



# Population dynamics and resting egg production in *Daphnia*: Interactive effects of mercury, population density and temperature



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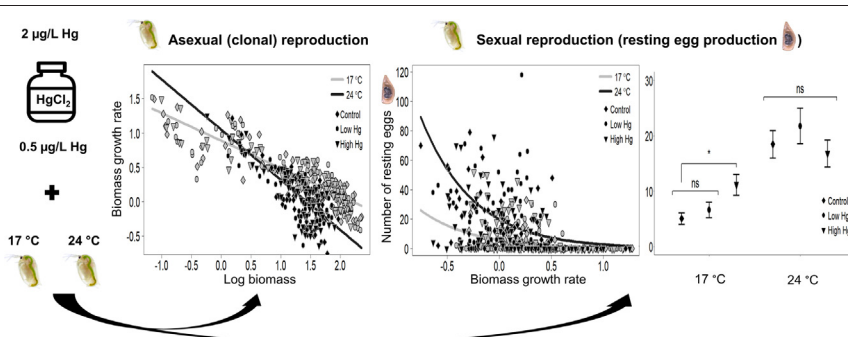
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## HIGHLIGHTS

- Biomass growth rate in *Daphnia* was unaffected by mercury but was density dependent
- Density dependence of biomass growth rate also increased at high temperature
- Sexual reproduction in *Daphnia* was density dependent
- Sexual reproduction also increased with mercury exposure at low temperature
- Sexual reproduction responds to lower mercury levels than biomass growth rate

## GRAPHICAL ABSTRACT



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## ABSTRACT

Toxicity studies on freshwater organisms are commonly conducted by quantifying effects on asexual (clonal) reproductive rates in *Daphnia*, whereas studies of effects on sexual reproductive rates remain relatively rare. Sexual reproduction in *Daphnia* and the associated production of resting eggs allows them to survive unfavorable environmental conditions and is thus a crucial component of their long-term fitness. It also maintains genetic diversity within *Daphnia* populations and hence their potential for adaptation to new environmental conditions. This aspect of their biology may therefore be important to consider in toxicity studies. The aim of this study was to investigate for the first time how mercury (Hg) affects sexual versus asexual reproduction in *Daphnia* under varying environmental conditions. Specifically, we experimentally tested the interactive effects of Hg and temperature on the population dynamics of *Daphnia magna*. For this purpose, we exposed *D. magna* to environmentally relevant concentrations (0 µg/L, 0.5 µg/L and 2 µg/L) of Hg (in the form of mercury (II) chloride) found in stream water and measured biomass growth rate resulting from asexual reproduction, and resting egg production resulting from sexual reproduction. This was done at both 17 °C and 24 °C. Biomass growth rate did not vary across Hg treatments and depended mainly on temperature and population density. Density dependence of biomass growth rate was indeed more pronounced at 24 °C than at 17 °C, as resource limitation from intraspecific competition was further exacerbated by the rise in feeding rates with temperature. Density dependence of resting egg production was unaffected by Hg and temperature, but resting egg production was higher under Hg exposure at low temperature. These findings show that depending on environmental conditions, rates of sexual reproduction in *D. magna* may respond to metal exposure at lower concentrations than those impacting population growth during the asexual phase.

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## 1. Introduction

Studies of metal toxicity in aquatic environments are commonly conducted using the freshwater keystone species *Daphnia* (Altshuler et al., 2011). *Daphnia* reproduce asexually (clonally) when environmental conditions are favorable and switch to production of resting eggs through sexual reproduction when environmental conditions deteriorate. In southern populations of *Daphnia* that generally experience hot and dry summers, a rise in temperature provides such a cue (Bernot et al., 2006). Other cues include high population density (Carvalho and Hughes, 1983), and low food abundance (Alekseev and Lampert, 2001). The dormant resting eggs survive stressful environmental conditions over long periods of time (Cáceres and Tessier, 2003), and are crucial for long-term fitness in *Daphnia* (Gerber et al., 2018; Hebert, 1978). Yet, few previous studies have looked at effects of metals on daphnid sexual reproduction (Araujo et al., 2019; Chen et al., 1999). One of the most toxic metals is mercury (Hg). Hg pollution in aquatic environments is a worldwide concern (Lavoie et al., 2013), with effects on aquatic biota that range from developmental and reproductive toxicity to neurotoxicity (Scheuhammer et al., 2007). Whereas Hg is known to have toxic effects on asexual reproduction in *Daphnia* (Doke et al., 2014; Fong et al., 2019; Tsui and Wang, 2005a), to our knowledge no studies have tested for effects of Hg on *Daphnia* sexual reproduction and the production of resting eggs.

Aquatic invertebrates, like *Daphnia*, regularly experience extensive spatial and temporal variation of environmental factors, which may interact with metals to influence important characteristics of individuals and populations (Issa et al., 2020; Wang, 1987). Temperature is one environmental factor that can largely alter the biochemistry and physiology of organisms, with population-level consequences (Atkinson, 1994; Somero, 2005). Within their natural range, a rise in temperature generally increases population growth of ectotherms when food is unlimited (Doorslaer et al., 2010; Sweeney et al., 2018). However, biological factors such as intraspecific competition for food can interact with temperature to affect population growth through density-dependent responses. As population density increases and resources become more limited, the positive effect of temperature on population growth decreases (Giebelhausen and Lampert, 2001; Orcutt and Porter, 1984). In the context of climate change, temperature mean and variance is expected to increase (IPCC, 2013), which can threaten the stability of aquatic ecosystems.

Temperature and population density interactions also play a role in determining species sensitivity to metals, although this is generally not included in classic toxicity tests (OECD, 2004, 2012). At elevated temperatures, species' sensitivity to metals increases (Rathore and Khangarot, 2002). One reason for this is that higher metabolic activity and cell membrane permeability lead to increased metal uptake from food and the aqueous environment (Dijkstra et al., 2013; Sokolova and Lannig, 2008). The rise in metabolic demand for energy at high temperature, and particularly if coupled with high intraspecific competition, reduces the per capita amount of resources available for allocation to detoxification and repair processes (Heugens et al., 2001). Hence, population-level effects of metals may be expected to be shaped by an interaction between temperature and population density. In aquatic environments, warmer temperatures in a climate change scenario are expected to increase the concentrations of bioavailable Hg (Dijkstra et al., 2013; Schartup et al., 2019). By increasing the bioaccumulation of Hg, higher temperatures can enhance Hg toxicity to aquatic biota (Dijkstra et al., 2013; Jordan et al., 2019), unless potentially offset by high food availability and hence higher energy available for detoxification (Jordan et al., 2019). Indeed, Hg interactions with temperature and food availability can affect the physiology and population dynamics of aquatic ectotherms. For example, in rotifers (*Proales similis* and *Brachionus plicatilis*), a temperature rise shortens generation times, thereby reducing the negative effects of Hg on population growth rate in the absence of competition for food (Rebolledo et al., 2018). High

food availability can also alleviate Hg-stressed populations of rotifers (*Brachionus patulus*) through higher longevity and fecundity (Ramírez-Pérez et al., 2004; Sarma et al., 2001). Hence, incorporating both temperature and population density effects in metal toxicity tests could provide a more comprehensive understanding of individual and population-level responses to Hg stress in aquatic ecosystems.

In this study, we investigated for the first time how mercury (Hg) affects sexual versus asexual reproduction in *Daphnia magna*, under varying environmental conditions. Specifically, we experimentally tested for the interactive effects of Hg and temperature on the population dynamics of *Daphnia magna*, through chronic exposure to environmentally relevant concentrations of Hg, during which biomass growth rate and resting egg production were quantified. We hypothesized that the metabolic costs of Hg detoxification would either induce stress that leads to increased sexual reproduction and the production of resting eggs, or if these costs were too high, would lower the amount of energy available for sexual reproduction and prevent the production of resting eggs. Furthermore, this would depend on the per capita amount of resources available, which varies with population density and temperature, motivating the inclusion of these factors in the current study.

## 2. Materials and methods

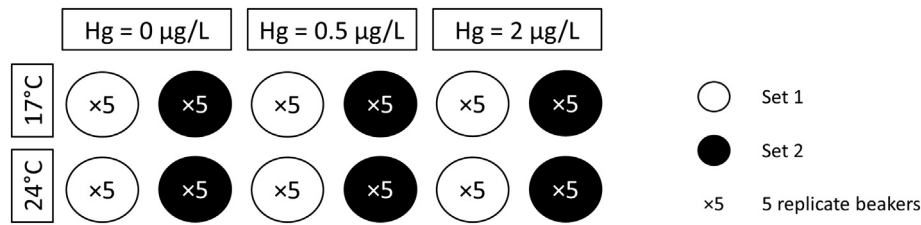
### 2.1. Study organisms

Ephippia containing resting eggs resulting from sexual reproduction of *D. magna* were collected in November 2014, in a pond at Værøy Island (1.0 ha, 67.687°N 12.672°E), northern Norway. Ephippial eggs were hatched in the laboratory and propagated clonally. For this experiment, juveniles of a single clone (clone EF7) of *D. magna* were asexually propagated for sixteen successive generations prior to use. During this period, a maximum of 30 *D. magna* individuals were cultured in 3 L aquaria at 17 °C and 24 °C in a modified "Aachener Daphnien Medium" (ADaM) (Klüttgen et al., 1994, SeO<sub>2</sub> concentration reduced by 50%), under long photoperiods (16 h L: 8 h D) using white fluorescent lamps. The medium was exchanged weekly to prevent poor medium quality, and the animals were fed three times a week with Shellfish Diet 1800® (Reed mariculture Inc.; Rikard and Walton, 2012) at a final concentration of  $2.4 \times 10^5$  algal cells/mL (75% ad lib at 20 °C).

### 2.2. Experimental design

Replicates were run in two identical experimental sets (Fig. 1) in parallel, which were sampled on different days. For each experimental set, a full factorial design with three different starting concentrations of Hg (0 µg/L, 0.5 µg/L and 2 µg/L) and two temperature treatments (17 °C and 24 °C) was applied, with five replicate beakers for each of the six combinations. The two exposure concentrations (0.5 µg/L and 2 µg/L) were selected for being environmentally relevant concentrations (Berzas Nevado et al., 2003; Gray et al., 2000) that are lower than the acute LC<sub>50</sub> of 2.2 µg/L Hg in cladocerans (Nichols et al., 1997). Moreover, a pilot study conducted prior to this experiment did not detect any effect of these concentrations on *D. magna* mortality or their capacity to undergo asexual reproduction (see Supporting information). Hg stock solutions (0.0016 g/L) were prepared at the onset of each experimental set, by dissolving 99.5% pure mercury (II) chloride (HgCl<sub>2</sub>) (Fluka, Switzerland) in Milli-Q water (18.2 MΩ cm) (Milli-Q Plus, Millipore Corp.). The exposure glass beakers (600 mL non-aerated borosilicate beakers, Fisherbrand) and equipment used for making Hg stock solutions were acid-washed overnight before use with 1 M HNO<sub>3</sub> suprapure quality prepared with a sub-boiling distillation system (Milestone, SubPUR) and subsequently washed with Milli-Q water. The stock solutions were added to ADaM to create the desired Hg exposure concentrations while controls (0 µg/L Hg) contained only ADaM.

Populations in each replicate beaker (containing 400 mL of medium) were founded by 5 female juveniles (< 48 h old) originating from the



**Fig. 1.** Schematic diagram of the experimental design.

The experiment was divided into two identical experimental sets run in parallel and sampled weekly on different days. Five replicates per set per treatment (x5). Treatments are defined by the Hg concentration (0 µg/L Hg versus 0.5 µg/L Hg and 2 µg/L Hg) and temperature (17 °C versus 24 °C). Replicates in set 1 are illustrated with white circles, with remaining replicates belonging to set 2.

same acclimation temperature as their experimental temperature (17 °C or 24 °C). These populations were thereafter observed for a period of eight weeks throughout which they were maintained under similar culture medium and feeding conditions as the aquaria cultures. The medium change was conducted weekly to renew exposure to Hg as well as prevent poor medium quality. The latter was needed to ensure that the crowding effect measured in the experiment was due to competition for food and not other factors that can originate from poor medium quality. The beakers were changed biweekly.

### 2.3. Sampling procedure

At each weekly medium renewal, the content of each replicate beaker was poured into a deep glass tray placed on a light plate with an overhanging video camera. The ephyppia were collected and counted, and a 15-second-long video recording was taken to be analyzed using the R package *trackdem* (Bruijning et al., 2018). The *trackdem* package estimates both the number of live individuals (based on moving particles) and their sizes (in pixels). Individual dry mass (mg) was calculated based on an empirical regression between pixel size and dry mass previously derived by Fossen et al. (2019) (Eq. (1)), and this allowed calculation of population biomass at each census (Eq. (2)).

$$\text{Mean dry mass} = -0.006351290 + (0.001003908 \times \text{pixels}) \quad (1)$$

$$\text{Biomass} = \text{Mean dry mass} \times \text{Population count} \quad (2)$$

Weekly rates of biomass growth were calculated on a log scale from mean population biomass estimated in two consecutive weeks ( $t$ ) as follows:

$$\text{Biomass growth rate}_t = \log \text{biomass}_{t+1} - \log \text{biomass}_t \quad (3)$$

### 2.4. Statistical analysis

All statistical analyses and graphic illustrations were performed in R v. 3.5.2. (R Core Team, 2020). For biomass growth rate<sub>t</sub>, we modelled the effect of log biomass<sub>t</sub>, Hg treatment, temperature, the three-way interaction and all two-way interactions between these, using a linear mixed effects (LME) model. For number of resting eggs produced per replicate beaker per week (RE<sub>t</sub>), we modelled the effect of Hg treatment, temperature, the direct effect of biomass growth rate during the week resting egg production was quantified, as well as a lagged effect of biomass growth in the preceding week. The reasoning behind including the lagged effect of biomass growth was that a low biomass growth rate may trigger resting egg production, but that resources for resting egg production may decline following a prolonged period of low biomass growth. The log of biomass<sub>t</sub> was also used as an offset in order to standardize RE<sub>t</sub> per unit of biomass, and the full model included all possible two- and three-way interactions among predictors. To deal with overdispersion and zero inflation, we used a zero-inflated negative binomial generalized linear mixed (ZINB GLM) model instead of a

zero-inflated Poisson generalized linear mixed model. For both dependent variables, set was added as a fixed effect and replicate beaker as a random effect.

Model selection followed a backwards selection procedure, where variables were removed sequentially, starting with random effects, using likelihood ratio tests (Zuur et al., 2009). For biomass growth rate<sub>t</sub>, models were implemented using the *lme* and *gls* functions in the package *nlme* (Pinheiro et al., 2020) and residuals were checked for homogeneous variance and for normal distribution. The *VarIdent* command from the *nlme* package was moreover used to allow residual variance to differ among Hg treatments, temperatures, sets and the two-way interactions between these (Pinheiro and Bates, 2000). For RE<sub>t</sub>, models were implemented with the *glmmTMB* package (Brooks et al., 2017). The regression results from the final models were plotted using the *visreg* package (Breheny and Burchett, 2017).

## 3. Results

Candidate models for testing effects of biomass<sub>t</sub>, the direct and lagged effects of biomass growth, Hg treatment, temperature and set on biomass growth rate<sub>t</sub> and number of resting eggs produced per replicate beaker per week (RE<sub>t</sub>) are depicted in Table A1. The summary statistics of the fitted final models are depicted in Table A2. Biomass growth rate<sub>t</sub> was similar across Hg treatments but differed between the two experimental sets and temperatures (Table A1, Fig. 2). At 17 °C, biomass increased steadily until sampling week seven and decreased in the final week of the experiment, while at 24 °C, biomass peaked around week five and decreased thereafter (Fig. 2). Density dependence was important, as growth rates decreased with increasing biomass. The strength of density dependence was more pronounced at 24 than at 17 °C (Table A1, Fig. 3). This caused the estimated carrying capacity (i.e. biomass at which growth rate = 0, based on the model parameters in Table A2) to be more than twice as high at 17 °C compared to at 24 °C (9.3 vs. 4.2 mg biomass). Biomass growth rate<sub>t</sub> was further higher in set 1 compared to set 2 (Table A1), yet the magnitude of this difference was relatively small (less than 5%, see Table A2).

In contrast to biomass growth rate<sub>t</sub>, the best model describing variation in RE<sub>t</sub> included an effect of Hg (Table A1). The effect of Hg depended however on temperature, with higher RE<sub>t</sub> under high Hg exposure at 17 °C only (Fig. 5). RE<sub>t</sub> was also overall higher at 24 than at 17 °C, initiating earlier at 24 (week three) than at 17 °C (week five) and thereafter increasing at both temperatures (Fig. 4). Population density was an additional important regulator of RE<sub>t</sub>, as RE<sub>t</sub> decreased with increasing biomass growth rate during the week resting egg production was quantified. This negative direct effect of biomass growth rate on RE<sub>t</sub> did not depend on temperature or Hg treatment but was strongly dependent on biomass growth rate in the week before resting egg production was quantified (Table A1, Fig. 6). Specifically, the negative direct effect of biomass growth rate on RE<sub>t</sub> became more pronounced as lagged biomass growth rate increased (Table A1, Fig. 6). Hence, a low biomass growth rate was associated with a high resting egg production if biomass growth rate was high during the preceding week (Fig. 6).

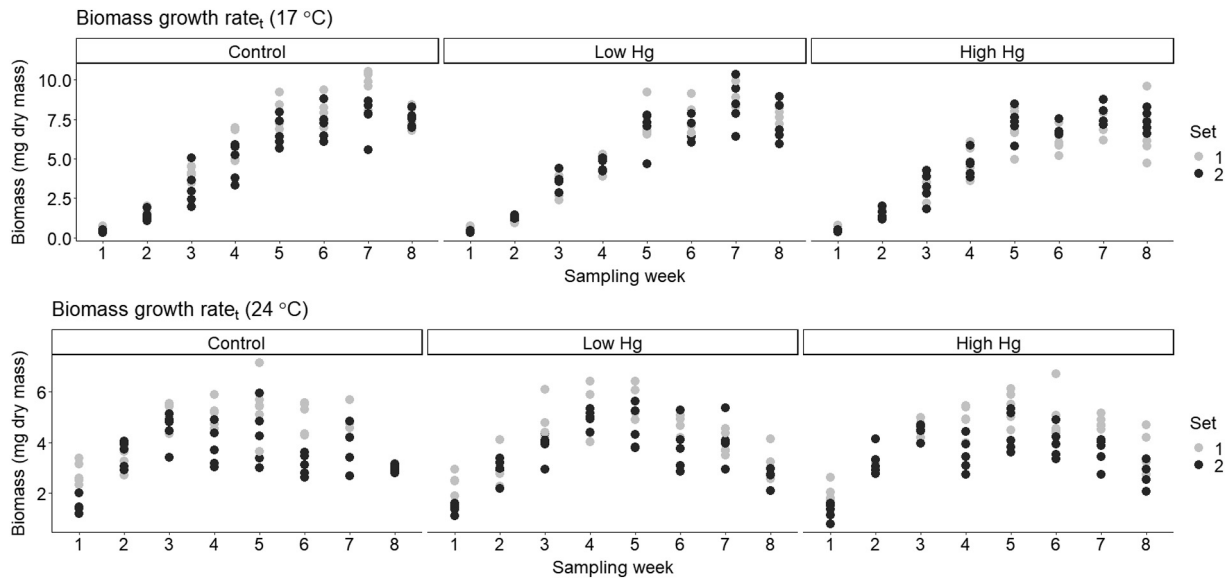


Fig. 2. Biomass growth rate<sub>t</sub> at 17 °C and 24 °C across Hg treatments (control versus low Hg and high Hg) and sets (1 versus 2). Scatter points represent measured data.

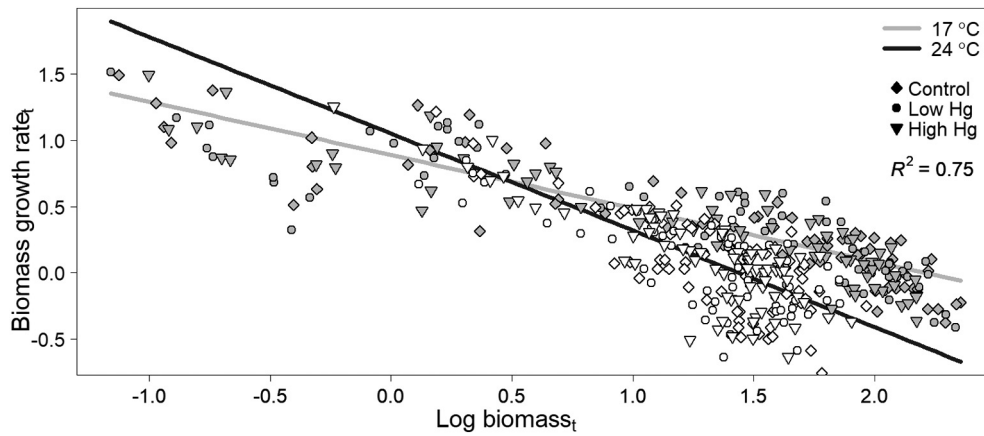


Fig. 3. Modelled effect of log biomass<sub>t</sub> (mg) on biomass growth rate<sub>t</sub> at 17 °C and 24 °C in the control, low Hg and high Hg treatments. Gray and white scatter points represent measured data at 17 °C and 24 °C, respectively. Efron's  $R^2$ , equal to the squared correlation between the predicted values and observed values, was 0.75.

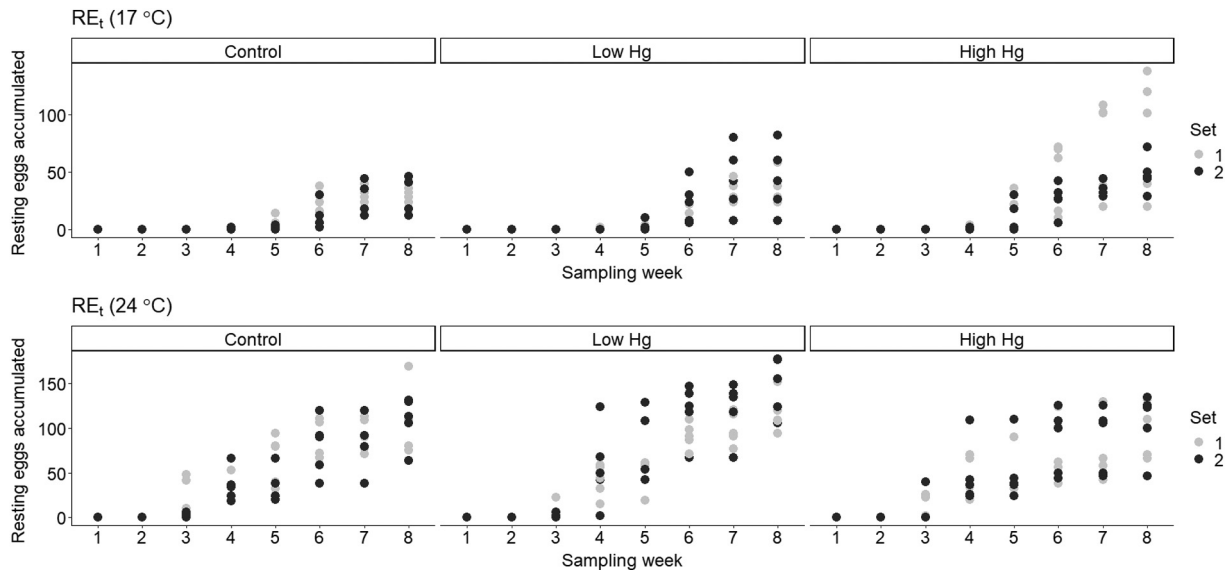
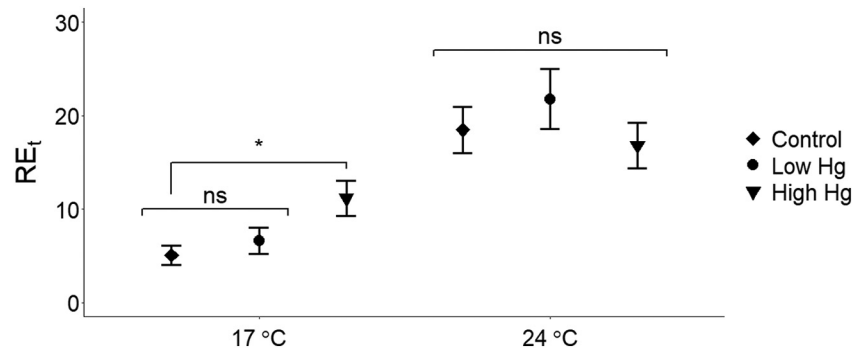


Fig. 4. Number of resting eggs produced per replicate beaker per week (RE<sub>t</sub>) at 17 °C and 24 °C across Hg treatments (control versus low Hg and high Hg) and sets (1 versus 2). Scatter points represent measured data.





**Fig. 5.** Effect of temperature on number of resting eggs produced per replicate beaker per week ( $RE_t$ ) in the control, low Hg and high Hg treatments. Scatter points represent measured data (error bars give 1 SE). A significant difference was observed between the high Hg and the control treatment at 17 °C only (\* $P < .05$ ; ns: not significant).

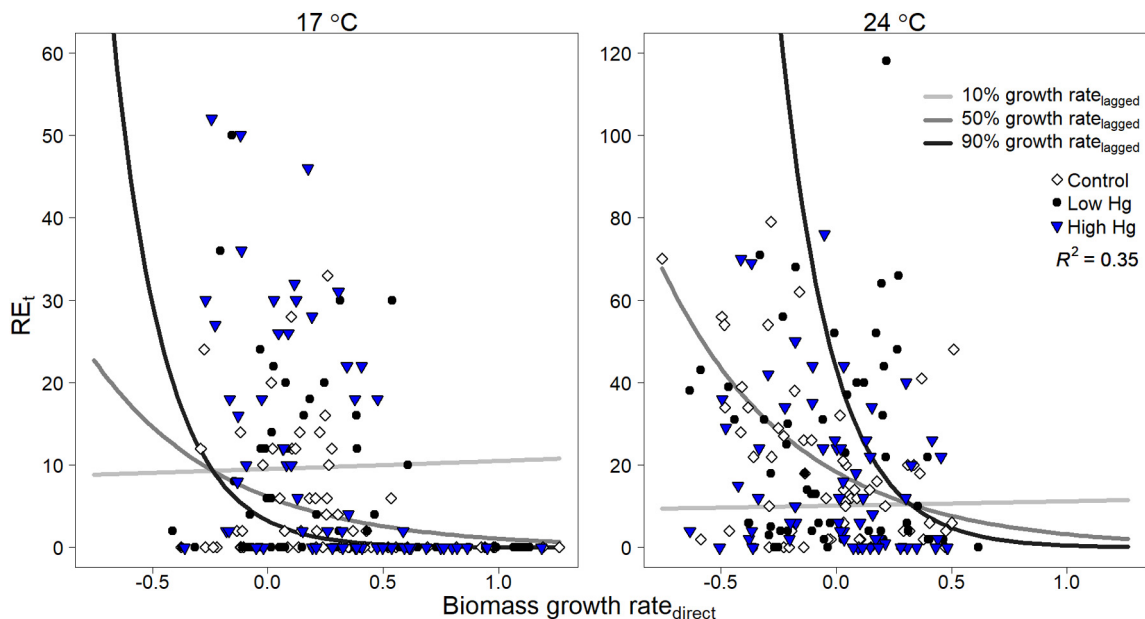
#### 4. Discussion

In this study, we examined the interactive effects of Hg, population density and temperature on the population dynamics and resting egg production of *Daphnia magna*. Biomass growth rate did not vary across Hg treatments and depended mainly on temperature and population density. This was however not the case for resting egg production, which responded differently to Hg exposure compared to control.

The missing response of biomass growth rate to Hg exposure was surprising, given that Hg stress can influence biomass growth through changes in body size and population size. Indeed, animals under metal stress direct more energy towards detoxification and recovery at the expense of other mechanisms such as feeding, somatic growth and reproduction (Muysen et al., 2006), which can directly decrease population growth rate through lower fecundity and survival rates (Fong et al., 2019; Muysen et al., 2006). Furthermore, studies show that metal stress can have contrasting effects on mean population body size through different modes of action that depend on the metal and its concentration. On one hand, metal stress can decrease the amount of resources available to *Daphnia* directly, by decreasing their filtration rate (Lopes et al., 2014), and indirectly, by impairing their swimming ability through oxidative stress (Bownik, 2017). A reduction in food uptake can subsequently result in a smaller mean body size for the population

(Enserink et al., 1995). On the other hand, metal stress may increase mean population body size as several studies show a negative relationship between body size and metal sensitivity (Alves et al., 2009; Bianchini et al., 2002; Vesela and Vijverberg, 2007), such that smaller individuals are eliminated through mortality. This is explained by smaller individuals having a higher mass-specific metabolic rate that enhances metal uptake (Yu and Wang, 2002). A possible explanation for the overall missing response of biomass growth rate to Hg treatment may be that exposure concentrations were too low to exert a strong effect on body size or population size. Indeed, while the highest exposure concentration used was within the range of concentrations found to significantly lower asexual reproduction in *D. magna* (Biesinger et al., 1982), this was not the case for adult survival and growth (Biesinger et al., 1982; De Coen and Janssen, 1997).

Other than toxic metals, temperature rise and low resource availability are factors that are also known to stress populations. A rise in temperature generally accelerates metabolic rates and hence population growth rate (Savage et al., 2004). However, as the population grows larger, intraspecific competition increases, thereby decreasing resource availability (Swanson et al., 2003), until the population reaches its carrying capacity, i.e. the maximum population size sustainable by the environment over time (Best et al., 2007). Once carrying capacity is exceeded, the population growth rate drops (Best et al., 2007). The



**Fig. 6.** Modelled direct effect of biomass growth rate (Biomass growth rate<sub>direct</sub>) on number of resting eggs produced per replicate beaker per week ( $RE_t$ ) at 17 °C and 24 °C. To visualize interactions, cross-sections were taken at the 10th, 50th and 90th percentiles of lagged biomass growth rate (growth rate<sub>lagged</sub>). White, black and blue scatter points represent measured data for each of the control, low Hg and high Hg treatments, respectively. Efron's  $R^2$ , equal to the squared correlation between the predicted values and observed values, was 0.35.

stronger density dependence of biomass growth rate at 24 °C compared to at 17 °C caused a significant reduction in carrying capacity at high temperature. Carrying capacity should indeed decrease with increasing temperature because of higher metabolic rates accelerating feeding rates and resource depletion (Savage et al., 2004). Surprisingly though, Hg did not interact with population density and temperature to affect the population dynamics of *D. magna* in this study. Previous studies have shown that temperature rise and low resource availability can enhance the negative effects of metals on *Daphnia* somatic growth and asexual reproduction (Heugens et al., 2001; Heugens et al., 2006). A high metal uptake rate at high temperature, coupled with intraspecific competition and the metabolic costs of metal detoxification, is indeed expected to lower the amount of energy available for allocation to somatic growth and/or overall reproduction and ultimately population growth (Heugens et al., 2003; Luna-Andrade et al., 2002; Sokolova and Lannig, 2008). Additional information on how temperature affects the bioavailability of Hg to *Daphnia* in this study may help explain the observed trends in population growth. The Biotic ligand model (BLM) is one tool that is commonly used for predicting metal bioavailability and toxicity. However, the BLM so far does not account for effects of temperature on the binding ability of metals and competing cations to sites of toxic action in organisms (Mebane et al., 2020). Therefore, it cannot be used to predict the bioavailability of Hg, hence nor its toxicity, in this study. Nonetheless, we suspect that, as discussed above, the Hg exposure concentrations were too low to exert effects on growth, which may explain the observed trends. It may also be that following their long term exposure to low Hg concentrations, the individuals in this study had acclimated across generations to Hg stress (Tsui and Wang, 2005b). This may have been driven by an increase in the concentration of metallothionein-like proteins that lower the availability of metals to cellular receptors (Tsui and Wang, 2007).

Despite the potential acclimation of exposed individuals in this study, metal stress was still high enough to affect sexual reproduction and the production of resting eggs in *D. magna*. Specifically, we observed a rise in resting egg production under high Hg exposure at low temperature. A higher investment in sexual reproduction under metal exposure has been previously observed in rotifers, as a strategy to overcome unfavorable environmental conditions (Aránguiz-Acuña and Pérez-Portilla, 2017; Aránguiz-Acuña and Serra, 2016). This was not the case at high temperature, where thermal stress was a more important factor at inducing resting egg production than Hg exposure. Thus, depending on environmental conditions, some stressors may be more important than others for inducing resting egg production.

Population density and temperature are additional well-known environmental cues that can trigger resting egg production. Temperatures close to species' upper and lower thermal tolerance limits promote the production of resting eggs (Holm et al., 2018; Wojtal-Frankiewicz, 2012), explaining the observed rise in resting egg production at 24 °C. Similarly, lower resource availability and higher encounter rates between females at high population density induce the production of resting eggs (Alekseev and Lampert, 2001; Ban and Minoda, 1994; Carvalho and Hughes, 1983). Resting egg production peaked under high lagged biomass growth, indicative of deteriorated food conditions. The sharp decrease in resting egg production the following week showed a worsening of food conditions with further population growth. High resource limitation can negatively affect sexual reproduction, if the energy demand for reproduction is not satisfied, or if maintenance is prioritized over ephippia production (Dinh et al., 2018; Smith et al., 2009). Hence, high resource limitation, whether from elevated temperatures, high population density or a combination of these, should under Hg stress reduce energy allocation to overall reproduction, in order to satisfy the energetic requirements of detoxification and repair processes (Fernández-González et al., 2011; Sokolova and Lannig, 2008), such as metallothionein synthesis (Amiard et al., 2006). However, this was not observed in this study.

In summary, both biomass growth rate and resting egg production, the main parameters measured in the asexual and sexual phases of *D. magna*, respectively, responded to population density and temperature, whereas only resting egg production responded to Hg exposure. Specifically, the strength of density dependence on biomass growth rate increased with temperature, as high metabolic rates worsened food conditions for competing individuals, significantly reducing the carrying capacity. Density dependence of resting egg production was on the other hand independent of temperature and Hg. However, Hg exposure prompted a higher investment in resting egg production at low temperature, indicative of stressful environmental conditions at low but environmentally relevant concentrations. Hence, we conclude that depending on temperature and population density, rates of sexual reproduction in *D. magna* may respond to metal exposure at lower concentrations than those impacting population growth during the asexual phase.

### CRedit authorship contribution statement

**Semona Issa:** Conceptualization, Methodology, Formal analysis, Writing - original draft. **Ane Simonsen:** Methodology, Formal analysis, Investigation. **Veerle L.B. Jaspers:** Conceptualization, Methodology, Formal analysis, Resources, Writing - review & editing. **Sigurd Einum:** Methodology, Formal analysis, Writing - review & editing.

### Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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### Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scitotenv.2020.143625>.

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