L}$ surviving to reproduce except two at the highest food ration. AgNPs are the most studied

nanoparticles when it comes to the toxicity of nanoparticles to *Daphnia*, and, according to a recent review, the most susceptible to AgNP toxicity (Liu et al. 2022). Other studies have similarly found that AgNPs are toxic at low, parts per billion concentrations to *Daphnia* (Garner et al. 2015; Griffitt et al. 2008; Hoheisel et al. 2012; Qin et al. 2015; Ribeiro et al. 2013; Stensberg et al. 2014; Ulm et al. 2015), including sublethal effects on growth and/or reproduction (Mackevica et al. 2015; Ribeiro et al. 2013; Sakamoto et al. 2015; Zhao and Wang 2011). For population predictions, the most important finding from Stevenson et al. (2017, see their Fig. 5)) was identifying a critical combination of food ration per daphnid and stressor concentration for a contaminated population to be viable (r close to zero).

To empirically test our interpretation of our data on individuals, we exposed small populations of *Daphnia pulicaria* to 200 $\mu\text{g/L}$ AgNPs at three food inputs and developed an Individual Based Model (IBM) that, apart from one calibration, utilized only parameters estimated from our individual-level data or supporting literature. We then simulated the population-level impact of AgNPs on these daphnid populations.

IBMs, also known as Agent Based Models, track individuals through time and population dynamics emerge as these individuals interact with each other and their shared environment. Multiple studies have used IBMs to extrapolate individual-level effects to the population-level for ecological (Martin et al. 2013a; Railsback and Grimm 2019) and ecotoxicological applications (Martin et al. 2013b; Preuss et al. 2010). Some studies have integrated IBMs into larger ecosystem models to extrapolate effects to higher levels of biological organization (Bartell et al. 2019; Forbes et al. 2019; Schmolke et al. 2019). Here, we incorporate a generalizable net production model (a variant of the model in Nisbet et al. 2004) into an IBM framework with modules to describe algal food and damage dynamics to connect individual to the population-

level effects of AgNPs we observed empirically. In our population experiments reported here, we found that 200 $\mu\text{g/L}$ AgNPs did not have a strong effect on populations of *Daphnia* except at the lowest food input after 50 days. The IBM we developed connects the individual and population levels: an IBM parameterized using the individual-level data and supporting literature was able to predict the equilibrium population biomasses observed in our experiments. This work highlights the predictive power of detailed studies of growth, reproduction, and mortality for the extrapolation of toxicant effects from the individual to population level.

Methods: Experimental

Silver nanoparticles characterization

We purchased 40 nm BioPure citrate-coated silver nanoparticles (AgNPs) from NanoComposix (San Diego, CA). We measured the size, dissolution, and reactive oxygen species (ROS) production of AgNPs in the light and in the dark (since these daphnid experiments were performed in the dark to control algal growth which would change the food concentration) and these data are reported in Stevenson et al. 2017. In summary, AgNPs at these exposures concentrations do not produce ROS, do not aggregate, and have very slow dissolution in the “low P” COMBO media (Kilham et al. 1998) used for the experiments reported here. Our data indicate that less than 1 $\mu\text{g/L}$ of the 200 $\mu\text{g/L}$ AgNP exposure is present after three days (the longest transfer interval) as ionic silver (Stevenson et al. 2017).

***Daphnia* individual experiments**

We exposed individual *Daphnia pulicaria* to 200 $\mu\text{g/L}$ AgNP and fed them four different food rations, as detailed in Stevenson et al. 2017. As described in that paper, these exposures

were comprised of multiple experiments: an experiment at the highest food ration (0.01 mgC/daphnid/day, which ran for 26 days) and two experiments at the other food rations (which ran for the entire lifespan of the organisms). We conducted two experiments at the food rations 0.0005, 0.001, and 0.0025 mgC/daphnid/day: the first experiment consisted of control and AgNP exposures ($n = 8$ for each treatment) and the second experiment consisted of six additional control individuals. We started additional control individuals because survival at the lower food rations was initially lower than we expected, and we wanted to ensure we had enough data to parameterize the control response of *D. pulicaria* to these lower food conditions.

***Daphnia* population experiment**

To test the effect of AgNPs at different food inputs to populations of *Daphnia*, we set up twelve small populations, half of which were kept as controls and half of which were dosed with 200 $\mu\text{g/L}$ AgNPs. The populations were fed one of three food inputs (0.07, 0.14, and 0.27 mgC/day), and there were two replicate populations per treatment. Populations were kept in 400 mL of autoclaved “low P” COMBO media (Kilham et al. 1998) and all glassware was autoclaved prior to the start of the experiment.

We started the populations at the approximate neonate:adult ratio of *Daphnia pulicaria* populations at equilibrium, based on data from past in experiments in our lab (unpublished data). This ratio is approximately 3 neonates or juveniles:1 adult and we started the populations with small neonates (0.69 – 0.71 mm), large neonates (0.71 – 0.81 mm), and adults (2.0 ± 0.5 mm). We removed *Daphnia* from our stock tanks 5 days prior to the experiment and placed them in tanks of fresh COMBO media for two days, then transferred them to another tank of fresh COMBO media for another 3 days to clean the individuals and minimize any carryover of algal

cells or other detritus into the experimental containers. We started the populations at total populations sizes proportional to the food input; the populations fed the highest food input (0.27 mgC/day) started with the most individuals (25 day-old neonates, 9 large neonates, and 11 adults), the middle food input started with approximately half that number of individuals (5 day-old neonates, 13 large neonates, and 5 adults), and the lowest food input started with approximately a quarter of the population size as the highest food input (2 day-old neonates, 7 large neonates, and 3 adults).

We sampled each population on a Monday-Wednesday-Friday schedule, resetting algal food densities, media, and AgNP exposure every 2, 2, and 3 days. For sampling, we poured the daphnid populations onto a 60-micron nylon net filter (Millipore NY60) and counted and identified the stage of all individuals using a dissecting scope (Leica M80). We identified developmental stages of the individuals under the microscopes using circles printed on a transparency film of known diameters to correspond to the stages of interest. There were two circles: neonates fit into the circle with diameter 1.0 mm, juveniles fit into the circle with diameter 1.8 mm, and any individuals larger than both circles were adults. We also identified pregnant adults and counted the number of eggs and embryos in the brood pouch. We then placed the individuals into fresh COMBO media dosed with 0 or 200 $\mu\text{g/L}$ AgNPs and fed the populations.

We fed the *Daphnia Chlamydomonas reinhardtii* cells from cultures 10-12 days old. We centrifuged a 500 mL algal batch culture on 7,000 rpm for 4 minutes, re-suspended the cells in nanopure water, and then measured the concentration of chlorophyll fluorometrically (see methods in Stevenson et al. 2013). We then converted the chlorophyll a concentration to a carbon concentration using a fixed mg C: μg chlorophyll ratio that was measured empirically in

our lab (0.22 mgC/ μ g chlorophyll a). We measured the concentration of algal carbon fed through time by removing 5 mL of sample and drying the samples down in a drying oven and analyzing them for total carbon and nitrogen on a CN analyzer (Thermo Scientific Flash 200 CN Analyzer). We also measured the amount of food left behind by the populations on a microplate reader and converted these concentrations to chlorophyll a concentrations (see methods in Stevenson et al. 2013).

We placed the experimental populations in the dark for the duration of the experiment to maintain the fed algal food input. The cultures were at a temperature of 21.8 ± 0.6 degrees Celsius (average $\pm$ standard deviation of hourly measurements taken by Maxim Integrated iButton DS1921G throughout experiment placed in 400 mL of water next to the experimental cultures). The experiment was run for 89 days for all treatments except the highest food input, AgNP treatments – those were concluded on Day 68.

Bacterial cell counts

Throughout the experiment, we noticed that AgNP-exposed populations had more buildup of detritus, especially shed carapaces, compared to controls, potentially indicating AgNPs may be toxic to the bacterial populations in our experiment and hindering breakdown of organic material. We measured the bacterial population through DAPI staining (4',6-diamidino-2-phenylindole), a commonly used method for counting cells as the stain binds to DNA and fluoresces. We removed 50 mL samples from media post-transfer (we removed the daphnid individuals and then took samples of the media left behind), fixed them with formalin, and then stained the samples within 72 hours of collection. We stained 5 mL of each sample with 0.5 mL of 5 μ g/mL DAPI (Acros Organics) for 3 minutes and then filtered via a vacuum filtration

system. We filtered the samples through 0.2 micron PC filters (Whatman Nuclepore Polycarbonate Track-Etched Membrane) stained with irgalan black (2 mg/mL) with a 0.8 micron backing filter (Fisherbrand General Filtration Membrane Filter) behind the 0.2 micron filter. We mounted the filters onto glass microscope slides and counted bacterial cells using epifluorescence microscopy (Olympus B202).

Methods: theory

Individual model description

We fit a variant of the net production biomass-based model of individual daphnid growth and reproduction from Nisbet et al. 2004 to the data in Stevenson et al. (2017) on the effect of AgNPs on *D. pulicaria* fed different food rations (Table 1). The only difference from Nisbet et al. (2004) was that we assumed ingestion rate of food for individuals is proportional to carbon mass rather than use a spline-based function as in the published paper. As our target population data came from transfer cultures where all food is eaten or sinks to the bottom of the culture flask (see below), this modification is expected to have small impact on model predictions of *biomass* and greatly eases fitting. It is, however, important to note that the omission of any allometry in net production removes any characterization of juvenile-adult competition for food which is likely critical for determining changes in population demography, notably any tendency to cycling (de Roos and Persson 2013). It thus also likely eliminates the likelihood that the IBM will correctly characterize the dynamics early in the experiment – even in biomass – where we anticipate the competition among individuals in the initial inoculum will be important. For this reason, our model tests focused on the biomass dynamics near the end of the population experiments.

The model has three state variables – algal food density, carbon weight of an individual, and the cumulative number of eggs produced. The model distinguishes between juveniles and adults – any individual less than the weight at reproductive maturity (estimated from the individual data) is a juvenile, and any individual above that weight is an adult. The transition to the “adult” stage occurs at the size where the *D. pulicaria* started releasing free-swimming neonates; we made this decision because our individual and population-level models do not include explicit molts or any delay between energy committed to reproduction and the production of neonates.

Since our experiments were transfer cultures (where the media and food were replaced on a Monday-Wednesday-Friday schedule), the algal food densities resets to the food ration every 2, 2, and 3 days. In between the transfer intervals, daphnid individuals feed at a rate proportional to their weight and with a Type 2 functional response to instantaneous food density. Maintenance rate per unit of body weight is the same for all stages. Adults allocate assimilated carbon to either growth and maintenance or to reproduction, and the proportion of carbon allocated to reproduction is defined by the function . Juveniles do not allocate any carbon to reproduction. Adults produce eggs at a rate proportional to the amount of carbon assimilated that is allocated to reproduction, and this amount of carbon is converted to eggs based on the amount of carbon that is required to produce one egg (). Individuals in this model were allowed to shrink in terms of weight but could not re-utilize energy already previously allocated towards reproduction.

We initialized the model with all individuals starting at the weight at birth (W_b). We converted the measured length data to carbon weight using the equation in Paloheimo et al. (1982) for *D. pulex* ($W = 0.00624L^{2.4}$ for W = weight in mg dry weight and L = body length in mm). We previously verified that this relationship is consistent with data on *D. pulicaria*

(unpublished data), a species with very similar physiological parameters. We converted dry weight to mgC assuming that the daphnid is 42% carbon (unpublished data on *D. pulicaria*).

Population model description

We modeled the dynamics of the empirical populations using an Individual Based Model (IBM) coded in NetLogo (version 6.2). Growth and reproduction of individual *Daphnia* were modeled using the net production model (Table 1) with parameters fit to the individual-level data from Stevenson et al. 2017 (Table 2) with a few additional parameters defined by the experimental setup or fit to the individual-level data (Table 3). The simulated populations are transferred into fresh food on Mondays, Wednesdays, and Fridays (any food leftover from the previous feeding is removed and food is supplied at the food supply rate for that population). Individuals release a neonate after allocating the appropriate amount of carbon for reproduction. New offspring are initialized with 0.0011 mgC. Any energy dedicated to egg production leftover after offspring release is saved until the next molt. Two new modules were added: a one-parameter “damage” module describing age and toxicant impacts on individual hazard rate (risk of death per unit time) and a module describing algal “sinking” (also called settling) to the bottom of the culture flasks where they become much less accessible to daphnids. We assumed that the sole physiological mode of action of the AgNPs is on mortality.

The IBM is initialized using the number and carbon mass of individuals that started the experiments which varied between the food supply rates (see earlier methods section). All individuals started without eggs. The populations were simulated for up to 89 days or until all individuals died and each food input and treatment (AgNP and control) was simulated 100 times because of the stochastic mortality term (discussed below).

The damage module is a simplified implementation of an approach to mortality estimation now widely used in ecotoxicology, notably in applications using GUTS (e.g. Jager et al. (2011)). In brief, we couple a maximally simple characterization of toxicokinetics, and assume that in response, an organism accumulates physiological “damage”, an abstract concept that potentially encompasses a vast range of biological mechanisms. Hazard rate is assumed to be proportional to damage. Here, we assume that *Daphnia* individuals absorb AgNPs at a rate proportional to their biomass and define Q = body burden of AgNP, W = biomass, $q = Q/W$ = damage density. Uptake rate of AgNP is assumed proportional to biomass and we assume no loss or transformation of absorbed AgNP.

$$\text{Then } \frac{dQ}{dt} = aW \text{ and } \frac{dq}{dt} = \underbrace{a}_{\text{uptake}} - \underbrace{q \left(\frac{1}{W} \frac{dW}{dt} \right)}_{\text{dilution by growth}} = a - g(t)q \text{ with } g = \frac{1}{W} \frac{dW}{dt} \quad (1)$$

The immediate implication is that damage density increases faster when growth is slower.

To simplify subsequent parameterization considerably, we assume no mother-to-neonate transfer of AgNP, so that for all individuals q is zero at birth. A mathematical consequence of this assumption is that the ODE for q has a solution of the form

$$q(t) = a \times \text{function independent of } a \text{ involving growth history} \quad (2)$$

We have no data on bioaccumulation and therefore cannot estimate the parameter a . However, we can rescale and work with a differential equation for the ratio q/a , (which has units of time) and which can be interpreted as a damage index. If $\tau = \frac{q}{a}$, then

$$\frac{d\tau}{dt} = 1 - g(t)\tau. \quad (3)$$

Hazard rate μ is now assumed to be the sum of a background and a term proportional to damage density and we can thus set

$$\mu = \mu_0 + A\tau \quad , \quad (4)$$

where A is a single parameter estimable from the data in Stevenson et al. (2017).

We incorporated an algal sinking module into the IBM because we found that the populations only consumed roughly half of the algal food they were provided. In the algal sinking module, the *C. reinhardtii* cells sink with an exponential rate α such that the cells are no longer available to the *Daphnia* when they have sunk to the bottom of the container.

Methods: statistical

We estimated parameters in a series of steps, executed sequentially.

- 1) Estimate control parameters for the model of growth and reproduction.
- 2) Assume that the population experimental results are impacted by sinking algae using a combination of data from our experiments and from published literature.
- 3) Estimate background mortality for controls using data from Stevenson et al. (2017) and modify with an estimate of the damage related parameter A by calibrating the estimated long run growth rate and comparison with Fig. 5 of Stevenson et al. (2017)
- 4) For 200 $\mu\text{g/L}$ treatments, estimate the new parameter A by calibrating to fit the 200 $\mu\text{g/L}$ estimates of r from Stevenson et al. (2017).

We describe each below.

- 1) We fixed parameters ε and β to values based on well-established estimates in the literature (summarized in Nisbet et al. (2004)). We calculated the value of b using the data in Stevenson et al. 2017, specifically growth at the food input of 0.001 mgC/daphnid/day. At this food ration, *Daphnia* did not reproduce and growth saturated around 1.9 mm (0.0122 mgC). We assumed an assimilation efficiency of 0.7, such that

the *Daphnia* were assimilation approximately 0.0007 mgC/daphnid/day. Assuming all of that energy went towards growth and respiration (since the individuals did not reproduce), we calculated a value of the maintenance rate (b) to be 0.06 1/day, a value consistent with past studies of *Daphnia*. We estimated the remaining parameters using a likelihood method coded in the package BYOM (“Bring Your Own Model”) platform for parameter estimation, developed by Tjalling Jager (<http://debttox.info>) for Matlab (Mathworks). Details on the fitting routines in this software can be found in Jager (2021). We estimated the values of parameters I , F_h , and ρ by fitting all food levels simultaneously. Negative log likelihood values the model fit is provided in the figure legend. Confidence intervals were calculated using likelihood profiles (95% probability from the χ^2 distribution).

- 2) Data from measurements of residual algae yielded an estimate of 2.0 day⁻¹ for the sinking rate α . Given our large uncertainty this estimate is consistent with a published estimate of 1.57 day⁻¹ (Kearns and Hunter 2001). We used this published value in the simulations reported here.
- 3) We estimated the background mortality rate using the individual-level data (Stevenson et al. 2007), assuming a constant, exponential decay function. We estimated the value of the damage accumulation rate for controls by adjusting the value of A until the final biomass in population controls with the highest food input was close to the equilibrium biomass we found in the population experiments. We then tested this value of A by computing the long-term population growth rate (r) applying the Euler-Lotka equation to simulations of the individual experiments with food inputs 0.01 and 0.0025 mgC/daphnid/day and comparing with the values calculated by (Stevenson et al. 2017 Fig. 5).

4) We used this method to estimate the value of A for the AgNP treatment – we adjusted the value of A until the calculated value for r matched the values found in Stevenson et al. 2017. For the AgNP treatments, only individuals at the highest food ration produced neonates, so only one value of r could be used. The damage accumulation rate for the AgNP treatment was estimated by matching this estimate of r .

To analyze for statistically significant differences between bacteria cell counts and to calculate mortality rates for the IBM, we performed linear regression using R (version 3.2.3). To calculate average background mortality rates, we assumed survival followed an exponential decay function and calculated the slope of this function by taking the natural log of survival and time and calculating the slope of the resulting line. Confidence intervals were calculated in R using ‘confint’.

Results: Parameter estimates

Individual-level model parameter values

We fed the *Daphnia* algal food at the nominal food rations (0.0005, 0.001, 0.0025, 0.01 mgC/daphnid/day) assuming a fixed chlorophyll to carbon ratio (Stevenson et al. 2017). We measured the carbon concentration of food fed throughout the experiment and the actual concentrations fed were 0.00064, 0.0013, 0.0032, and 0.0128 mgC/daphnid/day (unpublished data). We used the measured values converted to an algal carbon density at the start of each transfer interval as the model food inputs (Figure 2).

The fit of the individual-level model to our individual level data is shown in Figure 2. The model broadly matched the dynamics of growth and reproduction in *D. pulicaria* fed a wide range of food rations. There are some significant mismatches, specifically the model

underpredicts growth at the lower food rations but recognizing the importance of reproduction for population dynamics, we accepted this limitation of our individual model.

Population-level model parameter values

The IBM required a few parameters not estimated for the individual-level model, specifically the background hazard rate, damage accumulation rate, and algal sinking rate. We also explicitly measured concentration of algal carbon fed to the populations and used these values for the food input rate.

Hazard model

We calculated background hazard rate for unexposed individuals across the food rations (Figure S1) assuming that the individuals die exponentially. The parameter value and confidence interval are reported in Table 3.

We estimated the values for the damage accumulation rates for control and AgNP-exposed individuals and these values are reported in Table 3. We estimated these values by comparing the predicted r values for these estimates of A compared to the calculated r values in Stevenson et al. 2017. Values were only calculated for the two highest food rations, 0.01 and 0.0025 mgC/daphnid/day, because those are the only two food rations at which the *Daphnia* reproduced, and no individuals exposed to AgNPs reproduced at food rations less than the highest. In the controls, the r values calculated using the estimate of A reported here are 0.042 and 0.20 1/day for 0.0025 and 0.01 mgC/daphnid/day, respectively. For the AgNP-exposed individuals, the r value calculated using the estimate of A reported here is 0.18 per day for 0.01 mgC/daphnid/day. All of these values are within the variation reported in Stevenson et al. 2017.

Algal sinking rate

We found that the daphnid populations did not consume 18-63% of the algal food they were fed, and this difference varied across the number of transfer days that had elapsed and, to a lesser extent, between the control and AgNP treatments (Figure S2). We hypothesize that this is due to algal sinking, which we explicitly added to the IBM as described in the Methods section. Kearns and Hunter (2001) measured the sinking or settling rate of *C. reinhardtii* spectrophotometrically and found a value of 1.57 l/day. When plugged into the algal sinking module, this value roughly predicts the concentration of algal food left behind we observed in our experiment (Figure S2). With this value of α , the algal sinking model predicts: at the highest food input, the daphnid populations would eat roughly 50% and 40% of the algal food after 2 and 3 transfer days, respectively; at the medium food input, the daphnid populations would eat roughly 40% and 30% of the algal food after 2 and 3 transfer days, respectively; and at the lowest food input, the daphnid populations would eat roughly 40% and 36% of the algal food after 2 and 3 transfer days, respectively. An obvious deviation between our model and data (Figure S2) is that the model predicts that the daphnid populations would eat less of the food after the 3-day than the 2-day transfer interval, however our data show that the populations ate more of the food after the longer transfer interval. This is probably due to the daphnid populations starting to root around at the bottom of the beaker after an extended period without food, possibly eating some of the algal cells that the model assumes are unavailable because they have sunk. Although there is this mismatch, this simplified model using the single parameter value from Kearns and Hunter (2001) broadly captures the algal food dynamics observed in our population experiments.

Algal food input concentrations

We assumed the same fixed chlorophyll to carbon ratio as discussed for the individual-level data in order to calculate the concentration of algal food to feed the population exposures. We also measured the algal food throughout the experiment (Figure S3) and found that the concentration varied across the experiment. We used the average of these values as the input for the IBM: the concentration of algal food fed was 0.168, 0.34, and 0.675 mgC/(L*day) (Table 3).

Results: Bacterial measurements

The tested concentration (200 µg/L) of AgNPs has only a small effect on bacterial communities. We measured the bacterial population in all experimental cultures after a 2- and 3-day transfer interval to analyze for differences between AgNP and control cultures. We found no difference between control and AgNP bacterial populations after the three-day transfer interval. (Figure S4A), however samples taken after the two-day transfer interval were significantly different (Figure S4B). The food input ($p = 0.011$) and AgNP treatment ($p = 0.010$) both had significant effects on bacterial abundance.

These samples were taken on days 57 and 59 of the population experiment, as AgNP-exposed populations were recovering from the initial decline around day 50. It is unclear whether the significant or the not-significant result from the two- and three-day transfer results, respectively, more accurately describes the average state of the cultures throughout the experiment. However, 200 µg/L AgNPs is probably not a high enough concentration to kill most bacterial species – a meta-analysis of AgNP toxicity studies to a variety of taxa found that the average median lethal concentration (LC50) or half maximal effective concentration (EC50) of

AgNPs to bacteria is 7.10 mg/L, about 35 times higher than our exposure concentration (Bondarenko et al. 2013). A more recent review of silver nanoparticle toxicity found that EC50 or minimum inhibitory concentration (MIC) values of AgNPs to bacteria ranged from 0.27 – 250 mg/L with an average of 23.24 mg/L (Liu et al. 2022).

Results: Population impacts of exposure to AgNPs

200 µg/L AgNPs only impact populations substantially after 50 days to lowest food input

The tested concentration (200 µg/L) of AgNPs had no significant effect on the two highest food inputs, and only had an effect on populations fed the lowest food input after 50 days (Figures 1, 3, 4, S5-9). We can conjecture that the delay in responding is because the population starts with the “luxury” of undamaged adults who can produce many neonates before accumulating large levels of damage. The next generation starts accumulating damage from birth and hence has a much lower chance of surviving to reproduce. Although there is some variability, there are no large differences between control and AgNP treatments in the two highest food inputs (Figure 1). After 50 days, all AgNP-exposed treatments decline across all food inputs. This effect is stronger at lower food inputs – at 0.27 mgC/day, the population returns to control levels within 10 days and do not decline as sharply, while populations fed 0.14 mgC/day take about 15 days to return to control levels, and populations fed the lowest food input decline to extinction (Figure 1). Populations exposed to AgNPs at the two highest food inputs were able to recover to control levels, driven by boosts in fecundity in populations fed 0.14 and 0.27 mgC/day between days 50 and 70 (Figure S9), however populations fed the lowest food input never recovered. One AgNP-exposed replicate at the lowest food input went extinct immediately on day 54 while the other declined to one adult that continued to produce offspring

every transfer interval, however none of her offspring reached adulthood and the adult eventually died. While there are declines in the AgNP-treatments across all stages (Figures S6-8), adults seem to be the stage most affected by this decline in even the control treatments (Figure S8).

As noted earlier, we regard the most reliable test of the model to be biomass levels late in the experiment. Figure 3 displays the carbon biomass of the empirical daphnid populations through time compared to the IBM's prediction of the carbon biomass of the daphnid population (the mean of the values of the carbon biomass of the simulated populations after Day 68 from 100 IBM runs and the error bars represent their standard deviation).

A population model parameterized with individual data correctly predicts population persistence and long-term biomass.

We were able to predict the biomass towards the end of the population experiment using our IBM parameterized mostly using individual-level data (Figure 3). We assumed AgNP bioaccumulation resulted in the accumulation of damage which increased the hazard rate. We get general agreement between the predicted long-term biomasses of the model compared to the data collected from our experiment (Figure 3). Overall, the IBM underpredicted the biomass at the lowest food input in the control treatment but agreed with the middle and highest food inputs for the controls. Interestingly, the opposite was true for the AgNP treatments – the IBM underpredicted the highest food ration but matched the empirical populations better at the two lower food inputs. Importantly, the IBM successfully predicted that daphnid populations exposed to this concentration of AgNPs could go extinct at the lowest food input rate but could persist with more food: out of 100 IBM simulations, 0, 1, and 81 populations went extinct at the high, middle, and low food inputs, respectively.

The IBM does not accurately predict the size structure of the empirical populations

As anticipated in the model description, a simplifying assumption in the individual model removes the model's capacity to correctly describe detailed demography. The IBM overpredicts the number of neonates and juveniles present (Figures S11 and S12) and underpredicts the number of adults (Figure S13) at the end of the experiments.

Discussion:

Perhaps our most important conclusion for ecotoxicology is that estimation of extinction thresholds utilizing calculations of long run growth rates based on studies on *individuals* turn out to be reliable predictors of population outcomes. Our population outcome was broadly in line with what would be anticipated from the data on individuals. Stevenson et al. (2017) suggested a threshold with 200 µg/L exposure at around 0.01 mgC per day per animal. Our lowest food population experiments were given a little over 10 times that, with around half of the food sinking. This points to a population in single digits, obviously vulnerable to extinction due to stochasticity. Reliable estimates of r do require individual data over longer time periods that is required in current standardized toxicity tests (OECD 2012), but this is still much less time consuming and expensive than population experiments.

A second take home message is that other population metrics are harder to predict. Our predictions of final biomasses in the two viable exposed populations did not match the data well. This is a consequence of our somewhat heroic assumption that we could describe the combined effects of toxicokinetics and toxicodynamics with a single parameter. We also assumed a single physiological mode of action – mortality.

Our decision to focus on mortality was reached after considerable preliminary work on the individual growth/reproduction data where we assumed AgNPs impacted sublethal processes such that it took more energy for an individual daphnid exposed to AgNPs to grow and reproduce than an unexposed individual. However, after attempting to estimate individual-level parameters to describe this effect, we realized that 200 µg/L AgNP did not appear to strongly impact sublethal processes, but rather impacted mortality such that AgNP-exposed individuals grew to smaller sizes and did not reproduce as much or at all because individuals died before reaching maturity (Stevenson et al. 2017). This differs from past studies that found that silver decreases reproduction in *Daphnia* (Sakamoto et al. 2015; Volker et al. 2013), even at concentrations at which mortality was not impacted (Bianchini and Wood 2003; Hook and Fisher 2001; Qin et al. 2015; Ribeiro et al. 2013).

Another finding that may have considerable generality is that low food enhances AgNP toxicity to populations of *Daphnia*. 200 µg/L AgNPs did not have a significant effect on the daphnid populations until around Day 50, when all AgNP-exposed populations declined sharply across all food inputs (Figure 1). Populations at higher food inputs were able to survive the decline in AgNP-exposed populations around day 50 but the populations at the lowest food input went extinct, indicating the compounding effects of nano- and food stress. This conclusion follows our previous work (Stevenson et al. 2017) in addition to other studies that have found that decreased food availability increased the toxic effect of AgNPs (Mackevica et al. 2015; Sakka et al. 2016). This reinforces the message by Stevenson et al. (2017) that in spite of the practical challenges, data on toxicity at ecologically relevant food concentrations (commonly low) is critical. This requires deviation from standardized test standards (OECD 2012) by feeding *Daphnia* low, environmentally-relevant concentrations of food.

We end by commenting on the importance of the dynamics of the resource environment for determining population outcomes. One of the more interesting and striking results of this work is that 200 $\mu\text{g/L}$ AgNPs is largely toxic to individuals exposed as neonates at all food rations (Stevenson et al. 2017) but not when the neonates are part of a larger population, except when the food input is very low (Figure 1). We conjecture that this is because the transfer culture regime used in the population experiments resulted in some neonates being released into relatively high food environments. More broadly, the need to consider the dynamics of the resource environment, including feedbacks from an impacted population, needs further emphasis in all considerations of environmental stress. For example, Civitello et al. (in press) recently showed the importance of a snail's resource in determinizing human risk of schistosomiasis from parasite release; under some circumstances removing snails may increase risk. Schmolke et al. (2019) simulated the effect of reduced food availability on a fish population using a coupled IBM and aquatic food web model. Reducing different food items had differential effects on fish population and depended on the fish's diet preferences (Schmolke et al. 2019). Models such as these and the work presented here emphasize the importance of the incorporation of ecological mechanisms when quantifying the impact of anthropogenic change on natural populations.

530 *Tables:*

531 **Table 1: Individual net production model (variant on Nisbet et al. (2004))**

State variables:	
$F(t)$	Algal food density at time t (mgC/L)
$W(t)$	Carbon weight of an individual at time t (mgC)
$C(t)$	Cumulative eggs produced at time t
Functions:	
$\chi = \frac{1}{1 + \rho(W - W_p)}$	Size-dependent allocation to growth
$\phi(t) = \frac{IFW}{F + F_h}$	Algal food ingestion rate
$w_e = (1 + \gamma)W_b$	Weight of an egg
Balance equations:	
<i>Juveniles (If $W < W_p$)</i>	
$\frac{dF}{dt} = -\phi(t)V^{-1}$	Algal food
$\frac{dW}{dt} = \varepsilon\phi(t) - bW$	Juvenile growth
$\frac{dC}{dt} = 0$	Juvenile egg production
<i>Adults (If $W \geq W_p$)</i>	
$\frac{dF}{dt} = -\phi(t)V^{-1}$	Food
$\frac{dW}{dt} = \chi(\varepsilon\phi(t) - bW)$	Adult growth
$\frac{dC}{dt} = \frac{(1 - \chi)}{w_e}(\varepsilon\phi(t) - bW)$	Adult egg production ¹

532 ¹If maintenance costs were greater than assimilated energy, $\frac{dC}{dt} = 0$.

533

Table 2: Individual model parameters estimated from data in Stevenson et al. (2017) fit to control individuals fed four food rations. We distinguish between mgC of algae (mgC-A) and mgC of daphnid (mgC-D) biomass.

	Parameters	Units	Estimate	Confidence interval
I	Maximum specific ingestion rate	mgC-A/(mgC-D * day)	0.57	[0.55 0.60]
F _h	Half saturation constant in functional response	mgC-A/L	0.10	[0.094 0.10] ^a
ρ	Parameter in size-dependent allocation to growth function	1/mgC-D	1742	[1364 2246]
ε	Assimilation efficiency	mgC-D/mgC-A	0.7	
	Cost of egg production	--	0.5	
b	Maintenance rate	1/day	0.06	
W _b	Weight at birth	mgC-D	0.0011	
W _p	Weight at puberty	mgC-D	0.014	

^aHighest value of confidence interval restricted by range across which parameter was allowed to vary.

Table 3: Parameters for IBM including sources. See text for a description of estimation and methodological details.

	Parameter	Units	Value	Source
α	Algal sinking rate	1/day	1.57	Kearns and Hunter 2001
μ_0	Background hazard rate	1/day	0.04 CI: [0.038 0.043]	Individual data
A	Damage accumulation rate	1/day	0.0005 (Control) 0.005 (AgNP)	Individual data
V	System volume	L	0.4	Population experiment
X_R	Food concentration at transfer	mgC/(day*L)	0.168, 0.34, 0.675	Population experiment

Figures:

Figure 1: Total number of individuals in each of the daphnid populations fed different food inputs. Points are data from the daphnid population experiment: data points represent the average and the error bars their standard error. Each treatment (nanoparticle and food concentration) had 2 replicates. Note that the AgNP treatments at the highest food inputs were stopped on Day 68.

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Figure 4: Data (points) of total number of individuals in the empirical populations compared to the IBM simulations (lines). Points are data from the daphnid population experiment: data points represent the average and the error bars their standard error. Each empirical treatment (nanoparticle and food concentration) had 2 replicates. The IBM was run 100 times per treatment and then averaged. Error bars represent standard error for the empirical data and standard deviation for the IBM simulations.

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Statements and Declarations

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Competing Interests

The authors have no relevant financial or non-financial interests to disclose.

Author Contributions

All authors contributed to the study conception and design. Material preparation and data collection were performed by Louise M. Stevenson and Katherine E. Krattenmaker. Data analysis, model development, and model analysis were performed by Louise M. Stevenson and Roger M. Nisbet. The first draft of the manuscript was written by Louise M. Stevenson, and all

702 authors commented on previous versions of the manuscript. All authors approved the final
703 manuscript.

704 ***Data Availability***

705 The datasets generated during and/or analyzed during the current study are available from the
706 corresponding author on reasonable request.

Figures

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Supplementary Files

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