



Norwegian University of  
Science and Technology

# Interactive Effects of Inorganic Mercury and Temperature on Production of Resting Eggs and Population Dynamics in *Daphnia magna*

**Ane Simonsen**

Environmental Toxicology and Chemistry

Submission date: June 2018

Supervisor: Veerle Jaspers, IBI

Co-supervisor: Sigurd Einum, IBI  
Semona Issa, IBI

Norwegian University of Science and Technology  
Department of Biology



## Acknowledgments

This master's thesis was written at the Department of Biology, Norwegian University of Science and Technology (NTNU), Trondheim 2018. All experimental work was conducted at NTNU from August 2017 to January 2018.

Firstly, I wish to express my genuine gratitude to my supervisors, Associate Professor Veerle Jaspers, Department of Biology, NTNU; Professor Sigurd Einum, Department of Biology, Centre for Biodiversity Dynamics, NTNU; and Ph.D. candidate Semona Issa, Department of Biology, Centre for Biodiversity Dynamics, NTNU. Veerle, I am grateful for all your help in preparing and executing the project, for writing assistance, and quick response to all my questions. Sigurd, thank you for invaluable help from beginning to end, for always having an open door, and welcoming me with a smile every time I knock. Semona, thank you for providing constructive guidance, for assisting me with laboratory work and statistics, and for your patience and encouragement. I am grateful for all the help and support throughout the entire process, and for being a part of this interesting interdisciplinary project.

Secondly, other people who deserve to be mentioned are the entire *Daphnia* group, particularly Hanna-Kaisa Lakka, Manon Jeannot, and Erlend Ignacio Fleck Fossen. Further, without the help of Grethe Stavik Eggen, I would have been lost in the laboratory. Thank you, Katarina Utne, for proofreading and, more importantly, endless laughs and good conversations the last five years.

Finally, special thanks to my family, fellow students, and friends for inspiring and encouraging me.

Trondheim, June 2018

Ane Simonsen



## Abstract

Freshwater ecosystems are particularly vulnerable to the effects of climate change. In addition to the predicted increase in global surface temperatures, expected changes in patterns of precipitation and wind can significantly alter the structure and functioning of these ecosystems. The predicted changes in climate variables can also influence the properties of toxic chemicals. Of particular concern is the reported enhanced toxicity of various metals, including mercury, at elevated temperatures. Considering that atmospheric mercury concentrations have increased threefold since preindustrial times, knowledge about the combined effects of these two stressors are important to properly manage ecosystem and human health in the future.

The present study investigated the interactive effects of elevated temperature and inorganic mercury toxicity using *Daphnia magna* as a model organism, a key species in freshwater ecosystems with widespread use in toxicity studies. *Daphnia* are facultative parthenogenetic, reproducing clonally during favorable conditions, but switching to sexual reproduction with resting egg production when conditions deteriorate. Although knowledge about effects of environmental stressors on both clonal and sexual reproduction seems essential to assess population effects, studies typically only assess the former mode. Thus, population growth experiments were run with a duration of nine weeks, allowing for the animals to grow past their carrying capacity and commence sexual reproduction. A factorial design was used, with two temperatures and three environmentally relevant concentrations (including control) of mercuric chloride (HgCl<sub>2</sub>). Populations were monitored at weekly intervals using video recordings and tracking software to record population size (number of individuals), total biomass, and the number of resting eggs produced.

A significantly lower population size, as well as maximum and final biomass, was observed at 24°C than at 17°C. An explanation for this might be a faster manifestation of resource scarcity related to increased metabolic costs at higher temperatures. As expected, limiting resources at 24°C promoted higher resting egg production compared to 17°C in the present study. Albeit displaying a less prominent effect than that of temperature, HgCl<sub>2</sub> treatment increased resting egg production by the daphnids and decreased maximum biomass. No clear conclusion on the interaction of the two parameters can be drawn based on the current study, although an indication of interactive effects was observed on the total number of resting eggs produced. The underlying causes for this observation are not obvious. More research is therefore needed to confirm the results.



## Sammendrag

Ferskvannssystem er spesielt sårbare for virkningene av klimaendringer. I tillegg til de antatte globale økningene i overflatetemperaturer, kan forventede endringer i nedbørs- og vindmønstre betraktelig endre disse økosystemenes struktur og funksjon. Videre kan det forventes at disse variablene kan ha innvirkning på egenskapene til giftige forbindelser. Spesielt bekymringsfullt er økningen i toksisitet som er blitt dokumentert for ulike metaller, inkludert kvikksølv, ved høyere temperaturer. Tatt i betraktning at konsentrasjonene av kvikksølv i atmosfæren er tredoblet siden førindustriell tid, er kunnskap om den kombinerte effekten av disse to stressorene viktig for kunne beskytte økosystem og menneskelig helse på best mulig måte i framtida.

Denne studien undersøkte de kombinerte effektene av temperaturøkning og uorganisk kvikksølvtoksisitet ved bruk av *Daphnia magna* som modellorganisme, en nøkkelart i ferskvannssystem med utstakt bruk i toksikologi. *Daphnia* er fakultativ partenogenetiske, med klonal reproduksjon under gunstige forhold, og seksuell reproduksjon med produksjon av hvileegg ved ugunstige forhold. Selv om kunnskap om effektene av miljøstressorer på både klonal og seksuell reproduksjon virker essensielt for å vurdere populasjonseffekter, fokuserer studier hovedsakelig på førstnevnte metode. Med dette i tankene, ble populasjonsveksteksperimenter med en varighet på ni uker gjennomført. Dette åpnet for at dyrene kunne vokse forbi bæreevne og igangsette seksuell reproduksjon. Et faktorielt design med to temperaturer og tre miljømessig relevante konsentrasjoner (inkludert kontroll) av kvikksølvklorid ( $\text{HgCl}_2$ ) ble brukt. Populasjonene ble overvåket på ukentlig bases ved bruk av videoopptak og tracking programvare for å måle populasjonsstørrelse (antall individ), total biomasse og antall hvileegg.

En signifikant lavere populasjonsstørrelse, samt maksimum og endelig biomasse, ble observert ved  $24^\circ\text{C}$  sammenliknet med  $17^\circ\text{C}$ . En forklaring på dette kan være raskere manifestasjon av ressursknapphet relatert til økte metabolske kostnader ved høyere temperaturer. Som forventet promoterte ressursbegrensning ved  $24^\circ\text{C}$  i denne studien høyere produksjon av hvileegg sammenliknet med  $17^\circ\text{C}$ . Selv om effekten var mindre fremtredende enn effekten av temperatur, medførte  $\text{HgCl}_2$ -behandlingen økt hvileeggproduksjon hos daphniene og reduksjon i maksimum biomasse. Basert på resultatene fra denne studien, kan ingen entydig konklusjon trekkes om interaksjonen mellom de to variablene, selv om en indikasjon på interaksjon ble observert på totalt antall hvileegg produsert. De bakenforliggende årsakene til denne observasjonen er ikke åpenbare. Mer forskning er derfor nødvendig for å bekrefte resultatene.





# Contents

Acknowledgments .....	iii
Abstract .....	v
Sammendrag .....	vii
Abbreviations .....	xii
1 Introduction .....	1
1.1 Climate change and global pollution.....	1
1.1.1 Impacts of climate change on freshwater systems .....	2
1.1.2 Climate change impacts on metal toxicity .....	3
1.2 Mercury toxicity.....	3
1.2.1 Chemical species of mercury .....	3
1.2.2 Sources of release.....	4
1.2.3 Environmental transport.....	5
1.2.4 Increasing concentrations in the Arctic .....	6
1.2.5 Effects of mercury poisoning .....	7
1.3 <i>Daphnia magna</i> .....	7
1.3.1 Lifecycle of <i>D. magna</i> .....	8
1.3.2 Ehippia production .....	9
1.3.3 <i>Daphnia</i> in a warming climate .....	10
1.3.4 Mercury toxicity in <i>D. magna</i> .....	11
1.4 Aims .....	12
2 Materials and methods .....	13
2.1 Experimental preparations.....	13
2.1.1 Laboratory cultures of <i>D. magna</i> .....	13
2.1.2 Culturing stock animals.....	13
2.1.3 Fungus infection.....	13

2.2 Pilot study.....	14
2.2.1 Experimental setup.....	14
2.2.2 Procedure.....	15
2.2.3 Results and conclusion .....	15
2.3 Experimental setup.....	15
2.4 Experimental procedure .....	17
2.4.1 Experimental preparations and startup.....	17
2.4.2 Preparation of HgCl <sub>2</sub> start and working solutions.....	17
2.4.3 Ehippia sampling and treatment renewal .....	18
2.5 Statistics .....	19
3 Results .....	20
3.1 <i>D. magna</i> population size and biomass.....	20
3.1.1 Population and adult count over time.....	20
3.1.2 Biomass over time.....	22
3.1.3 Final biomass.....	23
3.1.4 Maximum biomass .....	25
3.2 Resting egg production.....	27
3.2.1 Cumulative number of resting eggs .....	27
3.2.2 Total number of resting eggs.....	28
3.2.3 Resting eggs per adult .....	30
4 Discussion .....	32
4.1 <i>D. magna</i> population size and biomass .....	32
4.1.1 Population and adult count over time.....	32
4.1.2 <i>D. magna</i> population biomass .....	34
4.1.3 The effects of temperature on body size .....	36
4.1.4 Effects of temperature and toxicity stress on population size and biomass .....	37
4.2 Resting egg production.....	38

4.2.1 Cumulative and total number of resting eggs.....	38
4.2.2 Resting eggs per adult .....	40
4.2.3 Temperature-dependent metal toxicity in aquatic ectotherms .....	41
4.3 Protective effects of selenium against mercury toxicity .....	44
4.4 Experimental reflections .....	46
4.4.1 Methodology, study design, and sources of error .....	46
4.4.2 Resting egg production as an important experimental endpoint .....	47
4.5 Future prospects .....	48
4.6 Conclusions .....	49
5 References .....	50
Appendix A .....	57
Appendix B .....	59
Appendix C .....	61

## Abbreviations

$\Delta\text{AICc}$	Difference from lowest score of AICc
ADaM	Aachener Daphnied Medium
AIC	Akaike Information Criterion
AICc	Corrected Akaike Information Criterion
AICc wt	Corrected Akaike Information Criterion weight
BBB	Blood-brain barrier
BPB	Blood-placenta barrier
$\text{CaCl}_2 \times 2\text{H}_2\text{O}$	Calcium chloride dihydrate
Cd	Cadmium
$(\text{CH}_3\text{Hg})_2\text{Se}$	Bis(methylmercuric) selenide
$\text{CO}_2$	Carbon dioxide
Cu	Copper
Cum. wt	Cumulative AICc weight
GEM	Gaseous elemental mercury
GLS	Generalized least squares
Hg	Mercury
$\text{Hg}^0$	Elemental mercury
$\text{Hg}^{2+}$	Mercuric cation
$\text{HgCl}_2$	Mercuric chloride
$\text{HNO}_3$	Nitric acid
HgS	Cinnabar
HgSe	Mercuric selenide
IPCC	Intergovernmental Panel on Climate Change
IQR	Interquartile range
K	Number of parameters
LC50	Lethal concentration 50
LL	Log-likelihood
MeHg	Monomethyl mercury
ML	Maximum likelihood
$n$	Number of observations
NaCl	Sodium chloride
$\text{NaHCO}_3$	Sodium bicarbonate

NOEC	No observed effect concentration
NTNU	Norwegian University of Science and Technology
REML	Restricted maximum likelihood
ROS	Reactive oxygen species
Se	Selenium
SeO <sub>2</sub>	Selenium dioxide
SeO <sub>3</sub> <sup>2+</sup>	Selenite
SS	Start solution
Std. E	Standard error
Temp	Temperature
TSR	Temperature size rule
WS	Working solution



# 1 Introduction

## 1.1 Climate change and global pollution

Since the first report was published in 1990, The United Nations Intergovernmental Panel on Climate Change (IPCC) has published four comprehensive assessments concerning evidence, impacts, and mitigation of climate change (Noyes *et al.*, 2009, IPCC, 2013). Their observations of the climate system, based on direct measurements and remote sensing, unambiguously demonstrate global warming since the 1950s related to human emissions of greenhouse gases (IPCC, 2013). Water vapor makes the largest contribution to the retention of heat, followed by carbon dioxide (CO<sub>2</sub>), methane, and ozone (Walker, 2014). In addition to the projected average global increase in surface temperatures of 1.5°C for the end of the 21<sup>st</sup> century (IPCC, 2013), widespread snow and ice melt, rising global sea levels, and changes in precipitation patterns are expected under future climate change scenarios (Noyes *et al.*, 2009, IPCC, 2013).

Habitat changes, declining species abundance and diversity, and shifts in animals' and plants' range, phenology, and interaction with other species are some of the climate-driven changes reported (Helmuth *et al.*, 2002, Beaugrand, 2004, Kendall *et al.*, 2016). Moreover, because changes in patterns of temperature, precipitation, and wind regimes can affect transport, transformation, mobility, and persistence of environmental pollutants, climate change-related shifts in these processes can also impact human and animal exposure to toxic substances (Balbus *et al.*, 2013). For instance, erosion can increase pollutant concentrations by remobilizing toxic substances in soil and sediment (Whitehead *et al.*, 2009). Other parameters including pH, salinity, hypoxia, and UV-light irradiation can also be altered by global climate change (Schiedek *et al.*, 2007). Together with changes in temperature and precipitation, these parameters might affect toxicants' distribution and toxicity in aquatic ecosystems (Kim *et al.*, 2010). Of further concern is the reported increased toxicity of various toxic chemicals, e.g. heavy metals (Holmstrup *et al.*, 2010), under future climate change scenarios (Kim *et al.*, 2010). The combined effects of these stressors are important to elucidate in order to properly manage ecosystem and human health in the future.

### 1.1.1 Impacts of climate change on freshwater systems

Freshwater systems are particularly vulnerable to climate change because of existing additional anthropogenic stress through exploitation for goods and services (Woodward, 2009). Furthermore, the majority of aquatic species are ectotherms, meaning that their body temperature depends on the surrounding temperatures (Cairns, Heath and Parker, 1975). Changes in environmental temperatures might therefore result in considerable disruption of the animals' physiology (Walther *et al.*, 2002). Being relatively isolated and fragmented further increases the difficulty of organisms inhabiting these ecosystems to disperse and adapt (Woodward, Perkins and Brown, 2010). Climate change-related shifts in temperature, precipitation, and wind patterns can affect physical, chemical, and biological aspects of freshwater systems (Kernan, Battarbee and Moss, 2011). For instance, changes in hydrological regimes are projected to impact frequency and intensity of precipitation events (IPCC, 2013). In addition to causing habitat destruction, the accompanying higher frequency of flood and drought events can increase mobility and deposition of sediments, contaminants, and organic matter, lower oxygen concentrations, and affect nutrient load in freshwater systems (Kernan, Battarbee and Moss, 2011).

Water temperatures are in close equilibrium with air temperature; as air temperatures rise, so will water temperatures (Whitehead *et al.*, 2009). Abrupt temperature changes can be a direct stress factor for aquatic organisms if they are unable to adapt at the same pace (Heugens *et al.*, 2003). Studies have shown that warming can affect the distribution and abundance of species (Harte and Shaw, 1995, Parmesan *et al.*, 1999). Outdoor freshwater micro and mesocosm experiments demonstrated that warming altered the size composition of the community by increasing the dominance of smaller organisms (Petchey *et al.*, 1999, Yvon-Durocher *et al.*, 2011). Additionally, chemical reactions and biological processes, such as primary production and decomposition (Friberg *et al.*, 2009), tend to run faster at higher temperatures (Whitehead *et al.*, 2009). Temperature is also an environmental cue that regulates animal behavior (Walther *et al.*, 2002). As the climate warms, it is expected that phenological events such as migration, reproduction, and flowering will change (Kendall *et al.*, 2016). These and related changes in the trophic structure can lead to alterations in ecosystem function (Friberg *et al.*, 2009).



### **1.1.2 Climate change impacts on metal toxicity**

Changes in climate variables can influence persistence, mobility, and toxicity of toxic chemicals, including metals (Balbus *et al.*, 2013). In addition to concerns related to remobilization and release of legacy contaminants from soil and sediment during high precipitation events, of major importance for toxic metals, such as mercury (Hg), is the reported increased toxicity to various organisms at elevated temperatures (Rathore and Khangarot, 2002, Heugens *et al.*, 2003, Tsui and Wang, 2006). Metal toxicity is generally enhanced with rising temperatures because higher temperatures increase the rate of uptake via changes in ventilation or feeding due to increased metabolism and energy demand (Boening, 2000, Sokolova and Lannig, 2008). Additionally, cellular pathways for metal uptake in ectotherms are affected by temperature, for instance, active transport via ion pumps, facilitated diffusion via ion channels, or membrane permeability (Sokolova and Lannig, 2008). If the higher uptake rate is not balanced by equal elimination of the metals, net accumulation in the organism occurs, and hence increased toxicity (Heugens *et al.*, 2003).

Particularly worrying for mercury is the concern with which net methylation of inorganic forms will be amplified under future climate change scenarios. For instance, in the Arctic, the large quantities of mercury stored in permafrost, soils, sediments, and glaciers might be remobilized to nearby environments and exposed to methylating promoting conditions (Rydberg *et al.*, 2010, AMAP, 2011). Being that the methylated chemical form of mercury is considered highly toxic (Klaassen and Amdur, 1996), this is of great concern for the Arctic biota. These points, in addition to the reported increase in atmospheric mercury concentrations since preindustrial times (Mason, Fitzgerald and Morel, 1994), clearly illustrate the importance of considering climate change impacts on mercury toxicity.

## **1.2 Mercury toxicity**

### **1.2.1 Chemical species of mercury**

Mercury can exist in many chemical forms, including elemental Hg (Hg<sup>0</sup>), inorganic Hg (e.g. Hg<sup>2+</sup> – mercuric cation), and organic Hg (e.g. CH<sub>3</sub>Hg<sup>+</sup> – monomethyl mercury [MeHg]) (Bank, 2012). Except for cinnabar (HgS), the most prominent mercury ore, all these forms are considered toxic to humans and wildlife on an acute or chronic basis (European Commission, 2017). Hg<sup>0</sup>, as gaseous elemental mercury (GEM), is the dominant form of mercury in the atmosphere (Schroeder and Munthe, 1998). It has great potential for toxicity as it is readily absorbed in the lungs and can cross both the blood-brain barrier (BBB) and the blood-placenta

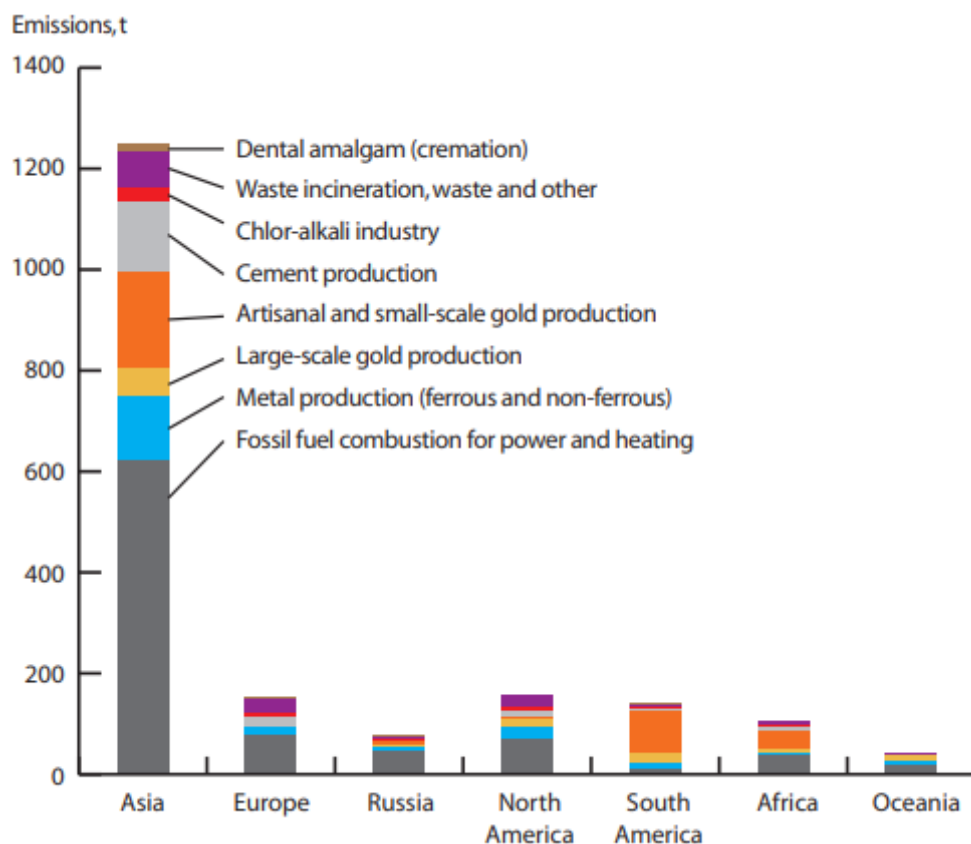
barrier (BPB) (Klaassen and Amdur, 1996). Different from  $\text{Hg}^0$ , divalent Hg, being more water-soluble, is more prevalent in water than in the atmosphere, particularly in complexes with other elements, e.g. as mercuric chloride ( $\text{HgCl}_2$ ) (Boening, 2000). Although it is poorly absorbed in the gastrointestinal tract (Klaassen and Amdur, 1996), it can exert toxic effects in the body because of high affinity for thiol groups (sulfhydryl groups) found in amino acids and proteins (Bank, 2012). This affinity has also been observed for other mercuric compounds (Aschner and Aschner, 1990). The kidneys are the major target for inorganic mercury toxicity (Klaassen and Amdur, 1996).

MeHg is an organometallic compound consisting of a methyl group bound to a Hg-ion (Bank, 2012). It is considered more toxic than the other forms of mercury because it is readily absorbed from ingestion, inhalation, and dermal contact, and lipophilic, as it can cross the BBB and the BPB, and can pass from mothers to children in breast milk (Klaassen and Amdur, 1996, EuropeanCommission, 2017). Another key point is its ability to bioaccumulate in organisms and biomagnify in the food chain, especially in aquatic ecosystems (Watras *et al.*, 1998). MeHg is the chemical form of Hg to which most humans are primarily exposed, although occupational exposure to  $\text{Hg}^0$  can be predominant for certain professions, for example, mine workers, dental workers, and workers in chloralkali industry (Aschner and Aschner, 1990, Schroeder and Munthe, 1998, EuropeanCommission, 2017).

### **1.2.2 Sources of release**

Mercury can be released to the environment from natural and anthropogenic sources (Bank, 2012). The sources of Hg to the atmosphere can further be classified as primary or secondary (Pacyna *et al.*, 2010, EuropeanCommission, 2017). Primary sources transfer mercury from long-lived reservoirs below the surface of the earth to the atmosphere. Examples include volcanic eruptions, geothermal vents, and fossil fuel combustion (Pirrone *et al.*, 2010, Bank, 2012). Upon its deposition to land and ocean, mercury can be re-emitted to the atmosphere (Schroeder and Munthe, 1998). These re-emission processes represent secondary sources, and do not, in contrast to primary sources, increase the global pool of mercury (European Commission, 2017). The major anthropogenic sources of Hg are burning of fossil fuels (primarily coal), small-scale artisanal gold-mining, metal manufacturing, and various industrial processes and products (Pacyna and Pacyna, 2002, Pacyna *et al.*, 2006) (Figure 1.1). Direct anthropogenic releases contribute to about one-third of the current emissions to the atmosphere (Mason and Sheu, 2002). Asia, particularly China, is the largest current global mercury polluter

(Pacyna *et al.*, 2006). Total global Hg emissions to the atmosphere were estimated in 2010 by Pirrone *et al.* (2010) to exceed 7500 megagrams year<sup>-1</sup>.



**Figure 1.1** Geographical trends in global mercury emissions to air from anthropogenic sources in 2005 (AMAP, 2011).

### 1.2.3 Environmental transport

Although atmospheric concentrations of Hg are relatively low, Hg can be labeled a global pollutant of concern because it is subject to long-range transport (Bank, 2012). Depending on the chemical form, Hg in the atmosphere can be deposited on different spatial scales (Schroeder and Munthe, 1998). Hg<sup>2+</sup> is generally short-lived and usually deposits locally with wet or dry deposition within one or two days after emission (Bank, 2012). The concern with emissions of Hg<sup>2+</sup> is therefore primarily on local and regional scales rather than global scale, for instance from point sources to locations of methylation. The atmospheric residence time of Hg<sup>0</sup> (GEM) can, however, exceed a year (Schroeder and Munthe, 1998), and it can therefore travel long distances in jet streams to remote locations far from its source, such as polar regions (Bank, 2012). This atmospheric transport of GEM is the most important mechanism for Hg dispersion at the earth's surface (Mason, Fitzgerald and Morel, 1994). It is also important to realize that more than half (53%) of the Hg emitted by human activity is released as GEM (Pacyna *et al.*,

2006). Atmospheric chemical reactions and meteorological processes can determine the rate of wet and dry deposition from the atmosphere to land and ocean (Bank, 2012).

#### **1.2.4 Increasing concentrations in the Arctic**

Lake sediment and glacial ice cores have been used as historical records of preindustrial deposition of various metals and pollutants (Bank, 2012). Sediment sampling and monitoring in the atmosphere indicate that the atmospheric concentrations and deposition rate of mercury has increased following the industrialization (Swain *et al.*, 1992, Mason, Fitzgerald and Morel, 1994). Mason, Fitzgerald and Morel (1994) estimated that current atmospheric Hg-concentrations are approximately three times higher than preindustrial levels. This is consistent with the estimated increase in annual deposition of Hg from 3.7 to 12.5  $\mu\text{g}$  per square meter since 1850 in Midcontinental North America reported by Swain *et al.* (1992). The increased levels are likely a consequence of anthropogenic activity, primarily mobilization and extraction of Hg from deep reservoirs in the ocean and on land through mining and coal combustion (Bank, 2012, EuropeanCommission, 2017).

Even in remote areas, atmospheric deposition of mercury has increased threefold since preindustrial times (European Commission, 2017). Long range atmospheric transport and subsequent deposition of Hg from lower latitudes to the Arctic poses environmental and human health risks in these areas, despite few local sources (AMAP, 2011, Bank, 2012). In the Arctic atmosphere,  $\text{Hg}^0$  is oxidized to chemical forms that readily deposits on the cryosphere (AMAP, 2011). When the snow melts, the mercury within it can be remobilized and converted to MeHg (European Commission, 2017). MeHg accumulation in Arctic seafood is concerning because of possible excessive exposure of indigenous communities for which this is a substantial protein source (Sonke, Heimbürger and Dommergue, 2013). A review by Dietz, Outridge and Hobson (2009) reported an increase in mercury concentrations in Arctic marine animals over the past 150 years. The highest biological concentrations in the marine environment are found within the upper trophic levels, particularly in polar bears, beluga whale, hooded seal, and some seabird species (AMAP, 2011, Dietz *et al.*, 2013). In several species concentrations exceed threshold values for biological effects, and there is a concern whether they may be approaching values that cause an impact on behavior and health (AMAP, 2011).

### 1.2.5 Effects of mercury poisoning

Hg poisoning can result from exposure to water-soluble forms (e.g.  $\text{Hg}^{2+}$ ), through inhalation (e.g. GEM), or by eating contaminated food (e.g. MeHg) (European Commission, 2017). Adverse effects have been documented in aquatic and terrestrial plants (Zhou, Wang and Yang, 2008), invertebrates (Tsui and Wang, 2006), birds (Scheuhammer, 1987), and mammals (Boening, 2000). It can affect multiple organ systems both during pre-natal and post-natal development, and in adulthood (European Commission, 2017). Exposure to mercury has a range of effects, which are dependent on the time and length of exposure, the developmental stage of the organism, the chemical form and concentration of Hg, and various external factors such as temperature, water salinity and hardness, pH, and dissolved organic matter (Boening, 2000, Ravichandran, 2004, Tan, Meiller and Mahaffey, 2009). Documented effects include damage to the kidneys (Buchet *et al.*, 1980), liver (Drevnick *et al.*, 2008), and lungs/gills (Asano *et al.*, 2000, de Oliveira Ribeiro *et al.*, 2002), oxidative stress (Aschner *et al.*, 2007), carcinogenicity (Schroeder and Munthe, 1998), reproductive impairment (Tan, Meiller and Mahaffey, 2009), and endocrine disruption (Tan, Meiller and Mahaffey, 2009).

The major target for Hg, especially MeHg, toxicity, is, however, the central nervous system (Bank, 2012). A range of associated responses have been reported, including changes in cognitive thinking, memory, attention, personality, mood, and language, ataxia (lack of motor coordination), paresthesia (numbness or tingling in limbs and extremities), visual deficits, hallucinations, and death (Klaassen and Amdur, 1996, European Commission, 2017). It has also been observed to cause a series of developmental abnormalities in children exposed *in utero*, for instance reduced IQ, defects in attention, memory, language, and motor function (European Commission, 2017).  $\text{Hg}^0$  has also produced neurotoxicity from long-term exposure, for example through occupational exposure for mine and smelting workers. Mechanisms of mercury neurotoxicity are not yet fully understood, but it has been suggested that the observed effects are related to mercury-induced changes in the redox state of nucleophilic groups, e.g. thiols, which might alter protein function and cause oxidative stress culminating in neurotoxicity (Aschner *et al.*, 2007, Farina, Aschner and Rocha, 2011, Farina *et al.*, 2013).

### 1.3 *Daphnia magna*

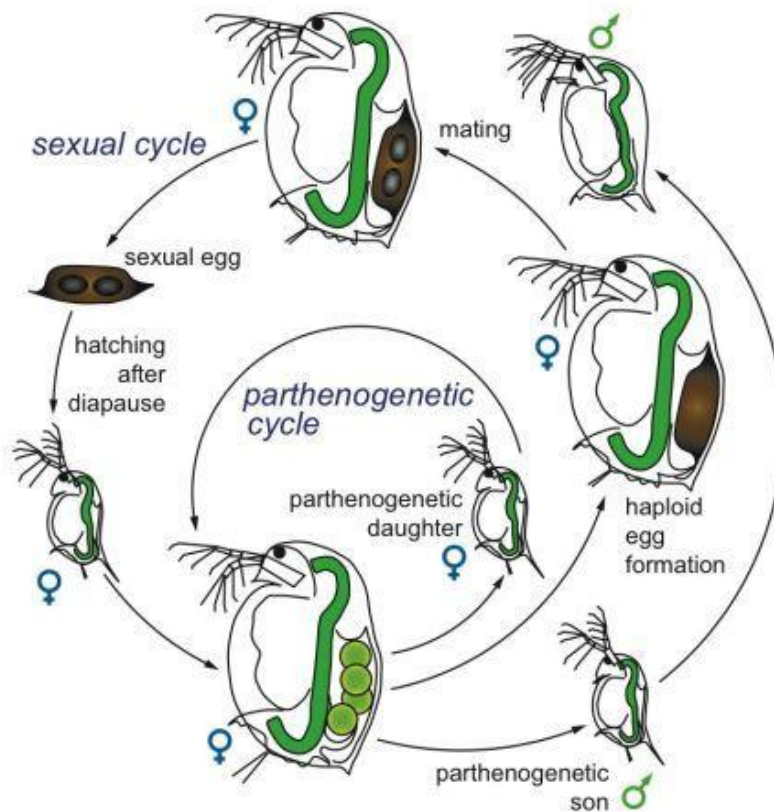
Crustaceans of the genus *Daphnia* are predominant zooplankton species in standing freshwater such as small lakes and ponds (Jonczyk and Gilron, 2005). This ubiquitous pelagic water flea is therefore an essential part of the food webs in these ecosystems and an important food source

for planktivorous fish. The genus *Daphnia* includes more than 100 known species, *D. magna* being one of the most well-known (Ebert, 2005). The body of *D. magna* is enclosed by an uncalcified shell, known as the carapace, and ranges from 0.5 to 6 mm in length. The animals have appendages used for swimming, as well as limbs on the trunk used for feeding and respiration (Ebert, 2005). *D. magna* are filter feeders, feeding primarily on suspended particles made up of planktonic algae and bacteria (Jonczyk and Gilron, 2005).

There are few other organisms whose ecology is as well-known and as extensively studied as that of *Daphnia* (Ebert, 2005). *Daphnia*, particularly *D. magna*, have been widely used as bioindicator species in both acute and chronic toxicity tests since the 1970s. Various assessment endpoints have been evaluated for toxic impact, including survival (Brković-Popović, 1990), immobility (Khangarot and Ray, 1987), reproduction (Biesinger, Anderson and Eaton, 1982), fecundity (i.e. young production per live adult female at test termination), and growth (e.g. dry weight or body length) (Biesinger and Christensen, 1972, Jonczyk and Gilron, 2005). Their broad distribution in a wide range of habitats, relatively short life cycle, large numbers of offspring, parthenogenic reproduction, sensitivity to toxic substances, and easy culturing and maintenance in the laboratory are rationale for the widespread experimental use (Bodar *et al.*, 1988, Khangarot and Das, 2009, Persoone *et al.*, 2009). Furthermore, *Daphnia*'s relatively large body size compared to other cladocerans makes them easy to monitor without the use of a microscope (Jonczyk and Gilron, 2005). Their widespread use has resulted in a large collection of toxicity data. This increases the confidence and reliability of use, and simultaneously creates comparable results and relative toxicity of various pollutants and chemicals (Jonczyk and Gilron, 2005).

### **1.3.1 Lifecycle of *D. magna***

In the laboratory, the lifespan of females can surpass two months, and she may produce a clutch of eggs every three to four days (Ebert, 2005). *D. magna* reproduce by cyclical (facultative) parthenogenesis, in which clonal and sexual reproduction alternates in time (Rouger *et al.*, 2016) (Figure 1.2). During asexual reproduction, the female produces a clutch of diploid eggs which develop and hatch into embryos in a brood chamber, before they are released after about three days (Ebert, 2005). In favorable environmental conditions, the offspring produced from clonal reproduction are usually female (Galimov, Walser and Haag, 2011). Clutch sizes vary depending on environmental conditions such as food availability and temperature (Jonczyk and Gilron, 2005). Clutch sizes exceeding 100 have been documented in *D. magna* (Ebert, 2005).



**Figure 1.2** Cyclic parthenogenetic life cycle of *D. magna*. Includes asexual (parthenogenetic) and sexual life cycles, in which the female produces diploid eggs that develop directly into females or males, or haploid eggs that require fertilization by males to produce resting eggs (sexual egg), respectively (Ebert, 2005).

### 1.3.2 Ehippia production

Depending on environmental conditions, clonal offspring of *D. magna* can develop into either males or females (Galimov, Walser and Haag, 2011). When conditions deteriorate, production of genetically identical males is initiated and the daphnids reproduce sexually (Doma, 1979, Galimov, Walser and Haag, 2011). During gametogenic reproduction, the males fertilize haploid eggs which develop into resting eggs in the female's brood chamber. The resting eggs are enclosed in a protective membranous shell called an ehippium, which is released during molting. Each ehippium usually contains two resting eggs (Ebert, 2005). Conditions promoting ehippia production in *D. magna* include high female population density (Carvalho and Hughes, 1983), low food supply (Doma, 1979, Carvalho and Hughes, 1983), predator cues (Pijanowska and Stolpe, 1996), temperature stress (Jonczyk and Gilron, 2005), and decreased photoperiod (Carvalho and Hughes, 1983). Because sexual reproduction is more energetically expensive under favorable environmental conditions and comes at the cost of ceased

parthenogenic reproduction, this strategy is only implemented under poorer environmental conditions (Korpelainen, 1986).

The resting eggs within the ephippia are able to survive under poor and stressful environmental conditions for long periods of time (Ebert, 2005). This ensures the long-term survival of the population in cases where all adult individuals die (Carvalho and Hughes, 1983). All daphnids hatched from the resting eggs develop into females (Ebert, 2005). As these females are the product of sexual reproduction, their genetic composition is different from their mother's (Hairston, 1996), and they may therefore respond differently to environmental stressors than the original population (Antunes, Castro and Gonçalves, 2003, Van Doorslaer *et al.*, 2009).

### **1.3.3 *Daphnia* in a warming climate**

*Daphnia* occur ubiquitously in environments experiencing great seasonal variability (Korpelainen, 1986). It can therefore be argued that the daphnids are good at adapting to a varying climate (e.g. through the production of dormant eggs) (Van Doorslaer *et al.*, 2009). In the future, however, these environments might experience increased seasonal variations and additional environmental stress related to climate change. Temperature is one of the factors expected to change (IPCC, 2013). As cladocerans are ectotherms, shifts in temperature may additionally influence the daphnids' ability to adapt and thus impact their behavior and distribution (Korpelainen, 1986).

Several studies have demonstrated significant temperature effects on *Daphnia* life history parameters such as reproduction, growth, and metabolism (Orcutt and Porter, 1983, Korpelainen, 1986, Paul *et al.*, 2004). For instance, reproductive parameters such as decreased mean generation time and age at first reproduction, declined fecundity, and a reduced proportion of male offspring, have been observed at higher temperatures (Orcutt and Porter, 1983, Korpelainen, 1986). As described above, temperature is also important for determining periods of sexual reproduction in *Daphnia*, as temperatures outside the optimum range to which the animals are adapted, have been associated with ephippia production (Jonczyk and Gilron, 2005). Temperature also affects somatic growth, with higher growth rates observed at higher temperatures (Dawidowicz and Loose, 1992).



#### 1.3.4 Mercury toxicity in *D. magna*

*D. magna* have been widely used as an experimental organism in both acute and chronic mercury toxicity tests (Jonczyk and Gilron, 2005). Compared to other taxa, *D. magna* have a relatively large body size, which enables them to carry higher body burdens of organic and inorganic Hg (Watras *et al.*, 1998). Furthermore, they are sensitive to metal toxicity and can therefore provide a conservative estimate of risk posed by the toxicant in aquatic systems (Fargasova, 1994, Mark and Solbé, 1998). In fact, in a study by Rodrigues *et al.* (2013), *D. magna* proved to be the most sensitive aquatic organism to mercury toxicity compared to multiple other taxonomic groups. Lastly, because of their intermediate placement in the food chain, they act as an important link between lower and higher trophic levels (Tsui and Wang, 2007). This renders them pivotal in the trophic transfer of toxic metals, e.g. MeHg, to higher trophic level organisms, such as fish, and makes them good predictors of Hg content higher in the food chain (Khangarot and Ray, 1987, Chen *et al.*, 2000, Tsui and Wang, 2004b).

Mercury, in both organic and inorganic form, has been regarded as the most toxic metal in several *Daphnia* toxicity tests (Khangarot and Ray, 1987, Fargasova, 1994, Okamoto, Yamamuro and Tatarazako, 2015). Multiple effects in various tissues and organ systems have been described. A selection of reported effects of sublethal mercury toxicity in *D. magna* include changes in carbohydrate metabolism (De Coen, Janssen and Segner, 2001), significantly decreased number of young produced per adult female (Biesinger, Anderson and Eaton, 1982), reduced growth (Biesinger and Christensen, 1972), induction of antioxidant enzyme activity, upregulation of antioxidant-related genes, and expression of stress response genes (Kim, Kim and Lee, 2017).

## 1.4 Aims

Ample evidence suggests a range of ecological responses to climate change impacts on the biosphere (Walther *et al.*, 2002). Vulnerable freshwater ecosystems are prone to adverse effects related to predicted alterations in environmental distribution, toxicity, and biological effects of pollutants (Ficke, Myrick and Hansen, 2007, Noyes *et al.*, 2009). In this context, the reported synergistic effect of elevated temperature and metal toxicity is particularly concerning because of the additional anticipated increased loading and remobilization of metals and similar chemical pollutants to aquatic ecosystems in the future (Heugens *et al.*, 2003, Kernan, Battarbee and Moss, 2011). The combined effects of these two stressors will in this study be assessed using *D. magna* as a model organism, a key species in the freshwater ecosystem.

It is generally accepted that occurrence of sexual reproduction in *D. magna* is controlled by environmental cues, such as temperature (Carvalho and Hughes, 1983, Korpelainen, 1986). No studies have, however, considered the potential consequences of climate change-related temperature increase and chronic mercury toxicity on the sexual reproduction of this ecologically significant species. As this topic is highly relevant in a rapidly changing environment, this study investigated the combined effect of these two stressors on the production of dormant eggs in *D. magna*, as well their population dynamics. To address these aims, the number of resting eggs produced, as well as the total number and biomass of individuals and adults, were registered during chronic exposure to different temperatures and mercury exposure concentrations. We hypothesized that environmentally relevant concentrations of HgCl<sub>2</sub> would increase the production of resting eggs in *D. magna* by creating unfavorable living conditions for the daphnids. Further, the toxicity of HgCl<sub>2</sub> would be intensified at higher temperatures as the parameters would interact synergistically, possibly negatively affecting resting egg production as conditions become too harsh for the normal reproductive cycle to be maintained.

## 2 Materials and methods

### 2.1 Experimental preparations

#### 2.1.1 Laboratory cultures of *D. magna*

Multiple clones of *D. magna* have been cultured in a laboratory at Norwegian University of Science and Technology (NTNU) for several years. Their original source is Sandtjønnna (67° 41' 12.8 " N, 12° 40' 19.2 " E), Værøy, Norway, where ephippia containing resting eggs were collected in November 2014. The stock culture clones are reared in multiple separate replicate jars (250 mL) at various temperatures. Regular maintenance of the laboratory cultures involves feeding three times a week and weekly medium renewal. The cultures' condition and development are closely monitored and registered.

Laboratory and experimental populations of *D. magna* are kept in a selenium dioxide (SeO<sub>2</sub>) altered Aachener Daphnien Medium (ADaM), an artificial freshwater medium for the culturing of zooplankton (Klüttgen *et al.*, 1994). The detailed recipe for the preparation of ADaM applied in the laboratory is given in Appendix A (Table A.1). The medium is prepared by laboratory technicians daily and stored at appropriate temperatures. All laboratory and experimental animals are fed three times a week with Shellfish Diet 1800<sup>®</sup> (Reed mariculture Inc.) prepared to reach a final concentration of  $2.4 \times 10^5$  algal cells mL<sup>-1</sup> medium.

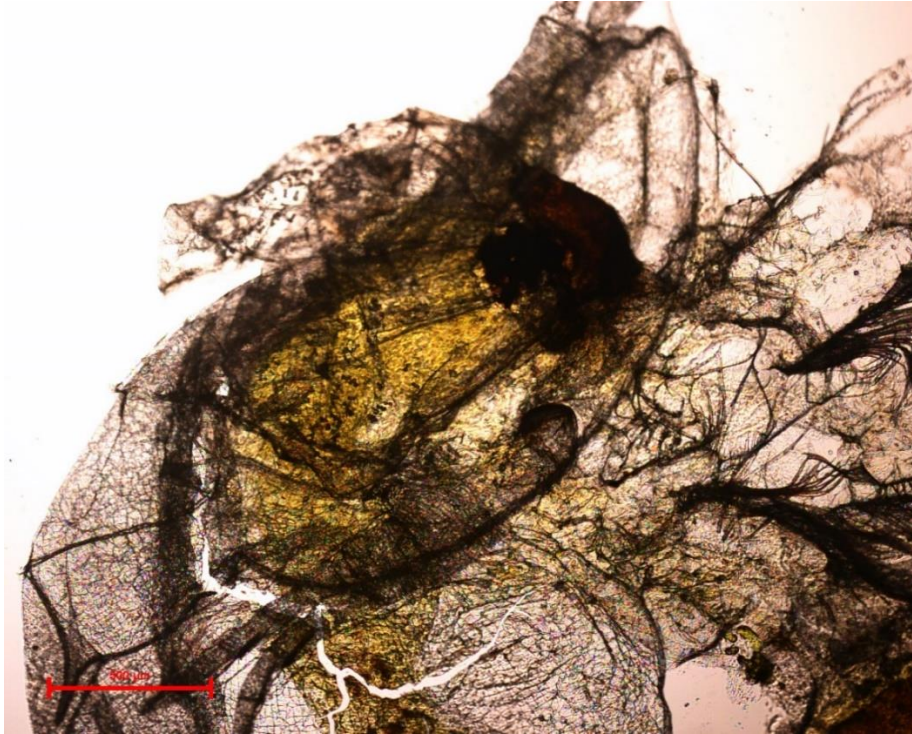
#### 2.1.2 Culturing stock animals

Experimental preparations were conducted at NTNU in Trondheim, Norway, during August-November 2017. Daphnids used for the experiments were collected from clone EF7 and cultured in 3 L aquaria kept at 17 and 24°C and a 16:8 L:D cycle for four months to produce the required number of acclimatized adult individuals. At each weekly medium renewal, animals were collected in individual sieves before the aquaria were filled with newly prepared tempered ADaM and ~200 mL of the old medium from the aquaria. The aquaria were changed biweekly to avoid buildup of biofilm.

#### 2.1.3 Fungus infection

One week before the scheduled start of the main experiment, fungi were discovered in all aquaria at 24°C and three aquaria at 17°C (Figure 2.1). Infected animals' reproduction, growth, and movement were impaired. All infected animals were discarded. The remaining aquaria at

17°C were subsequently used as the new starting population for 24°C. Medium renewal and feeding routine remained unchanged apart from only using newly prepared ADaM (2 L) in the aquaria.



**Figure 2.1** A black unknown fungus was detected in several aquaria prior to experimental startup. Infected animals nearly stopped reproducing and growing completely and were greatly immobilized by the fungus. All infected animals were discarded.

## 2.2 Pilot study

A pilot study was conducted from September 18<sup>th</sup> to October 2<sup>nd</sup>, 2017, the purpose of which was to determine the appropriate non-lethal HgCl<sub>2</sub> concentrations to be used in the main experiment.

### 2.2.1 Experimental setup

The pilot study was carried out in a similar manner to the main experiment, except for shorter exposure period and fewer replicates per treatment. *D. magna* were exposed to two different concentrations (0.5 and 1.75 µg L<sup>-1</sup>) of HgCl<sub>2</sub> at two different temperatures (17 and 24°C) for two weeks (Appendix A, Figure A.1). The pilot study consisted of three replicates per treatment, each replicate kept in separate beakers (600 mL). The mortality in each beaker was registered visually each week. Ehippia production and population growth were not registered. As the

exposure period was only two weeks, the medium, the HgCl<sub>2</sub>-treatment, and the beakers were not renewed during the exposure period.

### 2.2.2 Procedure

Start and working solutions of HgCl<sub>2</sub> were prepared according to the procedure given in section 2.4.2. Correct volumes (μL) transferred from the HgCl<sub>2</sub> working solution to the beakers were calculated from equation 2.1, where C<sub>2</sub> is the experimental HgCl<sub>2</sub> concentration, V<sub>2</sub> is the volume of ADaM in the beakers (400 mL), and C<sub>1</sub> is the concentration of the HgCl<sub>2</sub> working solution. After adding HgCl<sub>2</sub>, ADaM, and ten adult *D. magna* to each beaker, the beakers were covered with transparent plastic lids, and placed in climate cabinets. Mortality in the beakers was registered visually by weekly monitoring the animals' condition and development.

$$V_1 = \frac{c_2 V_2}{c_1} \quad [2.1]$$

### 2.2.3 Results and conclusion

The results from the pilot showed no effect of the treatment on the mortality of the daphnids. The animals reproduced during the entire exposure period. As the pilot study was carried out only a few weeks prior to the fungus infection was detected in the reared animals, it is possible that the infection was present also during the pilot experiment. However, infected animals tend to stop reproducing and die prematurely and this was not observed during the pilot experiment. Based on these results, the concentrations used in the main experiment were decided at 0.5 and 2.0 μg L<sup>-1</sup>.

## 2.3 Experimental setup

The main experiment was performed at NTNU, Trondheim, Norway, from November 2017 to January 2018. The experiment's six treatments, including two controls, varied in temperature (17 and 24°C) (Appendix A, Table A.2) and HgCl<sub>2</sub> concentration (0.5 and 2.0 μg L<sup>-1</sup>) (Figure 2.2). During the nine-week exposure period, the replicate beakers were kept in temperature and light-regulated (16:8 L:D cycle) climate cabinets. Their respective location within the cabinets was random and switched weekly. The beakers were covered with transparent plastic lids while kept in the climate cabinets.

The medium and treatment were renewed weekly, and the animals were fed three times a week. At each medium change, the ephippia produced in each beaker were counted and collected. Only free ephippia, i.e. ephippia deposited by the females to the medium, was counted. Ephippia still attached to the female was not counted. Additionally, the population density and age structure (based on individual size) in each beaker was estimated by recording the beaker content with a video camera, and subsequently analyzing the recordings in *R* using the package *trackdem* (Bruijning *et al.*, 2018). Similarly, total weekly population biomass (mg dry mass) was calculated from video recording analysis by estimating a relationship (using a standard curve) between the animals' mean body size (dry mass) and their pixel size in the recordings (i.e. mean size) (Appendix A, Equation A.1 and A.2). The beakers were changed biweekly.

To ease the workload at each sampling, the experiment was divided into two sets run in parallel and sampled on different days. The two sets were randomly mixed on each shelf in the climate cabinets. Rotation among the shelves occurred weekly.

[HgCl <sub>2</sub> ]	Temperature		○ → Set 1 → Set 2
	17°C	24°C	
0.5	①②③④⑤ 6 7 8 9 10	①②③④⑤ 6 7 8 9 10	
2.0	⑪⑫⑬⑭⑮ 16 17 18 19 20	⑪⑫⑬⑭⑮ 16 17 18 19 20	
Control	⑴⑵⑶⑷⑸ 26 27 28 29 30	⑴⑵⑶⑷⑸ 26 27 28 29 30	

**Figure 2.2** Experimental setup for the main experiment. *D. magna* were exposed to two different concentrations of HgCl<sub>2</sub> (0.5 and 2.0 µg L<sup>-1</sup>) plus control, at two different temperatures (17 and 24°C) for nine weeks. Each treatment consisted of ten replicates, represented by individual numbers on the figure. The control consisted of ADaM. The experiment was divided into two sets run in parallel and sampled weekly on different days. Replicates in set 1 are illustrated with circles on the figure. The remaining replicates belong to set 2.

## 2.4 Experimental procedure

### 2.4.1 Experimental preparations and startup

All glass equipment used in the experiment was acid washed prior to use. The equipment was soaked in a diluted nitric acid (HNO<sub>3</sub>, 65%) solution (1:100 volume concentration) for approximately 24 hours, then rinsed three times with Milli-Q water (> 18 MΩ) (Milli-Q Plus, Millipore Corp.) and set to dry before use. Washed equipment was placed in heating cabinets (45°C) while drying and subsequently wrapped in plastic foil.

48 hours before experimental startup, all juveniles in each aquarium were removed and discarded. This ensured that, at experimental startup, all juveniles present in the aquaria were approximately 48 hours old. At experimental startup, the sex of the juveniles was determined, and 75 females from each temperature were collected to be the experimental starting population for each set.

### 2.4.2 Preparation of HgCl<sub>2</sub> start and working solutions

The HgCl<sub>2</sub> solution was prepared in two successive steps; first preparing a start solution (SS) of a high varying concentration (< 0.4 g L<sup>-1</sup>), and subsequently preparing a working solution (WS) of a lower constant concentration (0.0016 g L<sup>-1</sup>). The SS and WS were prepared under a fume hood.

To create the SS, < 0.06 g HgCl<sub>2</sub> (Fluka, Switzerland) was added to a volumetric flask (100 mL). The accurate mass of the weighed HgCl<sub>2</sub> was used to calculate the mass of Hg<sup>2+</sup> and the concentration of the SS (equation 2.2 and 2.3, respectively). The volumetric flask was then filled with Milli-Q water (> 18 MΩ) (100 mL) and the solution was mixed well to make sure all HgCl<sub>2</sub> salt dissolved.

$$\text{mass Hg}^{2+} (g) = \frac{\text{weighed mass (g) of HgCl}_2 * \text{atomic mass Hg (u)}}{\text{molar mass HgCl}_2 (\frac{g}{mol})} \quad [2.2]$$

$$\text{concentration SS } \left(\frac{g}{L}\right) = \frac{\text{mass Hg (g)}}{\text{volume SS (mL)}} * 1000 \frac{mL}{L} \quad [2.3]$$

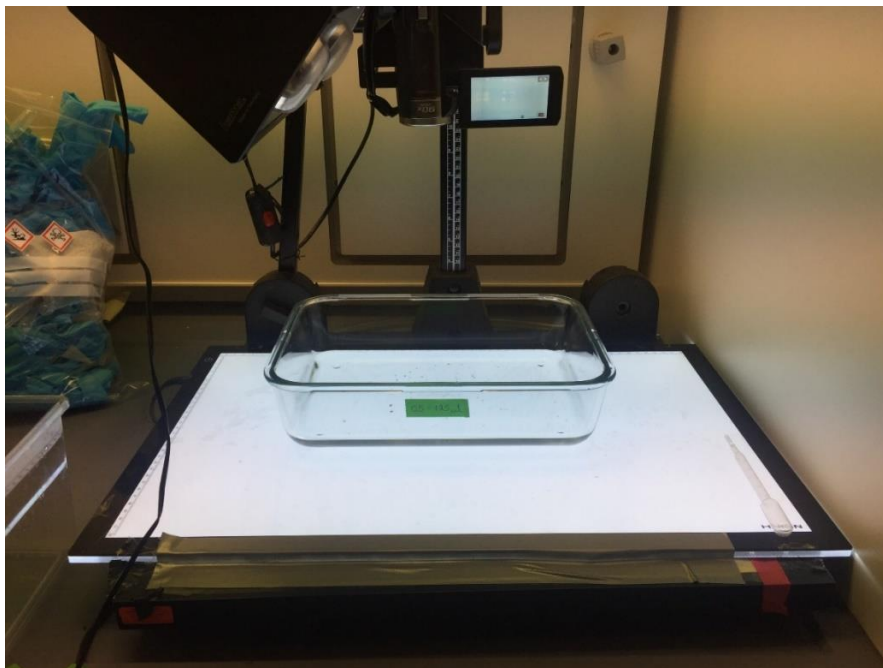
Next, the WS was prepared by transferring a specific amount from the SS into a second volumetric flask (100 mL) and subsequently filling it with Milli-Q water (> 18 MΩ). The correct volume transferred from the SS was calculated according to equation 2.1, where C<sub>2</sub> is the concentration of the WS, V<sub>2</sub> is the volume of the WS, and C<sub>1</sub> is the concentration of the SS (calculated from equation 2.3).

The volume transferred from the WS to the beakers was calculated according to equation 2.1 where  $C_2$  is the desired experimental treatment concentration in the beaker,  $V_2$  is the volume of medium in the beaker, and  $C_1$  is the WS concentration.

### 2.4.3 Ehippia sampling and treatment renewal

Ehippia sampling and data recording were carried out weekly at each treatment renewal. The sampling procedure was identical for both sets. All work was conducted in a fume hood.

The content of each beaker was poured into a deep glass tray placed on a light plate with an overhanging video camera (Figure 2.3). After counting and removing the ehippia, the content of the tray was recorded by the video camera for approximately 15 seconds. The ehippia were stored in ADaM at 4°C in SafeSeal microtubes (2 mL). For treatment renewal and medium change, the animals were collected in sieves while WS of HgCl<sub>2</sub> was added to the empty beakers. ADaM (400 mL) was subsequently used to transfer the animals back to the beakers.



**Figure 2.3** During ehippia sampling and data recording, the content of each beaker was poured into a deep glass tray placed on a light plate with an overhanging recording camera. The content was recorded by the camera (~15 seconds) and subsequently analyzed in *R* (*trackdem*) to estimate the population density and age structure of the population.



## 2.5 Statistics

All statistical analyses and graphical representation were performed in *R*, version 3.4.1 (R Development Core Team, 2017). The effect of temperature and HgCl<sub>2</sub> treatment on production of resting eggs, resting eggs per adult, biomass at experimental completion, and maximum biomass was tested with model selection using likelihood ratio tests based maximum likelihood (ML) and restricted maximum likelihood (REML) estimation, and Akaike Information Criterion (AIC). Following the principle of parsimony, reduced models were considered better, and thus carried forward, if dropping one term did not make the reduced model significantly different ( $p < 0.05$ ) from the full model and if the  $\Delta\text{AIC} < 2$ . Set was included as a fixed categorical explanatory variable in addition to temperature and HgCl<sub>2</sub> treatment to account for possible impacts on the results.

The total number of resting eggs produced over the entire exposure period (i.e. summed per sampling week), referred to as cumulative number of resting eggs, was used in the statistical analysis to study the effect of the treatments on total resting egg production by the daphnids. Moreover, the amount of resting eggs produced in each treatment throughout the experiment depends on the number of adult individuals present in the beakers as only adult individuals can produce resting eggs. To account for this when studying the effect of the treatments on resting egg production, and to investigate the relationship between the total number of resting eggs and adult quantity in each treatment, a new response variable was created by dividing cumulative resting eggs per week by cumulative adult count per week. The variable displayed the number of resting eggs produced per adult and was therefore a good indication of treatment effect on resting egg production independently of adult quantity.

The models were applied using generalized least squares (GLS) from the *nlme* package. Models were first compared with VarIndent variance structure with temperature, treatment, and set as variance covariates using *model.sel* from the *MuMIn* package and likelihood ratio tests based on REML estimation. The model selection criteria for these comparisons were corrected AIC (AICc), i.e. AIC adjusted for small sample sizes. The model with the lowest value of AICc was considered better. Next, carrying forward with the best variance covariate, backwards model selection based on procedure given by Zuur *et al.* (2009) was implemented using likelihood ratio tests based on ML. Starting with a full model including all main effects and possible interactions, terms were excluded sequentially until no further model simplification could significantly ( $p < 0.05$ ) improve the model. Assumptions of normality and homogeneity of residuals were tested graphically and satisfied for all chosen models.

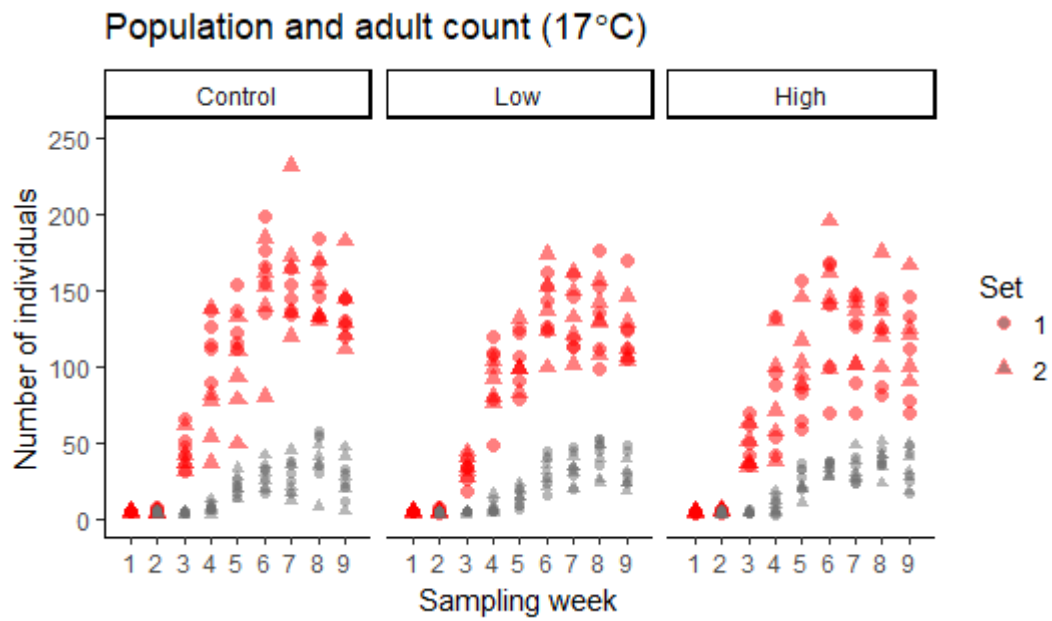
## 3 Results

### 3.1 *D. magna* population size and biomass

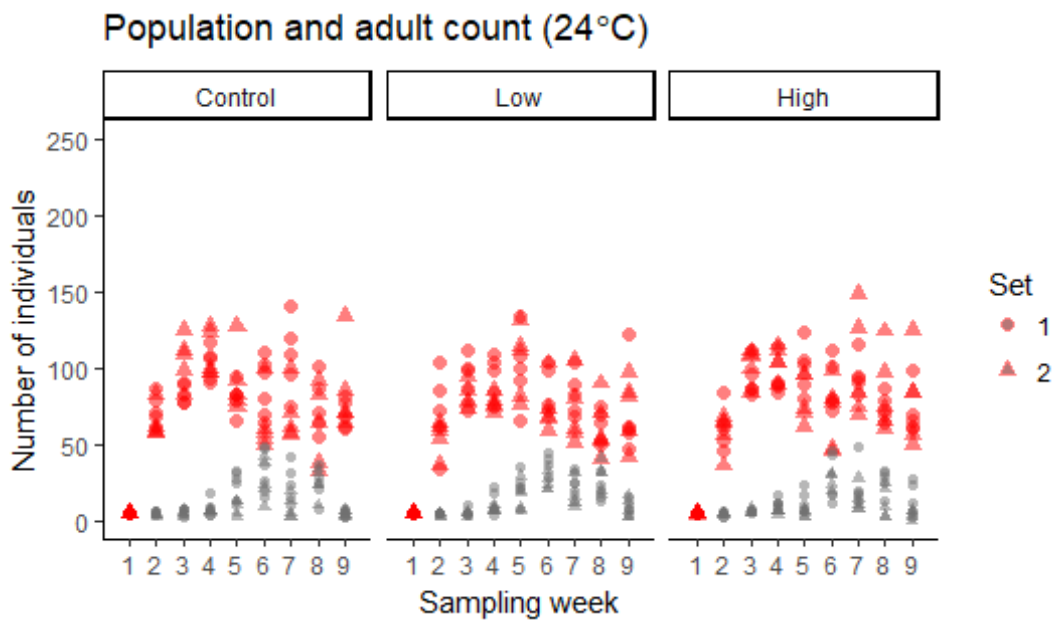
#### 3.1.1 Population and adult count over time

No observable difference in population development over time, i.e. per sampling week, could be found between the different HgCl<sub>2</sub> treatments or sets (Figure 3.1 and 3.2). Temperature, however, had an observable influence on population size and development over time. Differences in maximum population density, rate of population increase, and changes in population and adult count over time could be detected at the two temperatures.

Population count at 24°C increased rapidly, reaching its maximum density around sampling week two, and then declined gradually towards the end of the exposure period. A slight increase in population count was observed in the final sampling week. Adult count at 24°C increased more steadily than the population count, reached apex a few weeks after maximum population density, and declined thereafter towards baseline levels. At 17°C, the population count continued to increase for a longer period of time before reaching maximum population count around sampling week six. A decrease was subsequently observed in the final weeks of the experiment. Similarly to 24°C, adult count at 17°C reached a maximum a few weeks after maximum population count and decreased thereafter. Maximum population count was higher at 17°C compared to 24°C in all HgCl<sub>2</sub> treatments. At both temperatures, a sharp increase in the number of juveniles was followed by an increase in adults, before the population size finally decreased towards the end of the experiment.



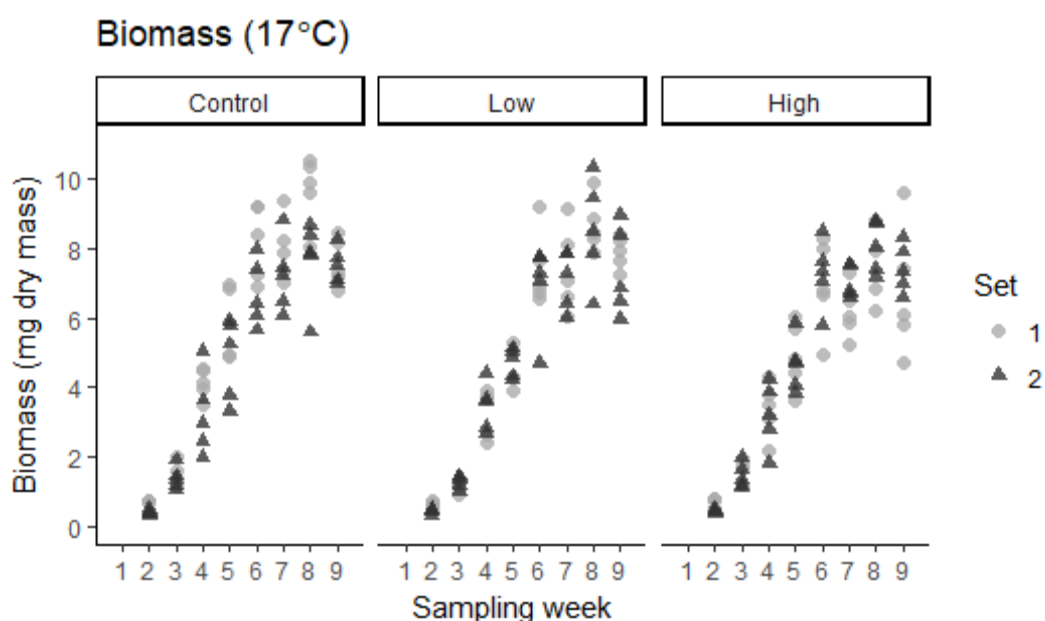
**Figure 3.1** Total population (red) and adult population (grey) size of *D. magna* at 17°C from both sets over a nine-week exposure period to control, low (0.5 µg L<sup>-1</sup>), and high (2.0 µg L<sup>-1</sup>) concentrations of HgCl<sub>2</sub>.



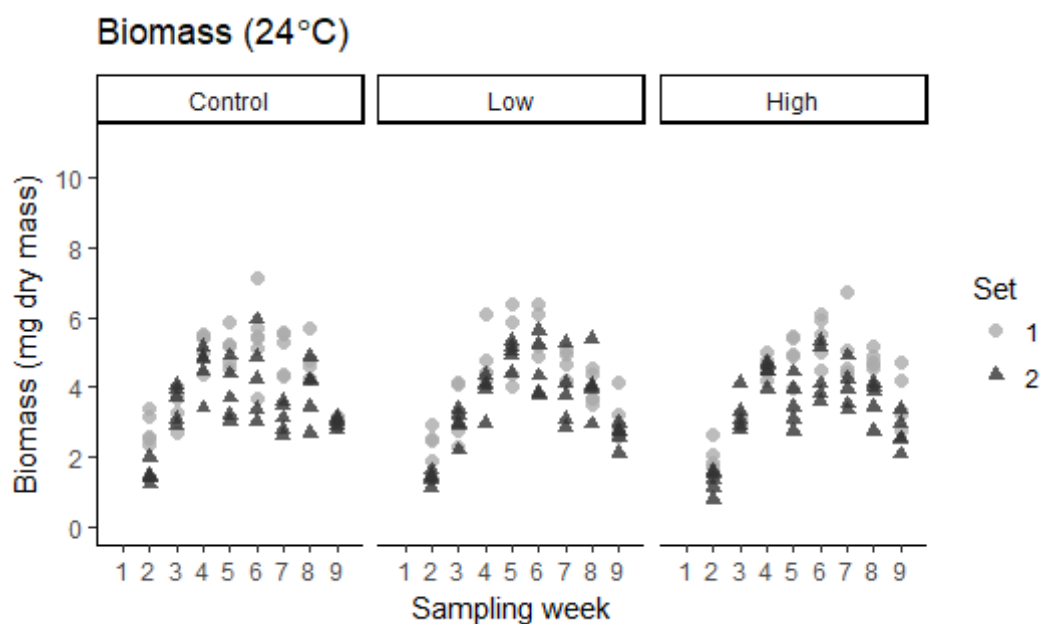
**Figure 3.2** Total population (red) and adult population (grey) size of *D. magna* at 24°C from both sets over a nine-week exposure period to control, low (0.5 µg L<sup>-1</sup>), and high (2.0 µg L<sup>-1</sup>) concentrations of HgCl<sub>2</sub>.

### 3.1.2 Biomass over time

Weekly population biomass (mg dry mass) showed observable differences between the two experimental temperatures (Figure 3.3. and 3.4). In the 17°C population, the biomass increased steadily until sampling week eight and decreased in the final week of the experiment. Maximum biomass obtained was ~10 mg dry mass at all HgCl<sub>2</sub> concentrations. Biomass increased rapidly at 24°C, reaching maximum biomass around week six and decreased immediately thereafter. The biomass continued to decrease throughout the exposure period. Maximum biomass obtained was ~7 mg dry mass in all treatments. There were no observable differences in biomass over time between HgCl<sub>2</sub> treatments at either temperature.



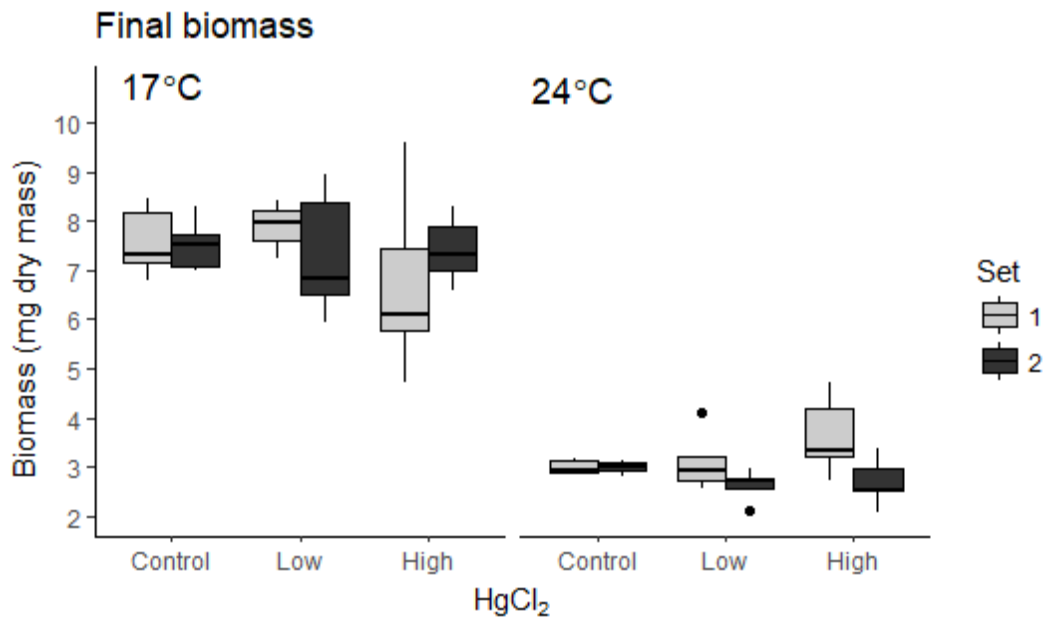
**Figure 3.3** Total biomass (mg dry mass) per week of *D. magna* from both sets at 17°C over a nine-week exposure period to control, low (0.5 µg L<sup>-1</sup>), and high (2.0 µg L<sup>-1</sup>) concentrations of HgCl<sub>2</sub>.



**Figure 3.4** Total biomass (mg dry mass) per week of *D. magna* from both sets at 24°C over a nine-week exposure period to control, low ( $0.5 \mu\text{g L}^{-1}$ ), and high ( $2.0 \mu\text{g L}^{-1}$ ) concentrations of  $\text{HgCl}_2$ .

### 3.1.3 Final biomass

*D. magna* biomass at the end of the experiment (i.e. sampling week nine in Figure 3.3 and 3.4) proved to be temperature dependent (Figure 3.5). Final biomass was best explained by the model including temperature and set as explanatory variables, with temperature as model variance covariate (Table 3.1). Thus, statistical analysis revealed no statistically significant effect of  $\text{HgCl}_2$  treatment or interaction between  $\text{HgCl}_2$  treatment and temperature on final biomass. There was a highly significant decrease in final biomass at 24°C compared to 17°C and in set 2 compared to set 1 (Appendix B, Table B.1).



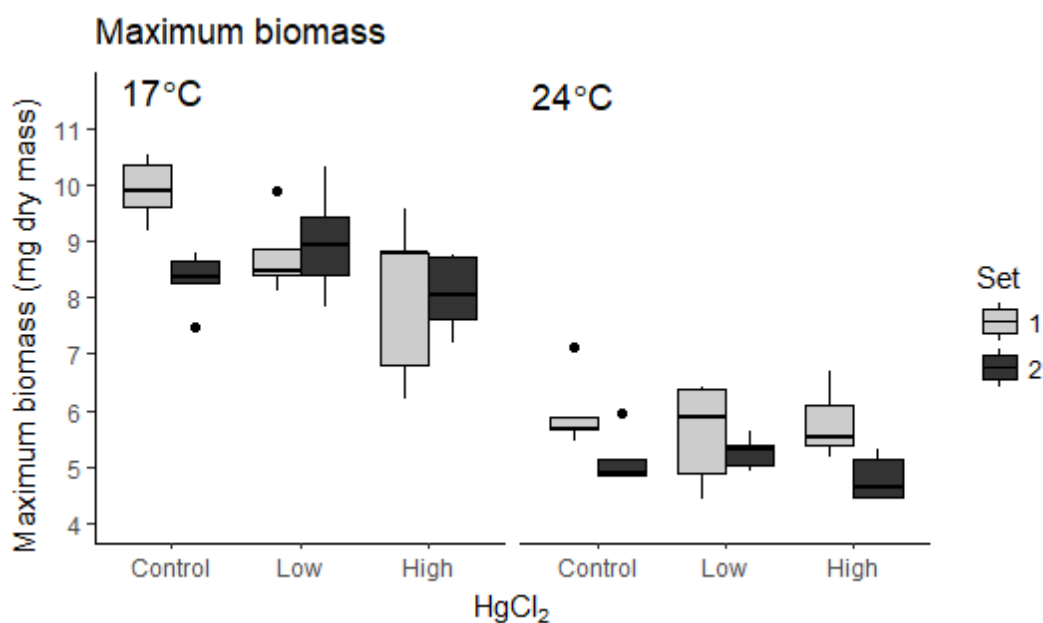
**Figure 3.5** Total *D. magna* population biomass obtained at experimental termination in set 1 and 2 following nine-week exposure to control, low ( $0.5 \mu\text{g L}^{-1}$ ), and high ( $2.0 \mu\text{g L}^{-1}$ ) concentrations of  $\text{HgCl}_2$  at 17 and 24°C ( $n = 60$ ). The middle, lower, and upper horizontal lines represent median, 25<sup>th</sup> and 75<sup>th</sup> percentile of the data, respectively. Whiskers extend up to  $1.5 \times$  the interquartile range (IQR) of the sample. The black dots are outlying values extending beyond  $1.5 \times$  IQR.

**Table 3.1** Model selection statistics for analysis of final biomass explained by explanatory variables temperature (temp; 17 and 24°C), HgCl<sub>2</sub> treatment (control, low [0.5 µg L<sup>-1</sup>], and high [2.0 µg L<sup>-1</sup>]), and set (1 and 2). Temperature was used as variance covariate. All models were fitted with maximum likelihood and tested with likelihood ratio tests. Models are presented in increasing order of the value of corrected Akaike Information Criterion (AICc). ΔAICc was calculated as the difference in AICc compared to best model (i.e. with lowest AICc). K: number of parameters, AICc wt: AICc weight, Cum. wt: cumulative AICc weight, LL: log likelihood. ‘×’ indicates interaction and main effect of variables, ‘+’ indicates main effects only.

Explanatory variables	K	AICc	ΔAICc	AICc wt	Cum. wt	LL
Temp + Set	5	139.55	0.00	0.66	0.66	- 64.22
Temp	4	142.48	2.94	0.15	0.81	- 66.88
HgCl <sub>2</sub> + Temp + Set	7	144.00	4.46	0.07	0.89	- 63.92
HgCl <sub>2</sub> + Temp × Set	8	145.12	5.57	0.04	0.93	- 63.15
Temp + HgCl <sub>2</sub> × Set	9	146.16	6.61	0.02	0.95	- 62.28
HgCl <sub>2</sub> + Temp	6	147.05	7.50	0.02	0.97	- 66.73
HgCl <sub>2</sub> × Set + Temp × Set	10	147.52	7.97	0.01	0.98	- 61.51
HgCl <sub>2</sub> × Temp + Temp × Set	10	147.64	8.10	0.01	0.99	- 61.58
HgCl <sub>2</sub> × Temp + HgCl <sub>2</sub> × Set	11	149.09	9.54	0.01	1.00	- 60.79
HgCl <sub>2</sub> × Temp + HgCl <sub>2</sub> × Set + Temp × Set	12	150.57	11.03	0.00	1.00	- 59.97
HgCl <sub>2</sub> × Temp × Set	14	152.98	13.43	0.00	1.00	- 57.82
Set	4	225.68	86.13	0.00	1.00	- 108.47
HgCl <sub>2</sub> + Set	6	228.71	89.16	0.00	1.00	- 107.56

### 3.1.4 Maximum biomass

Maximum biomass (mg dry mass) achieved for each replicate beaker during the experiment was calculated. A significantly higher maximum biomass was obtained at 17°C for all HgCl<sub>2</sub> treatments compared to 24°C (Figure 3.6). In addition to temperature, a significant effect of HgCl<sub>2</sub> treatment and set was also detected (Table 3.2). Within 17°C, a decrease in maximum biomass with increasing HgCl<sub>2</sub> treatment concentration was significant for both treatments compared to the control conditions (Appendix B, Table B.2). This trend was not observed at 24°C. At both temperatures, there was an observable and significant difference between the two sets. Except for low HgCl<sub>2</sub> treatment at 17°C, in which there was a significant increase in maximum biomass in set 2 compared to set 1, set 2 generally achieved lower maximum biomass compared to set 1 under the same experimental conditions.



**Figure 3.6** Maximum *D. magna* population biomass obtained in set 1 and 2 during nine-week exposure to control, low ( $0.5 \mu\text{g L}^{-1}$ ), and high ( $2.0 \mu\text{g L}^{-1}$ ) concentrations of  $\text{HgCl}_2$  at 17 and 24°C ( $n = 60$ ). The middle, lower, and upper horizontal lines represent median, 25<sup>th</sup> and 75<sup>th</sup> percentile of the data, respectively. Whiskers extend up to  $1.5 \times$  the interquartile range (IQR) of the sample. The black dots are outlying values extending beyond  $1.5 \times$  IQR.

**Table 3.2** Model selection statistics for analysis of maximum biomass explained by explanatory variables temperature (temp; 17 and 24°C),  $\text{HgCl}_2$  treatment (control, low [ $0.5 \mu\text{g L}^{-1}$ ], and high [ $2.0 \mu\text{g L}^{-1}$ ]), and set (1 and 2). No variance structure was included. All models were fitted with maximum likelihood and tested with likelihood ratio tests. Models are presented in increasing order of the value of corrected Akaike Information Criterion (AICc).  $\Delta\text{AICc}$  was calculated as the difference in AICc compared to best model (i.e. with lowest AICc). K: number of parameters, AICc wt: AICc weight, Cum. wt: cumulative AICc weight, LL: log likelihood. ‘×’ indicates interaction and main effect of variables, ‘+’ indicates main effects only.

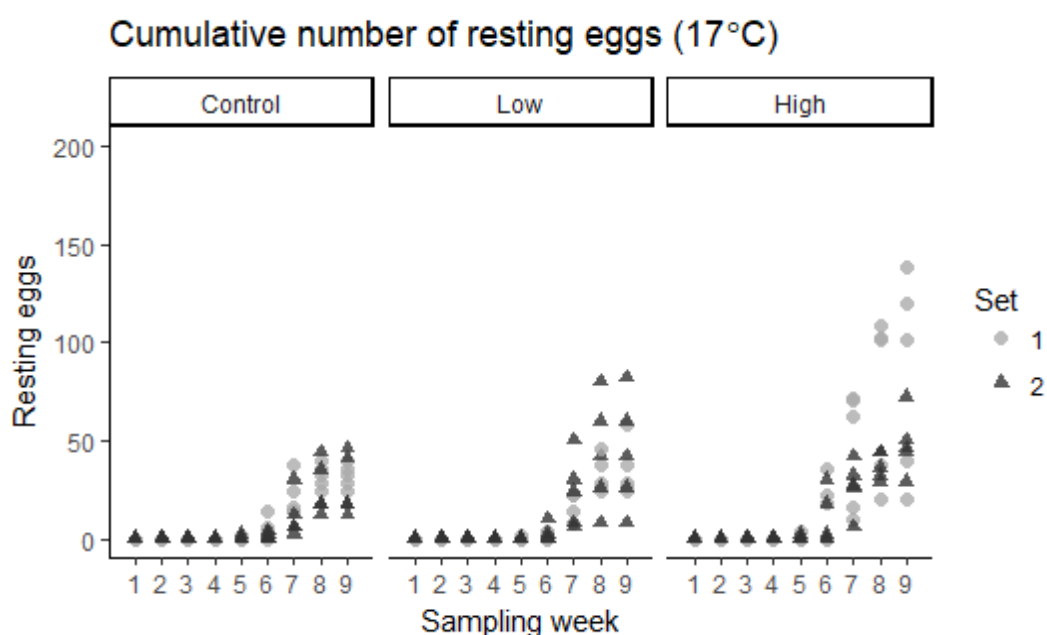
Explanatory variables	K	AICc	$\Delta\text{AICc}$	AICc wt	Cum. wt	LL
Temp + $\text{HgCl}_2 \times$ Set	8	149.85	0.00	0.36	0.36	- 65.51
$\text{HgCl}_2$ + Temp + Set	6	151.04	1.19	0.20	0.57	- 68.73
$\text{HgCl}_2 \times$ Temp + $\text{HgCl}_2 \times$ Set	10	151.70	1.85	0.14	0.71	- 63.60
$\text{HgCl}_2 \times$ Set + Temp $\times$ Set	9	152.07	2.22	0.12	0.83	- 65.23
Set + $\text{HgCl}_2 \times$ Temp	8	152.86	3.01	0.08	0.91	- 67.02
$\text{HgCl}_2 \times$ Temp + $\text{HgCl}_2 \times$ Set + Temp $\times$ Set	11	154.11	4.26	0.04	0.96	- 63.31
$\text{HgCl}_2 \times$ Temp + Temp $\times$ Set	9	155.10	5.25	0.03	0.98	- 66,75
$\text{HgCl}_2 \times$ Temp $\times$ Set	13	155.94	6.09	0.02	1.00	- 61.01
$\text{HgCl}_2 \times$ Set	7	255.91	106.06	0.00	100	- 119.88



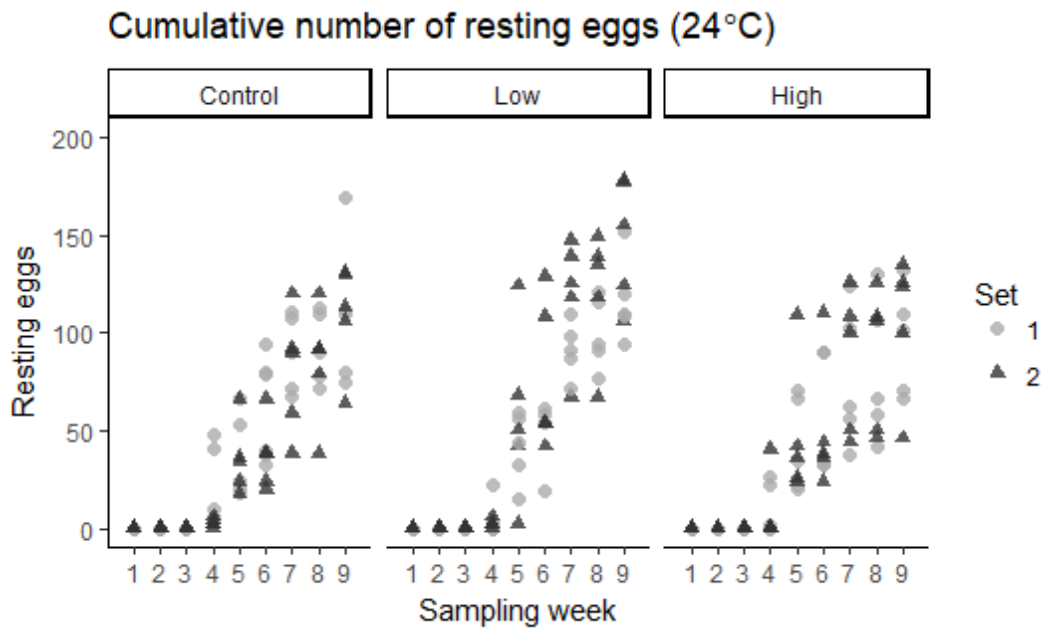
## 3.2 Resting egg production

### 3.2.1 Cumulative number of resting eggs

The number of resting eggs were summed for all nine sampling weeks to test for significant effects of the HgCl<sub>2</sub> and temperature treatments on the total number of resting eggs produced by the daphnids over the entire exposure period. Differences in total resting egg production over time was observed between the two experimental temperatures (Figure 3.7 and 3.8). Resting egg production was initiated earlier at 24°C (week four) compared to 17°C (week six). The total number of resting eggs produced was also higher at 24°C.



**Figure 3.7** Number of resting eggs (cumulative) from both sets produced at 17°C by *D. magna* over a nine-week exposure period to control, low (0.5 µg L<sup>-1</sup>), and high (2.0 µg L<sup>-1</sup>) concentrations of HgCl<sub>2</sub>.

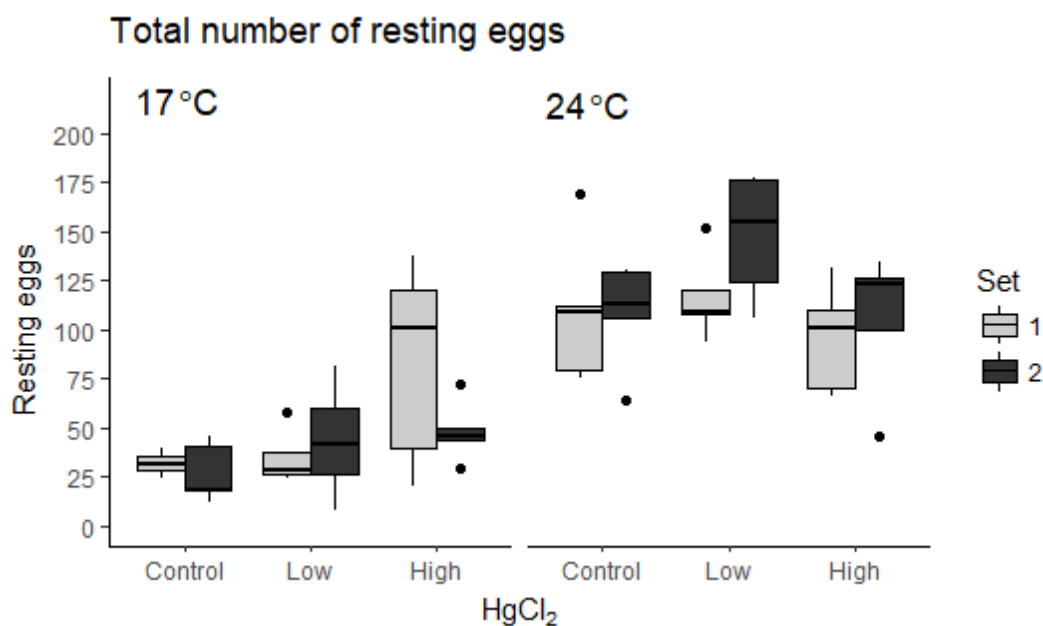


**Figure 3.8** Number of resting eggs (cumulative) from both sets produced at 24°C by *D. magna* over a nine-week exposure period to control, low (0.5 µg L<sup>-1</sup>), and high (2.0 µg L<sup>-1</sup>) concentrations of HgCl<sub>2</sub>.

### 3.2.2 Total number of resting eggs

The total number of resting eggs produced by *D. magna* throughout the experiment (equivalent to sampling week nine in Figure 3.7 and 3.8) are presented per temperature and HgCl<sub>2</sub> treatment (Figure 3.9).

The best model explained total number of resting eggs by temperature and HgCl<sub>2</sub> treatment (Table 3.3). The total number of resting eggs produced was significantly higher in the high HgCl<sub>2</sub> treatment at 17°C compared to the control treatment, and at 24°C compared to 17°C (Figure 3.9). A significantly lower total number of resting eggs was detected at 24°C in the high HgCl<sub>2</sub> treatment compared to control. No significant change in the total number of resting eggs produced was detected at either temperature in the low HgCl<sub>2</sub> treatment compared to the control treatment (Appendix B, Table B.3).



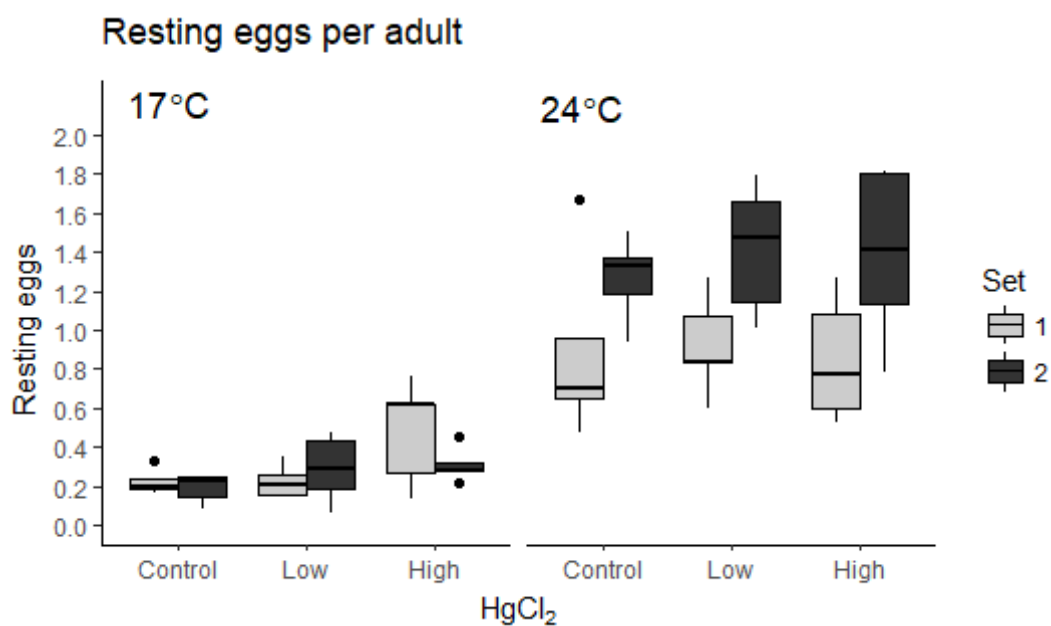
**Figure 3.9** Total number of resting eggs produced by *D. magna* at 17 and 24°C during nine-week exposure to control, low (0.5  $\mu\text{g L}^{-1}$ ), and high (2.0  $\mu\text{g L}^{-1}$ ) concentrations of  $\text{HgCl}_2$  ( $n = 60$ ). The middle, lower, and upper horizontal lines represent median, 25<sup>th</sup>, and 75<sup>th</sup> percentile of the data, respectively. Whiskers extend up to  $1.5 \times$  the interquartile range (IQR) of the sample. The black dots are outlying values extending beyond  $1.5 \times$  IQR.

**Table 3.3** Model selection statistics for analysis of total number of resting eggs explained by explanatory variables temperature (temp; 17 and 24°C),  $\text{HgCl}_2$  treatment (control, low [ $0.5 \mu\text{g L}^{-1}$ ], and high [ $2.0 \mu\text{g L}^{-1}$ ]), and set (1 and 2). No variance structure was included. All models were fitted with maximum likelihood and tested with likelihood ratio tests. Models are presented in increasing order of the value of corrected Akaike Information Criterion (AICc).  $\Delta\text{AICc}$  was calculated as the difference in AICc compared to best model (i.e. with lowest AICc). K: number of parameters, AICc wt: AICc weight, Cum. wt: cumulative AICc weight, LL: log likelihood. ‘ $\times$ ’ indicates interaction and main effect of variables, ‘+’ indicates main effects only.

Explanatory variables	K	AICc	$\Delta\text{AICc}$	AICc wt	Cum. wt	LL
$\text{HgCl}_2 \times \text{Temp}$	7	584.60	0.00	0.53	0.53	- 284.23
$\text{HgCl}_2 \times \text{Temp} + \text{Temp} \times \text{Set}$	9	586.99	2.39	0.16	0.69	- 282.70
$\text{Set} + \text{HgCl}_2 \times \text{Temp}$	8	587.22	2.62	0.14	0.83	- 284.20
$\text{HgCl}_2 \times \text{Temp} + \text{HgCl}_2 \times \text{Set} + \text{Temp} \times \text{Set}$	11	588.87	4.26	0.06	0.89	- 280.68
$\text{HgCl}_2 \times \text{Temp} + \text{HgCl}_2 \times \text{Set}$	10	589.07	4.46	0.06	0.95	- 282.29
$\text{HgCl}_2 + \text{Temp}$	5	590.62	6.02	0.03	0.98	- 289.76
$\text{HgCl}_2 + \text{Temp} + \text{Set}$	6	593.05	8.45	0.01	0.98	- 289.73
$\text{HgCl}_2 + \text{Temp} \times \text{Set}$	7	593.13	8.53	0.01	0.99	- 288.49
$\text{HgCl}_2 \times \text{Temp} \times \text{Set}$	13	593.70	9.10	0.01	1.00	- 279.89
$\text{HgCl}_2 \times \text{Set} + \text{Temp} \times \text{Set}$	9	595.28	10.68	0.00	1.00	- 286.84

### 3.2.3 Resting eggs per adult

Similarly to total resting eggs production, a highly significant increase in the number of resting eggs produced per adult was detected at 24°C compared to 17°C (Figure 3.10). The best model selected described resting eggs per adult as a function of HgCl<sub>2</sub> treatment, temperature, and set with temperature as model variance covariate (Table 3.4). Regression details are given in Appendix B (Table B.4). An observable increase in number of resting eggs per adult was apparent with increasing HgCl<sub>2</sub> treatment concentrations at both temperatures. A significantly higher resting egg production per adult was detected in set 2 compared to set 1 at 24°C. No significant effect of set was detected at 17°C.



**Figure 3.10** Total number of resting eggs produced per adult in set 1 and 2 by *D. magna* at 17 and 24°C during exposure to control, low (0.5 µg L<sup>-1</sup>), and high (2.0 µg L<sup>-1</sup>) concentrations of HgCl<sub>2</sub> ( $n = 60$ ). The middle, lower, and upper horizontal lines represent median, 25<sup>th</sup>, and 75<sup>th</sup> percentile of the data, respectively. Whiskers extend up to 1.5 × the interquartile range (IQR) of the sample. The black dots are outlying values extending beyond 1.5 × IQR.

**Table 3.4** Model selection statistics for analysis of resting eggs per adult explained by explanatory variables temperature (temp; 17 and 24°C), HgCl<sub>2</sub> treatment (control, low [0.5 µg L<sup>-1</sup>], and high [2.0 µg L<sup>-1</sup>]), and set (1 and 2). Temperature was used as variance covariate. All models were fitted with maximum likelihood and tested with likelihood ratio tests. Models are presented in increasing order of the value of corrected Akaike Information Criterion (AICc). ΔAICc was calculated as the difference in AICc compared to best model (i.e. with lowest AICc). K: number of parameters, AICc wt: AICc weight, Cum. wt: cumulative AICc weight, LL: log likelihood. ‘×’ indicates interaction and main effect of variables, ‘+’ indicates main effects only.

<b>Explanatory variables</b>	<b>K</b>	<b>AICc</b>	<b>ΔAICc</b>	<b>AICc wt</b>	<b>Cum. wt</b>	<b>LL</b>
HgCl <sub>2</sub> + Temp × Set	8	2.84	0.00	0.57	0.57	7.99
Temp × Set	6	5.14	2.29	0.18	0.75	4.22
HgCl <sub>2</sub> × Set + Temp × Set	10	5.49	2.64	0.15	0.90	9.50
HgCl <sub>2</sub> × Temp + Temp × Set	10	6.87	4.03	0.08	0.98	8.81
HgCl <sub>2</sub> × Temp + HgCl <sub>2</sub> × Set + Temp × Set	12	10.00	7.16	0.02	1.00	10.32
HgCl <sub>2</sub> + Temp + Set	7	13.62	10.78	0.00	1.00	1.27
HgCl <sub>2</sub> × Temp × Set	14	15.48	12.64	0.00	1.00	10.93
Temp + HgCl <sub>2</sub> × Set	9	15.94	13.10	0.00	1.00	2.83
HgCl <sub>2</sub> × Temp + HgCl <sub>2</sub> × Set	11	20.72	17.88	0.00	1.00	3.39

## 4 Discussion

The main aim of this study was to investigate the combined effects of temperature and chronic mercury toxicity using *D. magna* as a model organism. To address this aim, the number of resting eggs produced, as well as the total number and biomass of individuals and adults in the populations, were registered during chronic exposure to HgCl<sub>2</sub> at two concentrations plus control treatment under two temperature regimes. The results demonstrated a highly observable and significant temperature effect on all response variables, showing a significant decrease in biomass and increase in resting egg production at 24°C compared to 17°C. HgCl<sub>2</sub> treatment was found to significantly influence maximum biomass, the total number of resting eggs, and resting eggs per adult, albeit the effect was less prominent than that of temperature. Total resting egg production increased with increasing HgCl<sub>2</sub> treatment concentrations at 17°C and decreased in the high concentration exposure treatment at 24°C. The number of resting eggs per adult significantly increased with HgCl<sub>2</sub> concentration at both temperatures.

### 4.1 *D. magna* population size and biomass

#### 4.1.1 Population and adult count over time

It is well-known that temperature is a key factor in determining variations in *Daphnia* population dynamics and life history parameters (Schalau *et al.*, 2008). An experiment performed by Korpelainen (1986) found decreased lifespan and size at death in *D. magna* with increasing temperatures (from 14 to 19°C) for both sexes in multiple clones. The author further reported shorter mean generation time, an increased intrinsic rate of increase, and a lower proportion of male offspring at higher temperatures. Earlier maturation, time to first brood, broods at shorter intervals, decreased number of offspring per female, and decreased longevity is also expected at warmer temperatures (Schwartz, 1984, Bae *et al.*, 2016). Similarly, it has been reported that daphnids grown at a low temperature (10°C) mature later and reach a larger size at maturity, resulting in a higher number of offspring (Heugens *et al.*, 2006). Lastly, temperature is a major driver for interannual variability of *Daphnia* population dynamics in the field (Benndorf *et al.*, 2001, Schalau *et al.*, 2008).

In the current study, temperature had an observable influence on population and adult count over time. Populations at both temperatures experienced a sharp increase in the number of juveniles followed by an increase in adult quantity before the population sizes decreased at the end of the experiment. This indicates that the populations reached carrying capacity, i.e. the maximum population size sustainable by the environment over time (Rees, 1992). Carrying

capacity of a population can be determined by a number of limiting biotic and abiotic factors such as nutritional availability, light, space, available mates etc., depending on the given environment. The 24°C population reached carrying capacity faster than the population grown at 17°C. This is as expected considering that ectotherm animals grow and reproduce faster at higher temperatures, thereby attaining maximum density sustainable by the environment at an earlier stage (Weetman and Atkinson, 2004). Metabolic theory also states that organisms at warmer temperatures tend to have higher maximal population growth rate (Brown *et al.*, 2004). The value of maximum population density was observably higher at 17°C compared to 24°C. Reduced carrying capacity is expected at elevated temperatures because equal energy supply in these conditions must support animals with higher energy flux (i.e. higher energy demand) (Brown *et al.*, 2004). To balance this, a smaller number of individuals can be sustained. Other resources can also become less available at higher temperatures, promoting resource limitations at an earlier stage. Higher metabolic and reproductive rates accompany higher temperatures (Cairns, Heath and Parker, 1975), meaning that food and available space will become limiting factors more quickly at these temperatures (Heugens *et al.*, 2006). An earlier manifestation of food scarcity and density stress at the higher temperatures might thus restrict population growth.

The 24°C population curve was characterized by a rapid and steep increase followed by a gradual decrease until the population dropped close to its starting point (of increase). The findings are in accordance with previous literature on *Daphnia* population dynamics over time (Pratt, 1943), in which populations at a similar experimental temperature (25°C) exhibited oscillating responses in population count from baseline level to maximum density. The observed slight increase in population count in the final sampling week of the current experiment may suggest that the population was starting to grow again. This could be an indication of oscillating population response in the present study as well.

Pratt (1943) explained the high-amplitude oscillating population dynamic observed at higher temperatures by changes in birth and death rates related to the daphnids' previous crowding experiences. At the onset of a population increase, the population consists of a few individuals not previously exposed to a crowded environment. Their reproductive rate is therefore high. Coupled with low mortality, the population grows rapidly. At maximum density, the population consists of a few adults and many juveniles. The stressful effect of high population density will be exerted on the reproductive rate before it affects the death rate, resulting in a decrease in juvenile number relative to adult number, which continues to increase as the animals age. A slow decrease in total population size follows, in which the age structure of the population shifts from a majority of juveniles to mostly adults. This, in combination with a prolonged impact of

crowding stress, could help to explain why the population drops to near baseline before increasing, instead of quickly stabilizing around the mean of equal birth and death rates. Oscillating populations continuously overshoot and undershoot a theoretical equilibrium density likely because of a delay in the manifestation of density stress (Pratt, 1943).

The population curve at 17°C was characterized by a more gradual and long-lasting population increase, followed by a prominent peak, after which the population declined at about the same pace as its increase. Similarly to 24°C, this tendency of population development over time is in accordance with the results presented by Pratt (1943). His study reported population dynamics of a *D. magna* population at 18°C, in which a similar prominent peak was followed by a gradual decrease and virtual stabilization or continued oscillation of minor amplitude over time. This was not observed during the nine-week exposure period of the current study.

Temperature had an observable influence on population and adult count over time. Carrying capacity was accomplished at both temperatures, with an observable higher population count at 17°C than at 24°C. The population dynamics observed are in agreement with existing literature. No observable difference in population development over time was observed between the different HgCl<sub>2</sub> treatments at either temperature. There can be a number of reasons for this, such as antagonism with selenium (Se) or decreasing HgCl<sub>2</sub> concentrations in the medium over time, as further discussed below (sections 4.1.4, 4.2.3, 4.3, and 4.4.1).

#### **4.1.2 *D. magna* population biomass**

##### *Biomass over time*

Biomass over time displayed a similar response as population and adult count at the two experimental temperatures. At 17°C, the biomass increased steadily until sampling week eight and decreased in the last week of the experiment (excluding one sampling point from set 1 in sampling week nine at the high HgCl<sub>2</sub> treatment). Maximum biomass at 24°C was achieved around sampling week six, before and after which it increased and decreased gradually, displaying a bell-shaped response curve. The observed results are consistent with previous literature describing *Daphnia* population development at different temperatures (Atkinson, 1994; see section 4.1.3). Similarly to population count, there were no observable differences in biomass over time between control, low, and high HgCl<sub>2</sub> treatments. This is in contrast to existing literature (Atkinson, 1994, Weetman and Atkinson, 2004), predicting a decrease in mean body size related to a disruption of metabolic demand and oxygen supply following combined temperature and toxicity stress (see section 4.1.4).



### *Final biomass*

HgCl<sub>2</sub> treatment did not have a significant or observable effect on final population biomass at either temperature. However, a significant decrease in final biomass at 24°C compared to 17°C and in set 2 compared to set 1 was detected. The observed temperature effect is expected in light of the population and adult count and is in accordance with literature (Atkinson, 1994; see section 4.1.3). The detected influence of set on final biomass was surprising. As the experimental procedure was identical for the two sets, and considering that these results were from the final week of the experiment, the difference was possibly a result of random variation between replicates. In a sampling size of this quantity ( $n = 5$  per treatment group per set), it can be expected that random variation between replicates might be highly influential on the statistical analyses (see section 4.4.1). Although the difference between sets was statistically significant, the actual difference observed (Figure 3.5) was small ( $< 1$  mg dry mass), indicating that the biological differences were minor.

### *Maximum biomass*

Similarly to final biomass and population and adult count over time, a significantly higher maximum biomass was obtained at 17°C for all HgCl<sub>2</sub> treatments compared to 24°C. Decreased maximum body size with increasing temperature for zooplankton might be related to increased metabolic demand and limited oxygen supply at higher temperatures (Weetman and Atkinson, 2004). This is further discussed in section 4.1.3. In addition to temperature, a significant effect of HgCl<sub>2</sub> treatment was detected, in which a decrease in maximum biomass was observed with increasing HgCl<sub>2</sub> treatment concentrations at 17°C. As there was no difference between the different HgCl<sub>2</sub> treatments for population count or for final biomass, it seems unlikely that the decrease in maximum biomass was due to a lower number of animals in this treatment. These results might therefore indicate that the daphnids' growth was restricted by the HgCl<sub>2</sub> treatment at this temperature. Reduced body size in relation to toxicity stress has previously been reported (Atkinson, 1994, Bae *et al.*, 2016; see section 4.1.4). The same tendency was not observed at 24°C. This is unexpected as it was hypothesized that the negative effect of HgCl<sub>2</sub> would be intensified at the higher temperature. Based on the results of the current study, a synergistic effect of high temperature and HgCl<sub>2</sub> on maximum biomass in *D. magna* therefore seems unlikely.

The findings demonstrated that the effect of temperature on *D. magna* population size, including quantity and biomass, was greater than that of the mercury treatment. Final and maximum biomass was significantly elevated at 17°C compared to 24°C in all treatment groups. Within the 17°C treatment, maximum biomass decreased with increasing HgCl<sub>2</sub> exposure concentration. A similar result was not obtained at 24°C. Based on the results, it appears that the environmentally relevant HgCl<sub>2</sub> concentrations used in the present study restricted the growth of *D. magna*, but were insufficient to greatly affect total *D. magna* population size. No synergism between elevated temperature and HgCl<sub>2</sub> treatment was observed for the measure of population size, maximum, or final biomass.

#### **4.1.3 The effects of temperature on body size**

Physiological processes of ectothermic animals are strongly dependent on their surroundings because their body temperatures fluctuate with the environmental temperatures (Zuo *et al.*, 2012). Temperature is for instance an essential factor when describing ectotherms' body size. According to the temperature size rule (TSR), ectotherms generally grow and develop at a faster rate at higher temperatures (Atkinson, 1994). Because of this, they reach a smaller size at maturity, resulting in smaller animals at higher temperatures (Atkinson, 1994). This phenotypic plastic response has been observed both in the field and in laboratory experiments (Weetman and Atkinson, 2004, Rinke and Vijverberg, 2005, Bae *et al.*, 2016). In the present study, lower population count, final, and maximum biomass were observed at 24°C compared to 17°C.

The mechanisms underlying the TSR are highly debated among scientists (Weetman and Atkinson, 2004, Forster, Hirst and Atkinson, 2012, Horne, Hirst and Atkinson, 2015). One of the mechanisms discussed is related to differences in organisms' oxygen demand in warm and cold water, namely the idea that costly oxygen uptake in warm water limits the size of animals (Weetman and Atkinson, 2004, Horne, Hirst and Atkinson, 2015). Larger animals require more oxygen to maintain their aerobic scope, and as temperature increase, so does the animals' metabolic demand. Oxygen availability in water is, however, limited, and does not increase proportionally to animal body size at higher temperatures (Verberk *et al.*, 2011, Forster, Hirst and Atkinson, 2012). The oxygen-mediated pressure at elevated water temperatures might therefore cause selection towards smaller body sizes. This theory also explains why the negative temperature-size relationship is stronger for aquatic ectotherms compared to terrestrial species, to which oxygen is a ubiquitous resource (Verberk *et al.*, 2011, Forster, Hirst and Atkinson, 2012, Horne, Hirst and Atkinson, 2015). The relationship between metabolic rate, body size, and temperature is described in the metabolic theory of ecology (Brown *et al.*, 2004).

An inverse relationship between *Daphnia* body size and temperature has also been observed (Weetman and Atkinson, 2004, Heugens *et al.*, 2006, Havens *et al.*, 2015). In fact, an extensive study performed by Gillooly and Dodson (2000) found that temperature was the primary cause of variation in cladoceran body size. Reduced body and population sizes at higher temperatures can possibly be due to less energy available for somatic growth and reproduction because of increased metabolic costs at elevated temperatures (Brown *et al.*, 2004, Heugens *et al.*, 2006). This can cause a reduction in carrying capacity at elevated temperatures, as observed in the current study (Savage *et al.*, 2004). Moreover, declined reproductive output at elevated temperatures could be a result of less offspring being produced by individuals of smaller body sizes (Glazier, 1992, Heugens *et al.*, 2006).

Importantly, the above-described research on temperature and body size interactions in ectotherms are measured on an organismal level, not on a population level, which is the focus of the current study. On a population level, the degree of complexity increases. For instance, additional parameters, such as population density, food availability, and predation, can greatly influence the outcome of the experiment. In effect, results from different levels of biological organization might not be directly comparable. However, although the current study did not focus on organismal level endpoints or attributes, the trends described above might give an indication of what to expect at a higher level of organization as well. Moreover, body size and biomass are not synonymous terms. I chose to use them as comparable measures in the current section because body size was a component in calculating *D. magna* biomass in the present study. By extrapolating the findings, the reported changes in body size might give an indication as to how biomass can be affected by temperature.

#### **4.1.4 Effects of temperature and toxicity stress on population size and biomass**

Laboratory and field studies have shown that the combined stress of elevated temperature (usually  $\geq 25^{\circ}\text{C}$ ) and toxicity often cause reductions in mean body size of zooplankton, and alter the community composition to favor small-bodied individuals and species (Havens and Hanazato, 1993, Moore and Folt, 1993). For instance, Tsui and Wang (2006) reported a tolerance decrease related to body size of *D. magna*, where larger animals were more intrinsically sensitive to mercury toxicity than smaller animals. Moore and Folt (1993) argued that reduced body size could be caused by a number of reasons: Firstly, altered foraging behavior associated with exposure to sublethal toxicant concentrations can bring about a reduction in body size within a population. Secondly, growth rates can be directly suppressed in the presence of pollutants. Thirdly, and related to the previous point, toxicant-induced growth

retardation can be genetically determined, such that body sizes of different clones within a species can vary significantly. Clonal variation has also been documented related to temperature effects alone (Korpelainen, 1986). Increased costs of respiratory maintenance under toxicant stress could also explain selection towards small-bodied individuals and species. Lastly, an elevated death rate of larger individuals and species in comparison to smaller will reduce the size composition of a population or community. This can be related to a greater number of molts in larger species and increased toxicant sensitivity during molting events (Gliwicz and Sieniawska, 1986).

The results obtained in the present study do not support the above-described findings. No enhanced effect of mercury treatment could be detected on total population count, final, or maximum biomass in combination with high temperature. Given that no such results were obtained, it is possible that chronic exposure to environmentally relevant inorganic mercury concentrations does not induce a reinforced reduction in body size in *D. magna* at 24°C. More research is, however, needed to confirm these results. The observed significant reduction in maximum biomass with higher HgCl<sub>2</sub> treatment concentration at 17°C was likely caused by the effect of mercury toxicity alone. Declines in body weight of *D. magna* treated with HgCl<sub>2</sub> and other metal chlorides have previously been reported (Biesinger and Christensen, 1972). No explanation for the observed reduced body weight was given in the latter study, but it can be suggested that the increased energy allocation to detoxification and repair mechanisms during mercury exposure leaves less energy available for somatic growth (Bae *et al.*, 2016).

## **4.2 Resting egg production**

The current study measured the combined effects of HgCl<sub>2</sub> treatment and temperature on both total resting egg production and resting eggs per adult. Total resting egg production represents the ecological effect of the treatment as it combines the population size and the per capita resting egg production. It can thus be a measure of population fitness. Contrary, resting eggs per adult portrays the effect of the experimental treatment on an individual and mechanistic level.

### **4.2.1 Cumulative and total number of resting eggs**

The results showed an observable and significant difference in the cumulative and total number of resting eggs between the two experimental temperatures. Production of resting eggs at 24°C was initiated approximately two weeks prior to resting egg production at 17°C, and therefore reached a higher number of resting eggs produced in total. The production of resting eggs is

triggered by high population density and other similarly unfavorable environmental conditions (Carvalho and Hughes, 1983). Resting egg production started in sampling week four at 24°C. At this point, the *D. magna* 24°C population had already surpassed its maximum population density, i.e. carrying capacity (Figure 3.2). It is therefore likely that crowding stress in addition to resource (e.g. food) limitations provoked resting egg production soon after. Similarly, initiation of resting egg production at 17°C coincided with maximum population density attained at this temperature. As the populations were acclimatized to the experimental temperatures, it seems unlikely that a temperature effect alone would trigger resting egg production.

The production of resting eggs can be a measure of population fitness because it represents the means by which a population survives when faced with poor environmental conditions (Korpelainen, 1986). In nature, overwintering of *Daphnia* populations in temperate zones is usually as resting eggs in the sediments (Cáceres, 1998). Similarly, daphnids in temporary ponds may produce resting eggs in spring before the pond dries up (Wolf and Carvalho, 1989). *Ephippia* from different seasons may form a resting egg-pool in the sediments that enable future population survival and provide important means for maintaining genetic diversity in the population (Carvalho and Wolf, 1989, Cáceres, 1998). At the onset of a new growing season, a new population hatches from previously deposited resting eggs (Preston and Snell, 2001). Because only a fraction of the resting eggs develop and hatch (Carvalho and Wolf, 1989), substantial resting egg production each season is essential for maintaining a viable population size (Wolf and Carvalho, 1989). A persistent reduction in the resting egg-pool related to toxicity stress might reduce genetic variation and increase the likelihood of population crash (Preston and Snell, 2001, Navis *et al.*, 2013). This may render exposed populations more vulnerable to environmental changes (Navis *et al.*, 2013).

Illustrated by an increase in total resting egg production at 17°C, with a significantly higher number of resting eggs in total produced in the high HgCl<sub>2</sub> treatment compared to the control treatment, the findings imply deteriorating conditions for the population with increasing HgCl<sub>2</sub> concentrations. Contrary, at 24°C, the total resting egg production increased at the low HgCl<sub>2</sub> exposure compared to control, but decreased significantly in the high concentration treatment. The deviating response and opposite relationship between the low and high HgCl<sub>2</sub> treatment at the two temperatures might indicate an interaction between HgCl<sub>2</sub> treatment and elevated temperature, as further discussed in the next section (section 4.2.2).

Total resting egg production in the current study can be used as an indication of the population effect of the experimental treatments. The results showed a reduction in total resting egg production when the daphnids were exposed to the combined stress of elevated temperature and high HgCl<sub>2</sub> concentration. The ecological consequences of this might be reduced population fitness caused by a decline in population size in the future (e.g. next growing season) as expected following reduction in the total resting egg production. Contrary, the increasing total number of resting eggs observed at 17°C with increasing concentrations of HgCl<sub>2</sub>, might be able to sustain a viable population in the following season.

#### **4.2.2 Resting eggs per adult**

Similarly to the cumulative and total number of resting eggs, a significantly higher number of resting eggs produced per adult was observed at 24°C compared to 17°C. This further illustrates the above-mentioned effects of resource limitations and unfavorable conditions associated with elevated temperatures on resting egg production by *D. magna*. Mercury treatment had an observable effect on resting egg production per adult. As the HgCl<sub>2</sub> concentrations increased, the number of resting eggs produced per adult increased at both temperatures. As described in the previous section, this is possibly due to deteriorating environmental conditions.

A decline in the total number of resting eggs produced was observed at 24°C in the high HgCl<sub>2</sub> treatment group (section 4.2.1). As described in section 4.2.3, synergism is commonly observed between metal toxicity and elevated temperatures in ectotherms. In case of a synergism between the two experimental variables in the current study, the combined effect of high HgCl<sub>2</sub> concentration treatment and elevated temperature might have produced an environment too harsh for the population to maintain reproduction at a normal rate. This might have induced a decline in any reproductive measure, including resting egg production.

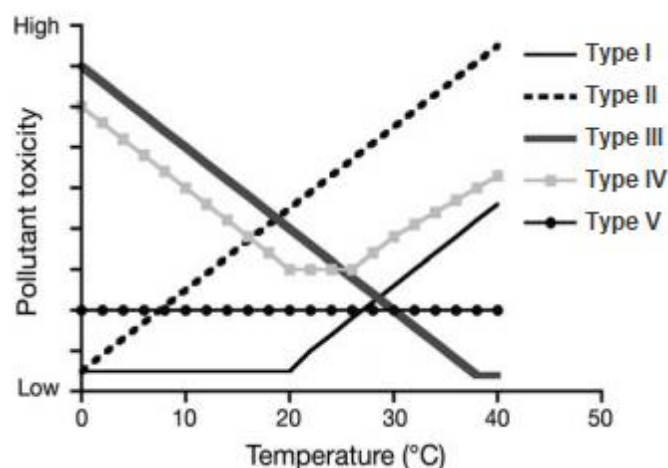
The decline in resting egg-number was, however, not apparent when adult quantity was accounted for. Given these results, it seems that the treatment primarily affected the number of adults and not the number of resting eggs produced, meaning that the decrease in total number of resting eggs at the high HgCl<sub>2</sub> concentration treatment at 24°C was caused by a decrease in the number of adults at this temperature. A plot of total adult count (cumulative number of adult individuals) per HgCl<sub>2</sub> treatment at both temperatures (Appendix C, Figure C.1) confirmed this. An observable decrease in the total number of adults was seen with increasing HgCl<sub>2</sub> exposure concentration. However, the within treatment variation and difference between sets was considerable, complicating the interpretation of the results. The underlying causes for the

contradicting results between cumulative number of resting eggs and resting eggs per adults at the high HgCl<sub>2</sub> concentration at 24°C are thus not clear.

Based on the results, it is unclear whether exposure to elevated temperature and toxic inorganic mercury stress during the sexual phase of *D. magna* life cycle limits their ability to produce resting eggs. An increase in resting egg production with increasing HgCl<sub>2</sub> treatment concentrations was observed in the total number of resting eggs at 17°C and at both temperatures for resting eggs per adult. These observations suggest deteriorating environmental conditions for the populations at higher mercury concentrations, promoting resting egg production by the daphnids. Expectedly, a significant temperature effect was detected. The cumulative number of resting eggs and number of resting eggs per adult was higher at 24°C compared to 17°C at all HgCl<sub>2</sub> treatments. Given the results, it can be expected that the population effects of HgCl<sub>2</sub> are weak compared to that of temperature.

#### **4.2.3 Temperature-dependent metal toxicity in aquatic ectotherms**

Generally, interactive effects of metal pollution and temperature result in enhanced toxicity of the metals on the organism at elevated temperatures (Figure 4.1; Heugens *et al.*, 2006, Sokolova and Lannig, 2008, Muysen, Messiaen and Janssen, 2010). This has been demonstrated in several studies. For instance, Tsui and Wang (2006) documented lethal concentrations with 50% mortality (LC50s) in the order 10 > 24 > 32°C in *D. magna* exposed acutely (24 hours) to mercury under different temperature conditions. They further revealed that at lower temperatures, Hg uptake rate was significantly reduced and accompanied by enhanced intrinsic tolerance in the daphnids. Their results are in accordance with Heugens *et al.* (2003), which showed a highly temperature-dependent effect of cadmium (Cd) on survival in *D. magna*. Heugens and colleagues suggested that the temperature-increased toxicity could be a result of alterations in metal accumulation and *D. magna* sensitivity at elevated temperatures. Additionally, at elevated temperatures, animals might be more vulnerable to additional stress because they are close to their environmental tolerance limits (Heugens *et al.*, 2001).



**Figure 4.1** Patterns of pollutant toxicity as a function of temperature. Metal toxicity generally follows type I and II response in which toxicity increases with increasing temperatures (Sokolova and Lannig, 2008).

The concept of temperature-dependent metal toxicity was further investigated by Sokolova and Lannig (2008) in their extensive review of the concept related to aquatic ectotherms. The authors identified two consequences of elevated temperature and trace metal exposure in aquatic ectotherms: 1) A sensitization of the organism to metal toxicity, and 2) a decrease in thermal tolerance limits (Cairns, Heath and Parker, 1975, Heugens *et al.*, 2001). In fact, Heugens *et al.* (2003) showed that Cd uptake rates were significantly higher at 20°C than at 10°C. If the higher uptake rate is not balanced by an equally large elimination of the metals, the internal concentrations could increase, resulting in elevated toxicity. In the same study, internal threshold concentrations were used as a measure of temperature-dependent sensitivity to metal toxicity by the daphnids. It was revealed that less Cd was needed to induce lethal effects at higher temperatures (Heugens *et al.*, 2003). Tsui and Wang (2004a) reported no effect of temperature on elimination rate in *D. magna*, supporting the hypothesis above.

Not only are the predominant routes of metal uptake in aquatic ectotherms, including active and facilitated transport, strongly affected by temperature, but changes in metabolic rates at elevated temperatures may also contribute to increased metal toxicity in the animals (Deb and Fukushima, 1999, Heugens *et al.*, 2003, Sokolova and Lannig, 2008). The survival and success of an organism under stressful conditions depends on its ability to balance energy demand and supply (Sibly and Calow, 1989, Sokolova and Lannig, 2008). It has been demonstrated that both temperature and metal toxicity alone can increase basal metabolic demand (De Coen and Janssen, 1997, Muysen, Messiaen and Janssen, 2010). Should their synergistic effect result in an additional increased energy demand for basal metabolism, it could, if not met by an increased



energy supply, have detrimental effects on the organism. Energy deficits during exposure might be due to elevated maintenance costs, because of for instance detoxification or repair mechanisms, or due to direct interference with energy conservation, for instance disrupting mitochondrial function or ATP-production pathways (Sibly and Calow, 1989). To satisfy the increased energy demand experienced, the animals' ventilation and/or feeding rates increase, which might in turn lead to higher exposure to, and uptake of, contaminants from the surroundings. Hence, an increase in metabolic rates at elevated temperatures may contribute to metal accumulation in ectotherms due to higher energy demand and increased metal uptake (Sokolova and Lannig, 2008). Additionally, a higher metabolic rate may increase active transport of metals across the membranes, possibly increasing metal accumulation rates (Cairns, Heath and Parker, 1975, Heugens *et al.*, 2003). Lastly, as mentioned previously, increased metabolic costs under stressful conditions might result in less energy available for other physiological processes such as growth and reproduction (Heugens *et al.*, 2006, Bae *et al.*, 2016).

The second issue with temperature-dependent metal toxicity in aquatic ectotherms stressed by Sokolova and Lannig (2008), was a decrease in thermal tolerance limits during exposure. The theory of oxygen-limited thermal tolerance states that an organism's thermal tolerance limits, between which growth and reproduction primarily occur, is determined by its aerobic scope, i.e. the organism's ability to balance oxygen demand and supply (Pörtner, 2001, Sokolova and Lannig, 2008). This is related to the principles described above. Metal toxicity contributes to limitations in oxygen supply indirectly by for instance disrupting energy metabolism (i.e. metal induced rise in oxygen demand), and directly by impairing circulatory and ventilatory performance, mitochondrial functioning, and oxygen carrying capacity of respiratory pigments (Sokolova and Lannig, 2008). A decrease in thermal tolerance limits can therefore occur under metal toxicity because of impaired aerobic capacities and subsequent oxygen deficiency. The implications of such a decrease can be serious under future global warming predictions if the animals are unable to adapt.

Based on results from Muysen, Messiaen and Janssen (2010), it seems that the temperature-induced increased metal toxicity previously reported (cf. above) is restricted to acute exposure and specific endpoints only (e.g. mortality). In fact, their results showed no synergistic effect of elevated temperature (24°C) and Cd on total reproductive output or time to first brood compared to the control treatments. They also failed to detect a decrease in thermal tolerance limit in the metal-exposed animals, and saw no synergism between temperature and Cd on energy reserves (i.e. no effect on basal metabolic demand). They did, on the other hand, observe

a decrease in *acute* Cd tolerance of the daphnids from 20 to 24°C, confirming the proposed temperature-induced increase in metal sensitivity for acute exposure. The results obtained by Muysen, Messiaen and Janssen (2010) may be relevant in explaining the results observed in the present study.

A possible explanation for the deviation between the chronic and acute experiments could be large differences in exposure concentrations used in chronic and acute studies. For instance, in the acute toxicity study performed by Heugens *et al.* (2003), in which synergism was detected, the exposure concentrations exceeded 100 µg L<sup>-1</sup> Cd. Contrary, a concentration of 5 µg L<sup>-1</sup> Cd was used in the chronic experiments performed by Muysen, Messiaen and Janssen (2010). Based on this, it can be hypothesized that high metal concentrations are required to enhance metal toxicity at elevated temperatures. Furthermore, during long-term experiments, the test animals might become acclimatized to the experimental conditions (Tsui and Wang, 2005). Muysen, Messiaen and Janssen (2010) proposed that an increase in cellular detoxification mechanisms, for instance, expression of heat shock proteins and antioxidant enzymes, in the acclimatized animals might protect them from reinforced toxicity at high temperatures. Similarly, Tsui and Wang (2007) suggested higher metal, including Hg, tolerance from multigenerational exposure compared to single-generation exposure could be attributed to induction of metallothionein-like protein.

Recently, Bae *et al.* (2016) reported temperature-dependent copper (Cu) toxicity in *D. magna*. The authors found that elevated temperature alone and in combination with Cu induced a significant increase in production of reactive oxygen species (ROS) and lipid peroxidation in the animals. Other metals, including Hg, are known to promote ROS formation and cause oxidative stress in organisms (Verlecar, Jena and Chainy, 2007). Oxidative stress might thus be an additional pathway for synergism between temperature and metal toxicity that needs further exploration (Bae *et al.*, 2016).

#### **4.3 Protective effects of selenium against mercury toxicity**

Se is generally considered to protect against mercury toxicity (Cuvin-Aralar and Furness, 1991). Multiple studies have demonstrated a protective effect of selenium against mercury toxicity in various organisms, with mitigating effects observed for instance in mortality (Ganter *et al.*, 1972), oxidative stress (Hoffman and Heinz, 1998), growth rate (Potter and Matrone, 1974), mercury poisoning symptoms (Sell and Horani, 1974), and total mercury body burden (Chen, Belzile and Gunn, 2001). The exact mechanisms for Se antagonism are poorly

understood (Dang and Wang, 2011). Cuvin-Aralar and Furness (1991) suggested five possible mechanisms for the observed protective role of Se against Hg toxicity that might function independently or in concert: 1) Redistribution of mercury from more sensitive targets in the body, e.g. the kidneys, to less sensitive tissue, e.g. muscle, in presence of selenium; 2) Se and Hg competition for binding sites and carrier proteins; 3) Formation of less toxic mercury-selenium complexes, for example mercuric selenide (HgSe) and bis(methylmercuric) selenide  $[(\text{CH}_3\text{Hg})_2\text{Se}]$ , which are inert and can no longer penetrate the cell membranes to cause intracellular damage, or will prevent mercury from binding to active sites (Yang *et al.*, 2008); 4) Conversion of more toxic forms of mercury to less toxic forms, for instance, demethylation of MeHg to inorganic Hg; and 5) Counteraction of oxidative stress caused by mercury because selenium is a component of several antioxidant enzymes, for instance, glutathione peroxidase (Yang *et al.*, 2008).

The chemical forms of mercury and selenium are important in determining the outcome of their interaction (Cuvin-Aralar and Furness, 1991). Selenite ( $\text{SeO}_3^{2+}$ ) has been suggested to be the most effective Se-compound in both  $\text{Hg}^{2+}$  and MeHg antagonism (Yang *et al.*, 2008), but contradicting results have also been observed possibly due to different modes of action of the distinct chemical forms (Cuvin-Aralar and Furness, 1991, Dang and Wang, 2011). Variable concentrations and administration methods employed further complicates the picture (Dang and Wang, 2011). According to Yang *et al.* (2008), antagonism is best achieved when the elements are co-administered. In fact, Se administration *after* Hg exposure has shown to eliminate the protective effect, likely because rapid removal from the blood prevented interaction between the elements from occurring (Naganuma, Ishii and Imura, 1984). Administration of selenium *prior* to mercury caused dimethylselenide intoxication as dimethylselenide can act synergistically with  $\text{Hg}^{2+}$  (Parizek *et al.*, 1980, Naganuma, Ishii and Imura, 1984).

The protective effect of Se have been demonstrated for both inorganic and organic mercuric compounds and by different chemical forms of selenium (Ganther *et al.*, 1972, Dang and Wang, 2011). In the current study, the daphnids in all the experimental treatments were kept in a  $\text{SeO}_2$  altered artificial freshwater medium (Appendix A, Table A.1). It is possible that the  $\text{SeO}_2$  had a mitigating effect on the toxicity of  $\text{HgCl}_2$  to the daphnids through any of the mechanisms outlined above. Relevant comparable studies were difficult to obtain, as most studies investigating Se-Hg antagonism have used  $\text{SeO}_3^{2+}$  and few have studied the effects on aquatic invertebrates or lower trophic level organisms (Belzile *et al.*, 2006). However, Kim, Birks and Heisinger (1977) reported decreased mortality of northern creek chubs pretreated with  $\text{SeO}_2$  (48 hours) before acute high-concentration exposure to  $\text{HgCl}_2$  (48 hours). Similarly, acute

toxicity tests revealed that  $\text{SeO}_2$  was a strong antagonist against  $\text{HgCl}_2$  toxicity in goldfish (Heisinger, Hansen and Kim, 1979). Belzile *et al.* (2006) demonstrated reduced bioassimilation of mercury as total Hg and MeHg in the tissue of zooplankton, mayflies, amphipods, and young-of-the-year perch related to selenium deposition from metal smelters.

Finally, it should be noted that contradicting results have also been found, in which no observed effect or increased mercury toxicity (i.e. synergism) have resulted from co-treatment with selenium (Khan and Wang, 2009, Dang and Wang, 2011). It appears that the sensitivity of the organ or organism exposed and the relative concentrations of Hg and Se are decisive for the outcome of the interaction (Ralston, Blackwell and Raymond, 2007, Khan and Wang, 2009, Sørmo *et al.*, 2011). The controversy of the results suggests that the interactive effects between the two elements are poorly understood. It is therefore difficult to evaluate if Se had any influence on the toxicity of  $\text{HgCl}_2$  in the current study.

## **4.4 Experimental reflections**

### **4.4.1 Methodology, study design, and sources of error**

The present study consisted of six experimental treatments, including two controls. Each treatment consisted of ten replicates, separated into two sets. In such a small sample size ( $n = 5$  per treatment group per set), treatment effects can be hard to detect, and random variation may constitute a considerable percentage of the obtained results. This is illustrated by the high variation observed among replicates within treatment groups in several measurements, for instance in the total number of resting eggs and resting eggs per adult. Some treatment groups also displayed skews and outlying values. The spread in the data can reduce the statistical power and create difficulty in trusting the statistical output because it affects the center and shape of the data. The causes for the data dispersion are not clear. One explanation can be sampling and experimental errors, for instance, imprecision in preparing  $\text{HgCl}_2$  start and working solutions, or disturbances of the video recording thereby disrupting the data analysis. Lastly, errors might have occurred in counting the resting eggs and ephippia as they tended to cluster together.

Both prior to experimental startup and during the final four weeks of the experimental period, problems with unwanted fungi and algae growth in the beakers occurred. As mentioned in section 2.1.3, the fungus infection rendered the animals immobile and unhealthy, restricting their movement, reproduction, and growth. The fungus infection might have impacted the results by affecting the outcome of the experimental treatment, by perhaps affecting the daphnids reproduction. Similarly, in addition to possibly affecting the daphnids health, the algae

might have disrupted the video recording analysis by being wrongfully interpreted as moving animals. This would have influenced biomass and population count.

Biesinger, Anderson and Eaton (1982) determined the chronic effects of inorganic and organic mercury compounds on survival and reproduction of *D. magna* using renewed static and flow-through systems. The study revealed that in the renewed static tests (similar procedure as the present study), mercury water concentrations were drastically reduced following addition. In fact, only 20-30% of the total mercury content remained in the water phase after one week. The authors approximate that 25% of the mercury was adsorbed to the glassware, 20-25% volatilized, while the remainder was lost due to biological activity, including uptake by bacteria, algae, or the daphnids. This observation is crucial as it can severely alter the mercury concentrations to which the animals are exposed. In this study is it particularly concerning because of the fungus and algae problems discussed in the previous paragraph.

#### **4.4.2 Resting egg production as an important experimental endpoint**

A highly interesting aspect of this study was the use of resting egg production as a final endpoint of toxic impact. Reproduction is a frequently used endpoint in toxicity studies. Various endpoints such as young production, time to first brood, and clutch size have been investigated in previous studies using aquatic invertebrates (Jonczyk and Gilron, 2005). Often, in species with both sexual and asexual reproduction, such as *D. magna*, toxicity tests primarily focus on asexual reproduction (Shurin and Dodson, 1997). However, sexual reproduction has been suggested a highly sensitive endpoint for aquatic invertebrates exposed to various toxic chemicals (Snell and Carmona, 1995, Preston and Snell, 2001). Resting egg production was for example found to be the most sensitive endpoint (based on no observed effect concentrations [NOECs]) for pentachlorophenol (a pesticide) and Cu after acute (48 and 96 hours) exposure in rotifers (Preston and Snell, 2001). In the same study, similar results, showing a disproportionately higher inhibition of resting egg production than that of the effect on asexual reproduction, were also obtained using cadmium, lead, mercury, nonylphenol, and testosterone. Because cladocerans and rotifers have similar life cycles, cladocerans may be expected to respond similarly in toxicity tests (Shurin and Dodson, 1997).

Chronic *Daphnia* reproductive tests rarely take sexual reproduction into consideration, and may thus fail to detect an ecologically relevant stress response (Preston and Snell, 2001). This can render them ineffective in ecological risk assessment. Furthermore, it is important to consider toxicity impact on various aspects of an organism's life cycle, as they can be exposed at various

stages or even throughout their whole life cycle in nature. Exposure to toxic substances can coincide with other unfavorable environmental circumstances that induce sexual reproduction. It can therefore be hypothesized that *Daphnia* will be more sensitive to toxic stress during the sexual phase because the stress that induces sexual reproduction may reduce its resilience (Shurin and Dodson, 1997). This is important to take into consideration when planning studies using aquatic invertebrates, in order to avoid underestimating the toxicity of pollutants to these organisms. Lastly, because sexual reproduction is a strategy that enables long-term population survival, disturbing this process can potentially have detrimental consequences for the entire population (Shurin and Dodson, 1997). A decline in population size, and a possible increase in likelihood of local population extinctions, is expected following a reduction in resting egg production under persistent toxicant stress (Preston and Snell, 2001).

#### **4.5 Future prospects**

Declining zooplankton body sizes with elevated temperatures and under exposure to toxic chemicals can have serious consequences at the population and ecosystem level. It can affect population growth because smaller females usually produce fewer and smaller eggs, sustaining a smaller population (Lampert, 1993). Moreover, a decline in the zooplankton community size structure can have serious consequences for freshwater ecosystem structure and functioning because of rippling effects to adjacent trophic levels in the food chain. Primary production, the transfer of energy between trophic levels, and ecosystem nutrient cycling are examples of important ecosystem processes that can possibly be disrupted by this modification (Moore and Folt, 1993). This includes for instance reduced grazing capacity by smaller-sized zooplankton populations, altered water clarity because of changes in algal density, and changes in fish abundance because of less-valuable and nutritious food for predators (Moore and Folt, 1993).

A long-term study of Lake Washington (USA) from 1962 to 2002 revealed a mismatch of favorable environmental conditions for growth between algae and zooplankton (*Daphnia*) communities produced by elevated temperatures related to climate change (Winder and Schindler, 2004). As daphnids are crucial in linking energy transfer between primary producers and secondary consumers in aquatic food webs (Persson *et al.*, 2007), a disruption of this relationship may be transmitted to higher trophic levels, causing drastic ecological consequences (Winder and Schindler, 2004). This highlights the importance of considering the effects of global warming on this keystone species. Moreover, as temperature is known to act synergistically with toxic metals (Heugens *et al.*, 2003), the combined effect of these two stressors should be further investigated.

## 4.6 Conclusions

The present study revealed important temperature effects on *D. magna* resting egg production and population dynamics. A significantly lower population size, as well as a decreased final and maximum biomass, was observed at 24°C compared to 17°C. An explanation for this might be a faster manifestation of resource scarcity at the higher temperature. Similarly, unfavorable environmental conditions associated with the elevated temperature caused higher resting egg production by the daphnids at 24°C. Although not as prominent as for temperature, effects of HgCl<sub>2</sub> treatment were also found on maximum biomass and resting egg production. Based on research performed by for instance Heugens *et al.* (2003) and Tsui and Wang (2006), an expected increased mercury toxicity was expected in presence of elevated temperatures. No clear conclusion on the interaction of the two parameters can be drawn based on the current study, although an indication of interactive effects was observed on the total number of resting eggs produced. The underlying causes for this observation are not obvious.

Based on the above-mentioned methodological reflections, caution is needed when interpreting the result from the present study. Further research is needed to confirm the observed results. Additionally, care should be taken when interpreting laboratory results in an ecological perspective because of substantial differences in laboratory and field conditions. Extrapolations should therefore be considered with caution. Nevertheless, to our knowledge, this is the first study to examine the interactive effects of elevated temperature and chronic mercury toxicity on resting egg production by *D. magna*. The results from the present study therefore add valuable information to a field of research with great importance for the future.

## 5 References

- AMAP (2011) *AMAP assessment 2011: Mercury in the Arctic*. (9788279710684). Oslo: Arctic Monitoring and Assessment Programme.
- Antunes, S. C., Castro, B. B. and Gonçalves, F. (2003) Chronic responses of different clones of *Daphnia longispina* (field and ehippia) to different food levels, *Acta Oecologica*, 24, pp. S325-S332. doi: 10.1016/S1146-609X(03)00026-2.
- Asano, S., Eto, K., Kurisaki, E., Gunji, H., Hiraiwa, K., Sato, M., Sato, H., Hasuike, M., Hagiwara, N. and Wakasa, H. (2000) Acute inorganic mercury vapor inhalation poisoning, *Pathology International*, 50(3), pp. 169-174.
- Aschner, M. and Aschner, J. L. (1990) Mercury neurotoxicity: mechanisms of blood-brain barrier transport, *Neuroscience & Biobehavioral Reviews*, 14(2), pp. 169-176.
- Aschner, M., Syversen, T., Souza, D., Rocha, J. B. T. d. and Farina, M. (2007) Involvement of glutamate and reactive oxygen species in methylmercury neurotoxicity, *Brazilian Journal of Medical and Biological Research*, 40(3), pp. 285-291.
- Atkinson, D. (1994) Temperature and Organism Size—A Biological Law for Ectotherms?, *Advances in Ecological Research*, 25, pp. 1-58. doi: 10.1016/S0065-2504(08)60212-3.
- Bae, E., Samanta, P., Yoo, J. and Jung, J. (2016) Effects of multigenerational exposure to elevated temperature on reproduction, oxidative stress, and Cu toxicity in *Daphnia magna*, *Ecotoxicology and environmental safety*, 132, pp. 366-371. doi: 10.1016/j.ecoenv.2016.06.034.
- Balbus, J. M., Boxall, A., Fenske, R. A., McKone, T. E. and Zeise, L. (2013) Implications of global climate change for the assessment and management of human health risks of chemicals in the natural environment, *Environmental Toxicology and Chemistry*, 32(1), pp. 62-78.
- Bank, M. S. (2012) *Mercury in the environment: pattern and process*. University of California Press.
- Beaugrand, G. (2004) The North Sea regime shift: evidence, causes, mechanisms and consequences, *Progress in Oceanography*, 60(2-4), pp. 245-262.
- Belzile, N., Chen, Y.-W., Gunn, J. M., Tong, J., Alarie, Y., Delonchamp, T. and Lang, C.-Y. (2006) The effect of selenium on mercury assimilation by freshwater organisms, *Canadian Journal of Fisheries and Aquatic Sciences*, 63(1), pp. 1-10.
- Benndorf, J., Kranich, J., Mehner, T. and Wagner, A. (2001) Temperature impact on the midsummer decline of *Daphnia galeata*: an analysis of long-term data from the biomanipulated Bautzen Reservoir (Germany), *Freshwater biology*, 46(2), pp. 199-211.
- Biesinger, K. E. and Christensen, G. M. (1972) Effects of various metals on survival, growth, reproduction, and metabolism of *Daphnia magna*, *Journal of the Fisheries Board of Canada*, 29(12), pp. 1691-1700.
- Biesinger, K. E., Anderson, L. E. and Eaton, J. G. (1982) Chronic effects of inorganic and organic mercury on *Daphnia magna*: Toxicity, accumulation, and loss, *Archives of environmental contamination and toxicology*, 11(6), pp. 769-774.
- Bodar, C., Van Leeuwen, C., Voogt, P. and Zandee, D. (1988) Effect of cadmium on the reproduction strategy of *Daphnia magna*, *Aquatic Toxicology*, 12(4), pp. 301-309.
- Boening, D. W. (2000) Ecological effects, transport, and fate of mercury: a general review, *Chemosphere*, 40(12), pp. 1335-1351.
- Brković-Popović, I. (1990) Effect of mercury on the survival of *Daphnia magna*, *Water science and technology*, 22(5), pp. 241-246.
- Brown, J. H., Gillooly, J. F., Allen, A. P., Savage, V. M. and West, G. B. (2004) Toward a metabolic theory of ecology, *Ecology*, 85(7), pp. 1771-1789.
- Bruijning, M., Visser, M. D., Hallmann, C. A. and Jongejans, E. (2018) trackdem: Automated particle tracking to obtain population counts and size distributions from videos in r, *Methods in Ecology and Evolution*, 9(4), pp. 965-973.
- Buchet, J.-P., Roels, H., Bernard, A. and Lauwerys, R. (1980) Assessment of renal function of workers exposed to inorganic lead, calcium or mercury vapor, *Journal of occupational medicine.: official publication of the Industrial Medical Association*, 22(11), pp. 741-750.
- Cáceres, C. E. (1998) Interspecific variation in the abundance, production, and emergence of *Daphnia* diapausing eggs, *Ecology*, 79(5), pp. 1699-1710.
- Cairns, J., Heath, A. G. and Parker, B. C. (1975) The effects of temperature upon the toxicity of chemicals to aquatic organisms, *Hydrobiologia*, 47(1), pp. 135-171.



- Carvalho, G. R. and Hughes, R. N. (1983) The effect of food availability, female culture-density and photoperiod on ehippia production in *Daphnia magna* Straus (Crustacea: Cladocera), *Freshwater biology*, 13(1), pp. 37-46.
- Carvalho, G. R. and Wolf, H. G. (1989) Resting eggs of lake-Daphnia I. Distribution, abundance and hatching of eggs collected from various depths in lake sediments, *Freshwater biology*, 22(3), pp. 459-470.
- Chen, C. Y., Stemberger, R. S., Klaue, B., Blum, J. D., Pickhardt, P. C. and Folt, C. L. (2000) Accumulation of heavy metals in food web components across a gradient of lakes, *Limnology and Oceanography*, 45(7), pp. 1525-1536. doi: 10.4319/lo.2000.45.7.1525.
- Chen, Y.-W., Belzile, N. and Gunn, J. M. (2001) Antagonistic effect of selenium on mercury assimilation by fish populations near Sudbury metal smelters?, *Limnology and Oceanography*, 46(7), pp. 1814-1818.
- Cuvin-Aralar, M. L. A. and Furness, R. W. (1991) Mercury and selenium interaction: a review, *Ecotoxicology and environmental safety*, 21(3), pp. 348-364.
- Dang, F. and Wang, W.-X. (2011) Antagonistic interaction of mercury and selenium in a marine fish is dependent on their chemical species, *Environmental science & technology*, 45(7), pp. 3116-3122.
- Dawidowicz, P. and Loose, C. J. (1992) Metabolic costs during predator-induced dielvertical migration of *Daphnia*, *Limnology and Oceanography*, 37(8), pp. 1589-1595.
- De Coen, W. M. and Janssen, C. (1997) The use of biomarkers in *Daphnia magna* toxicity testing. IV. Cellular energy allocation: a new methodology to assess the energy budget of toxicant-stressed *Daphnia* populations, *Journal of Aquatic Ecosystem Stress and Recovery*, 6(1), pp. 43-55.
- De Coen, W. M., Janssen, C. and Segner, H. (2001) The use of biomarkers in *Daphnia magna* toxicity testing V. In vivo alterations in the carbohydrate metabolism of *Daphnia magna* exposed to sublethal concentrations of mercury and lindane, *Ecotoxicology and environmental safety*, 48(3), pp. 223-234.
- de Oliveira Ribeiro, C., Belger, L., Pelletier, E. and Rouleau, C. (2002) Histopathological evidence of inorganic mercury and methyl mercury toxicity in the arctic charr (*Salvelinus alpinus*), *Environmental Research*, 90(3), pp. 217-225.
- Deb, S. and Fukushima, T. (1999) Metals in aquatic ecosystems: mechanisms of uptake, accumulation and release-Ecotoxicological perspectives, *International Journal of Environmental Studies*, 56(3), pp. 385-417.
- Dietz, R., Outridge, P. M. and Hobson, K. A. (2009) Anthropogenic contributions to mercury levels in present-day Arctic animals—a review, *Science of The Total Environment*, 407(24), pp. 6120-6131.
- Dietz, R., Sonne, C., Basu, N., Braune, B., O'Hara, T., Letcher, R. J., Scheuhammer, T., Andersen, M., Andreasen, C. and Andriashek, D. (2013) What are the toxicological effects of mercury in Arctic biota?, *Science of The Total Environment*, 443, pp. 775-790.
- Doma, S. (1979) Ehippia of *Daphnia magna* Straus—a technique for their mass production and quick revival, *Hydrobiologia*, 67(2), pp. 183-188.
- Drevnick, P. E., Roberts, A. P., Otter, R. R., Hammerschmidt, C. R., Klaper, R. and Oris, J. T. (2008) Mercury toxicity in livers of northern pike (*Esox lucius*) from Isle Royale, USA, *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*, 147(3), pp. 331-338.
- Ebert, D. (2005) *Ecology, epidemiology, and evolution of parasitism in Daphnia*. National Library of Medicine.
- EuropeanCommission (2017) *Tackling mercury pollution in the EU and worldwide*. (Science for environment policy. In-depth report 15 2363-28019789279440007). Luxembourg: Luxembourg : Publications Office.
- Fargasova, A. (1994) Toxicity of metals on *Daphnia magna* and *Tubifex tubifex*, *Ecotoxicology and environmental safety*, 27(2), pp. 210-213.
- Farina, M., Aschner, M. and Rocha, J. B. (2011) Oxidative stress in MeHg-induced neurotoxicity, *Toxicology and applied pharmacology*, 256(3), pp. 405-417.
- Farina, M., Avila, D. S., Da Rocha, J. B. T. and Aschner, M. (2013) Metals, oxidative stress and neurodegeneration: a focus on iron, manganese and mercury, *Neurochemistry international*, 62(5), pp. 575-594.

- Ficke, A. D., Myrick, C. A. and Hansen, L. J. (2007) Potential impacts of global climate change on freshwater fisheries, *Reviews in Fish Biology and Fisheries*, 17(4), pp. 581-613. doi: 10.1007/s11160-007-9059-5.
- Forster, J., Hirst, A. G. and Atkinson, D. (2012) Warming-induced reductions in body size are greater in aquatic than terrestrial species, *Proceedings of the National Academy of Sciences*, 109(47), pp. 19310-19314.
- Friberg, N., Dybkjær, J. B., Olafsson, J. S., Gislason, G. M., Larsen, S. E. and Lauridsen, T. L. (2009) Relationships between structure and function in streams contrasting in temperature, *Freshwater biology*, 54(10), pp. 2051-2068. doi: 10.1111/j.1365-2427.2009.02234.x.
- Galimov, Y., Walser, B. and Haag, C. R. (2011) Frequency and inheritance of non-male producing clones in *Daphnia magna*: evolution towards sex specialization in a cyclical parthenogen?, *Journal of Evolutionary Biology*, 24(7), pp. 1572-1583. doi: 10.1111/j.1420-9101.2011.02288.x.
- Ganther, H., Goudie, C., Sunde, M., Kopecky, M., Wagner, P., Oh, S.-H. and Hoekstra, W. (1972) Selenium: relation to decreased toxicity of methylmercury added to diets containing tuna, *Science*, 175(4026), pp. 1122-1124.
- Gillooly, J. F. and Dodson, S. I. (2000) Latitudinal patterns in the size distribution and seasonal dynamics of new world, freshwater cladocerans, *Limnology and Oceanography*, 45(1), pp. 22-30.
- Glazier, D. S. (1992) Effects of food, genotype, and maternal size and age on offspring investment in *Daphnia magna*, *Ecology*, 73(3), pp. 910-926.
- Gliwicz, M. Z. and Sieniawska, A. (1986) Filtering activity of *Daphnia* in low concentrations of a pesticide, *Limnology and Oceanography*, 31(5), pp. 1132-1138.
- Hairton, N. G. (1996) Zooplankton egg banks as biotic reservoirs in changing environments, *Limnology and Oceanography*, 41(5), pp. 1087-1092.
- Harte, J. and Shaw, R. (1995) Shifting Dominance Within a Montane Vegetation Community: Results of a Climate-Warming Experiment, *Science*, 267(5199), pp. 876-880. doi: 10.1126/science.267.5199.876.
- Havens, K. E. and Hanazato, T. (1993) Zooplankton community responses to chemical stressors: a comparison of results from acidification and pesticide contamination research, *Environmental Pollution*, 82(3), pp. 277-288.
- Havens, K. E., Pinto-Coelho, R. M., Beklioglu, M., Christoffersen, K. S., Jeppesen, E., Lauridsen, T. L., Mazumder, A., Méthot, G., Alloul, B. P. and Tavşanoğlu, U. N. (2015) Temperature effects on body size of freshwater crustacean zooplankton from Greenland to the tropics, *Hydrobiologia*, 743(1), pp. 27-35.
- Heisinger, J., Hansen, C. and Kim, J. H. (1979) Effect of selenium dioxide on the accumulation and acute toxicity of mercuric chloride in goldfish, *Archives of environmental contamination and toxicology*, 8(3), pp. 279-283.
- Helmuth, B., Harley, C. D., Halpin, P. M., O'donnell, M., Hofmann, G. E. and Blanchette, C. A. (2002) Climate change and latitudinal patterns of intertidal thermal stress, *Science*, 298(5595), pp. 1015-1017.
- Heugens, E. H., Hendriks, A. J., Dekker, T., Straalen, N. M. v. and Admiraal, W. (2001) A review of the effects of multiple stressors on aquatic organisms and analysis of uncertainty factors for use in risk assessment, *Critical reviews in toxicology*, 31(3), pp. 247-284.
- Heugens, E. H., Jager, T., Creyghton, R., Kraak, M. H., Hendriks, A. J., Van Straalen, N. M. and Admiraal, W. (2003) Temperature-dependent effects of cadmium on *Daphnia magna*: accumulation versus sensitivity, *Environmental science & technology*, 37(10), pp. 2145-2151.
- Heugens, E. H., Tokkie, L. T., Kraak, M. H., Hendriks, A. J., van Straalen, N. M. and Admiraal, W. (2006) Population growth of *Daphnia magna* under multiple stress conditions: joint effects of temperature, food, and cadmium, *Environmental Toxicology and Chemistry*, 25(5), pp. 1399-1407.
- Hoffman, D. J. and Heinz, G. H. (1998) Effects of mercury and selenium on glutathione metabolism and oxidative stress in mallard ducks, *Environmental Toxicology and Chemistry*, 17(2), pp. 161-166.
- Holmstrup, M., Bindesbøl, A.-M., Oostingh, G. J., Duschl, A., Scheil, V., Köhler, H.-R., Loureiro, S., Soares, A. M., Ferreira, A. L. and Kienle, C. (2010) Interactions between effects of

- environmental chemicals and natural stressors: a review, *Science of The Total Environment*, 408(18), pp. 3746-3762.
- Horne, C. R., Hirst, A. and Atkinson, D. (2015) Temperature-size responses match latitudinal-size clines in arthropods, revealing critical differences between aquatic and terrestrial species, *Ecology letters*, 18(4), pp. 327-335.
- IPCC (2013) *Summary for Policymakers. Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change.*
- Jonczyk, E. and Gilron, G. (2005) Acute and Chronic Toxicity Testing with Daphnia Sp *Small-scale freshwater toxicity investigations.* Springer, pp. 337-393.
- Kendall, R. J., Lacher, T. E., Cobb, G. C. and Cox, S. B. (2016) *Wildlife toxicology: emerging contaminant and biodiversity issues.* CRC Press.
- Kernan, M., Battarbee, R. W. and Moss, B. R. (2011) *Climate change impacts on freshwater ecosystems.* John Wiley & Sons.
- Khan, M. A. K. and Wang, F. (2009) Mercury-selenium compounds and their toxicological significance: Toward a molecular understanding of the mercury-selenium antagonism, *Environmental Toxicology and Chemistry*, 28(8), pp. 1567-1577. doi: 10.1897/08-375.1.
- Khargarot, B. and Ray, P. (1987) Correlation between heavy metal acute toxicity values in Daphnia magna and fish, *Bulletin of environmental contamination and toxicology*, 38(4), pp. 722-726.
- Khargarot, B. and Das, S. (2009) Toxicity of mercury on in vitro development of parthenogenetic eggs of a freshwater cladoceran Daphnia carinata, *Journal of Hazardous Materials*, 161(1), pp. 68-73.
- Kim, H., Kim, J.-S. and Lee, Y.-M. (2017) Changes in activity and transcription of antioxidant enzymes and heat shock protein 90 in the water flea, Daphnia magna-exposed to mercury, *Toxicology and Environmental Health Sciences*, 9(5), pp. 300-308.
- Kim, J., Park, J., Kim, P.-G., Lee, C., Choi, K. and Choi, K. (2010) Implication of global environmental changes on chemical toxicity-effect of water temperature, pH, and ultraviolet B irradiation on acute toxicity of several pharmaceuticals in Daphnia magna, *Ecotoxicology*, 19(4), pp. 662-669.
- Kim, J. H., Birks, E. and Heisinger, J. (1977) Protective action of selenium against mercury in northern creek chubs, *Bulletin of environmental contamination and toxicology*, 17(2), pp. 132-136.
- Klaassen, C. D. and Amdur, M. O. (1996) *Casarett and Doull's toxicology: the basic science of poisons.* 8th edn.
- Klüttgen, B., Dülmer, U., Engels, M. and Ratte, H. T. (1994) ADaM, an artificial freshwater for the culture of zooplankton, *Water Research*, 28(3), pp. 743-746. doi: 10.1016/0043-1354(94)90157-0.
- Korpelainen, H. (1986) The effects of temperature and photoperiod on life history parameters of Daphnia magna (Crustacea: Cladocera), *Freshwater biology*, 16(5), pp. 615-620.
- Lampert, W. (1993) Phenotypic Plasticity of the Size at First Reproduction in Daphnia: The Importance of Maternal Size, *Ecology*, 74(5), pp. 1455-1466. doi: 10.2307/1940074.
- Mark, U. and Solbé, J. (1998) Analysis of the ecetoc aquatic toxicity (EAT) database V — The relevance of Daphnia magna as a representative test species, *Chemosphere*, 36(1), pp. 155-166. doi: 10.1016/S0045-6535(97)10027-3.
- Mason, R. P., Fitzgerald, W. F. and Morel, F. M. (1994) The biogeochemical cycling of elemental mercury: anthropogenic influences, *Geochimica et Cosmochimica Acta*, 58(15), pp. 3191-3198.
- Mason, R. P. and Sheu, G. R. (2002) Role of the ocean in the global mercury cycle, *Global biogeochemical cycles*, 16(4), pp. 40-41-40-14.
- Moore, M. and Folt, C. (1993) Zooplankton body size and community structure: effects of thermal and toxicant stress, *Trends in ecology & evolution*, 8(5), pp. 178-183.
- Muysen, B. T., Messiaen, M. and Janssen, C. R. (2010) Combined cadmium and temperature acclimation in Daphnia magna: physiological and sub-cellular effects, *Ecotoxicology and environmental safety*, 73(5), pp. 735-742.
- Naganuma, A., Ishii, Y. and Imura, N. (1984) Effect of administration sequence of mercuric chloride and sodium selenite on their fates and toxicities in mice, *Ecotoxicology and environmental safety*, 8(6), pp. 572-580.
- Navis, S., Waterkeyn, A., Voet, T., De Meester, L. and Brendonck, L. (2013) Pesticide exposure impacts not only hatching of dormant eggs, but also hatchling survival and performance in the water flea Daphnia magna, *Ecotoxicology*, 22(5), pp. 803-814.

- Noyes, P. D., McElwee, M. K., Miller, H. D., Clark, B. W., Van Tiem, L. A., Walcott, K. C., Erwin, K. N. and Levin, E. D. (2009) The toxicology of climate change: Environmental contaminants in a warming world, *Environment International*, 35(6), pp. 971-986. doi: 10.1016/j.envint.2009.02.006.
- Okamoto, A., Yamamuro, M. and Tatarazako, N. (2015) Acute toxicity of 50 metals to *Daphnia magna*, *Journal of Applied Toxicology*, 35(7), pp. 824-830.
- Orcutt, J. D. and Porter, K. G. (1983) Diel vertical migration by zooplankton: Constant and fluctuating temperature effects on life history parameters of *Daphnia*, *Limnology and Oceanography*, 28(4), pp. 720-730. doi: 10.4319/lo.1983.28.4.0720.
- Pacyna, E. and Pacyna, J. (2002) Global emission of mercury from anthropogenic sources in 1995, *Water, Air, and Soil Pollution*, 137(1-4), pp. 149-165.
- Pacyna, E. G., Pacyna, J. M., Steenhuisen, F. and Wilson, S. (2006) Global anthropogenic mercury emission inventory for 2000, *Atmospheric environment*, 40(22), pp. 4048-4063.
- Pacyna, E. G., Pacyna, J., Sundseth, K., Munthe, J., Kindbom, K., Wilson, S., Steenhuisen, F. and Maxson, P. (2010) Global emission of mercury to the atmosphere from anthropogenic sources in 2005 and projections to 2020, *Atmospheric environment*, 44(20), pp. 2487-2499.
- Parizek, J., Kalouskova, J., Benes, J. and Pavlik, L. (1980) Interactions of selenium-mercury and selenium-selenium compounds, *Annals of the New York Academy of Sciences*, 355(1), pp. 347-360.
- Parmesan, C., Ryrholm, N., Stefanescu, C., Hill, J. K., Thomas, C. D., Descimon, H., Huntley, B., Kaila, L., Kullberg, J., Tammaru, T., Tennent, W. J., Thomas, J. A. and Warren, M. (1999) Poleward shifts in geographical ranges of butterfly species associated with regional warming, *Nature*, 399, pp. 579. doi: 10.1038/21181.
- Paul, R., Lamkemeyer, T., Maurer, J., Pinkhaus, O., Pirow, R., Seidl, M. and Zeis, B. (2004) Thermal acclimation in the microcrustacean *Daphnia*: a survey of behavioural, physiological and biochemical mechanisms, *Journal of Thermal Biology*, 29(7-8), pp. 655-662.
- Persoone, G., Baudo, R., Cotman, M., Blaise, C., Thompson, K. C., Moreira-Santos, M., Vollat, B., Törökne, A. and Han, T. (2009) Review on the acute *Daphnia magna* toxicity test—Evaluation of the sensitivity and the precision of assays performed with organisms from laboratory cultures or hatched from dormant eggs, *Knowledge and management of aquatic ecosystems*, (393), pp. 01p02-01p29.
- Persson, J., Brett, M. T., Vrede, T. and Ravet, J. L. (2007) Food quantity and quality regulation of trophic transfer between primary producers and a keystone grazer (*Daphnia*) in pelagic freshwater food webs, *Oikos*, 116(7), pp. 1152-1163. doi: 10.1111/j.0030-1299.2007.15639.x.
- Petchey, O. L., McPhearson, P. T., Casey, T. M. and Morin, P. J. (1999) Environmental warming alters food-web structure and ecosystem function, *Nature*, 402, pp. 69. doi: 10.1038/47023.
- Pijanowska, J. and Stolpe, G. (1996) Summer diapause in *Daphnia* as a reaction to the presence of fish, *Journal of Plankton Research*, 18(8), pp. 1407-1412. doi: 10.1093/plankt/18.8.1407.
- Pirrone, N., Cinnirella, S., Feng, X., Finkelman, R. B., Friedli, H. R., Leaner, J., Mason, R., Mukherjee, A. B., Stracher, G. B. and Streets, D. G. (2010) Global mercury emissions to the atmosphere from anthropogenic and natural sources, *Atmospheric Chemistry and Physics*, 10(13), pp. 5951-5964.
- Potter, S. and Matrone, G. (1974) Effect of selenite on the toxicity of dietary methyl mercury and mercuric chloride in the rat, *The Journal of nutrition*, 104(5), pp. 638-647.
- Pratt, D. M. (1943) Analysis of population development in *Daphnia* at different temperatures, *The Biological Bulletin*, 85(2), pp. 116-140.
- Preston, B. and Snell, T. (2001) Full life-cycle toxicity assessment using rotifer resting egg production: implications for ecological risk assessment, *Environmental Pollution*, 114(3), pp. 399-406.
- Pörtner, H. (2001) Climate change and temperature-dependent biogeography: oxygen limitation of thermal tolerance in animals, *Naturwissenschaften*, 88(4), pp. 137-146.
- Ralston, N. V., Blackwell, J. L. and Raymond, L. J. (2007) Importance of molar ratios in selenium-dependent protection against methylmercury toxicity, *Biological Trace Element Research*, 119(3), pp. 255-268.
- Rathore, R. S. and Khangarot, B. (2002) Effects of temperature on the sensitivity of sludge worm *Tubifex tubifex* Müller to selected heavy metals, *Ecotoxicology and environmental safety*, 53(1), pp. 27-36.

- Ravichandran, M. (2004) Interactions between mercury and dissolved organic matter—a review, *Chemosphere*, 55(3), pp. 319-331.
- Rees, W. E. (1992) Ecological footprints and appropriated carrying capacity: what urban economics leaves out, *Environment and urbanization*, 4(2), pp. 121-130.
- Rinke, K. and Vijverberg, J. (2005) A model approach to evaluate the effect of temperature and food concentration on individual life-history and population dynamics of *Daphnia*, *Ecological Modelling*, 186(3), pp. 326-344.
- Rodrigues, A. C., Jesus, F. T., Fernandes, M. A., Morgado, F., Soares, A. M. and Abreu, S. N. (2013) Mercury toxicity to freshwater organisms: extrapolation using species sensitivity distribution, *Bulletin of environmental contamination and toxicology*, 91(2), pp. 191-196.
- Rouger, R., Reichel, K., Malrieu, F., Masson, J. P. and Stoeckel, S. (2016) Effects of complex life cycles on genetic diversity: cyclical parthenogenesis, *Heredity*, 117, pp. 336. doi: 10.1038/hdy.2016.52.
- Rydberg, J., Klaminder, J., Rosén, P. and Bindler, R. (2010) Climate driven release of carbon and mercury from permafrost mires increases mercury loading to sub-arctic lakes, *Science of The Total Environment*, 408(20), pp. 4778-4783.
- Savage, V. M., Gillooly, J. F., Brown, J. H., West, G. B. and Charnov, E. L. (2004) Effects of body size and temperature on population growth, *The American Naturalist*, 163(3), pp. 429-441.
- Schalau, K., Rinke, K., Straile, D. and Peeters, F. (2008) Temperature is the key factor explaining interannual variability of *Daphnia* development in spring: a modelling study, *Oecologia*, 157(3), pp. 531-543.
- Scheuhammer, A. (1987) The chronic toxicity of aluminium, cadmium, mercury, and lead in birds: a review, *Environmental Pollution*, 46(4), pp. 263-295.
- Schiedek, D., Sundelin, B., Readman, J. W. and Macdonald, R. W. (2007) Interactions between climate change and contaminants, *Marine pollution bulletin*, 54(12), pp. 1845-1856.
- Schroeder, W. H. and Munthe, J. (1998) Atmospheric mercury—an overview, *Atmospheric environment*, 32(5), pp. 809-822.
- Schwartz, S. S. (1984) Life history strategies in *Daphnia*: a review and predictions, *Oikos*, pp. 114-122.
- Sell, J. and Horani, F. (1974) Influence of selenium on toxicity and metabolism of methylmercury in chicks and quail, *Nutr. Rep. Int.:(United States)*, 14(4).
- Shurin, J. B. and Dodson, S. I. (1997) Sublethal toxic effects of cyanobacteria and nonylphenol on environmental sex determination and development in *Daphnia*, *Environmental Toxicology and Chemistry*, 16(6), pp. 1269-1276.
- Sibly, R. and Calow, P. (1989) A life-cycle theory of responses to stress, *Biological Journal of the Linnean Society*, 37(1-2), pp. 101-116.
- Snell, T. W. and Carmona, M. J. (1995) Comparative toxicant sensitivity of sexual and asexual reproduction in the rotifer *Brachionus calyciflorus*, *Environmental Toxicology and Chemistry*, 14(3), pp. 415-420.
- Sokolova, I. M. and Lannig, G. (2008) Interactive effects of metal pollution and temperature on metabolism in aquatic ectotherms: implications of global climate change, *Climate Research*, 37(2/3), pp. 181-201.
- Sonke, J. E., Heimbürger, L.-E. and Dommergue, A. (2013) Mercury biogeochemistry: paradigm shifts, outstanding issues and research needs, *Comptes Rendus Geoscience*, 345(5-6), pp. 213-224.
- Swain, E. B., Engstrom, D. R., Brigham, M. E., Henning, T. A. and Brezonik, P. L. (1992) Increasing rates of atmospheric mercury deposition in midcontinental North America, *Science*, 257(5071), pp. 784-787.
- Sørmo, E. G., Ciesielski, T. M., Øverjordet, I. B., Lierhagen, S., Eggen, G. S., Berg, T. and Jenssen, B. M. (2011) Selenium moderates mercury toxicity in free-ranging freshwater fish, *Environmental science & technology*, 45(15), pp. 6561-6566.
- Tan, S. W., Meiller, J. C. and Mahaffey, K. R. (2009) The endocrine effects of mercury in humans and wildlife, *Critical reviews in toxicology*, 39(3), pp. 228-269.
- Team, R. C. (2017) R: a language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing; 2017.
- Tsui, M. T. and Wang, W.-X. (2004a) Temperature influences on the accumulation and elimination of mercury in a freshwater cladoceran, *Daphnia magna*, *Aquatic Toxicology*, 70(3), pp. 245-256. doi: 10.1016/j.aquatox.2004.09.006.

- Tsui, M. T. and Wang, W.-X. (2004b) Uptake and elimination routes of inorganic mercury and methylmercury in *Daphnia magna*, *Environmental science & technology*, 38(3), pp. 808-816.
- Tsui, M. T. and Wang, W.-X. (2005) Multigenerational acclimation of *Daphnia magna* to mercury: relationships between biokinetics and toxicity, *Environmental Toxicology and Chemistry*, 24(11), pp. 2927-2933. doi: 10.1897/05-085R.1
- Tsui, M. T. and Wang, W.-X. (2006) Acute toxicity of mercury to *Daphnia magna* under different conditions, *Environmental science & technology*, 40(12), pp. 4025-4030.
- Tsui, M. T. and Wang, W. X. (2007) Biokinetics and tolerance development of toxic metals in *Daphnia magna*, *Environmental Toxicology and Chemistry*, 26(5), pp. 1023-1032.
- Van Doorslaer, W., Stoks, R., Duvivier, C., Bednarska, A. and De Meester, L. (2009) Population dynamics determine genetic adaptation to temperature in *Daphnia* *Evolution*, 63(7), pp. 1867-1878. doi: 10.1111/j.1558-5646.2009.00679.x.
- Verberk, W. C., Bilton, D. T., Calosi, P. and Spicer, J. I. (2011) Oxygen supply in aquatic ectotherms: partial pressure and solubility together explain biodiversity and size patterns, *Ecology*, 92(8), pp. 1565-1572.
- Verlecar, X., Jena, K. and Chainy, G. (2007) Biochemical markers of oxidative stress in *Perna viridis* exposed to mercury and temperature, *Chemico-biological interactions*, 167(3), pp. 219-226.
- Walker, C. (2014) *Ecotoxicology: effects of pollutants on the natural environment*. CRC Press.
- Walther, G.-R., Post, E., Convey, P., Menzel, A., Parmesan, C., Beebee, T. J. C., Fromentin, J.-M., Hoegh-Guldberg, O. and Bairlein, F. (2002) Ecological responses to recent climate change, *Nature*, 416, pp. 389. doi: 10.1038/416389a.
- Watras, C. J., Back, R. C., Halvorsen, S., Hudson, R. J. M., Morrison, K. A. and Wentz, S. P. (1998) Bioaccumulation of mercury in pelagic freshwater food webs, *Science of The Total Environment*, 219(2), pp. 183-208. doi: 10.1016/S0048-9697(98)00228-9.
- Weetman, D. and Atkinson, D. (2004) Evaluation of alternative hypotheses to explain temperature-induced life history shifts in *Daphnia*, *Journal of Plankton Research*, 26(2), pp. 107-116.
- Whitehead, P., Wilby, R., Battarbee, R., Kernan, M. and Wade, A. J. (2009) A review of the potential impacts of climate change on surface water quality, *Hydrological Sciences Journal*, 54(1), pp. 101-123.
- Winder, M. and Schindler, D. E. (2004) Climate change uncouples trophic interactions in an aquatic ecosystem *Ecology*, 85(8), pp. 2100-2106. doi: 10.1890/04-0151.
- Wolf, H. G. and Carvalho, G. R. (1989) Resting eggs of lake-Daphnia II. In situ observations on the hatching of eggs and their contribution to population and community structure, *Freshwater biology*, 22(3), pp. 471-478.
- Woodward, G. (2009) Biodiversity, ecosystem functioning and food webs in fresh waters: assembling the jigsaw puzzle, *Freshwater biology*, 54(10), pp. 2171-2187.
- Woodward, G., Perkins, D. M. and Brown, L. E. (2010) Climate change and freshwater ecosystems: impacts across multiple levels of organization, *Philosophical Transactions of the Royal Society B: Biological Sciences*, 365(1549), pp. 2093-2106. doi: 10.1098/rstb.2010.0055.
- Yang, D.-Y., Chen, Y.-W., Gunn, J. M. and Belzile, N. (2008) Selenium and mercury in organisms: interactions and mechanisms, *Environmental Reviews*, 16(NA), pp. 71-92.
- Yvon-Durocher, G., Montoya, J. M., Trimmer, M. and Woodward, G. U. Y. (2011) Warming alters the size spectrum and shifts the distribution of biomass in freshwater ecosystems, *Global Change Biology*, 17(4), pp. 1681-1694. doi: 10.1111/j.1365-2486.2010.02321.x.
- Zhou, Z. S., Wang, S. J. and Yang, Z. M. (2008) Biological detection and analysis of mercury toxicity to alfalfa (*Medicago sativa*) plants, *Chemosphere*, 70(8), pp. 1500-1509.
- Zuo, W., Moses, M. E., West, G. B., Hou, C. and Brown, J. H. (2012) A general model for effects of temperature on ectotherm ontogenetic growth and development, *Proceedings of the Royal Society of London B: Biological Sciences*, 279(1734), pp. 1840-1846.
- Zuur, A. F., Ieno, E. N., Walker, N., Saveliev, A. A. and Smith, G. M. (2009) *Mixed effects models and extensions in ecology with R*. Springer New York : Imprint: Springer.

## Appendix A

**Table A.1** Protocol for preparing selenium dioxide altered Aachener Daphnien Medium (ADaM) using distilled water and the described chemicals. Stock solutions of calcium chloride dihydrate, sodium bicarbonate, selenium dioxide, and sodium chloride are prepared in advance in distilled water (1 L) and stored in a cold storage (~4°C). Concentration (g/L) and volume (ml/L) of the stock solutions are presented per 1 L distilled water.

Chemical	Concentration (g/L)	Volume (mL/L)
Sodium chloride (NaCl)	123	1.23
Calcium chloride dihydrate (CaCl <sub>2</sub> × 2H <sub>2</sub> O)	117.6	2.3
Sodium bicarbonate (NaHCO <sub>3</sub> )	25.2	2.2
Selenium dioxide (SeO <sub>2</sub> )	0.07	0.1

[HgCl <sub>2</sub> ]	Temperature	
	17 °C	24 °C
0.5	1 2 3	1 2 3
1.75	4 5 6	4 5 6
Control	7 8 9	7 8 9

**Figure A.1** Experimental setup for the pilot study. *D. magna* were exposed to two concentrations of HgCl<sub>2</sub> (0.5 and 1.75 µg L<sup>-1</sup>) at two temperatures (17 and 24°C) for two weeks. Each treatment consisted of three replicates, represented by individual numbers on the figure. The control treatment consisted of ADaM.

Total weekly population biomass was calculated from video recording analysis using *R* (*trackdem*) by estimating a relationship between the animals' mean body size (dry mass) and their pixel size. Equation A.1 and A.2 calculate the animals' mean dry mass based on the standard curve and their biomass based on the dry mass, respectively. "Pixels" is measured as mean size of the daphnids. "Population count" is measured as the number of moving particles within the recording.

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

$$\text{Biomass} = \text{Mean dry mass} \times \text{Population count} \quad [\text{A.2}]$$

**Table A.2** Nominal and measured temperatures (mean  $\pm$  standard deviation [SD]) in the medium of beakers in the climate cabinets throughout the nine-week exposure period.

Nominal temperatures	Measured temperatures
17°C	15.5 $\pm$ 0.6°C
24°C	23.3 $\pm$ 0.6°C



## Appendix B

**Table B.1** Summary output, including coefficient values, standard error (Std. E), t-value, and *p*-value for best model fit by maximum likelihood describing final biomass as a function of temperature (17 and 24°C) and set (1 and 2).

Model: Final biomass ~ Temperature + Set

<b>Coefficients</b>	<b>Value</b>	<b>Std. E</b>	<b>t-value</b>	<b><i>p</i>-value</b>
Intercept	7.6062	0.2085	36.4726	< 0.001
24°C	- 4.3988	0.2122	- 20.7299	< 0.001
Set 2	- 0.3975	0.1647	- 2.4137	0.019

**Table B.2** Summary output, including coefficient values, standard error (Std. E), t-value, and *p*-value for best model fit by maximum likelihood describing maximum biomass obtained during the experiment as a function of temperature (17 and 24°C), HgCl<sub>2</sub> treatment (control, low [0.5 µg L<sup>-1</sup>], and high [2.0 µg L<sup>-1</sup>]), and set (1 and 2).

Model: Maximum biomass ~ Temperature + (HgCl<sub>2</sub> × Set)

<b>Coefficients</b>	<b>Value</b>	<b>Std. E</b>	<b>t-value</b>	<b><i>p</i>-value</b>
Intercept	9.5729	0.2620	36.5313	< 0.001
24°C	- 3.2642	0.1981	- 16.4782	< 0.001
Low HgCl <sub>2</sub>	- 0.7670	0.3431	- 2.2355	0.0296
High HgCl <sub>2</sub>	- 1.0391	0.3431	- 3.0285	0.0038
Set 2	- 1.2248	0.3431	- 3.5697	0.0008
Low HgCl <sub>2</sub> × Set 2	1.1703	0.4852	2.4120	0.0194
High HgCl <sub>2</sub> × Set 2	0.7691	0.4852	1.5664	0.1232

**Table B.3** Summary output, including coefficient values, standard error (Std. E), t-value, and *p*-value for best model fit by maximum likelihood describing total resting egg production as a function of temperature (17 and 24°C) and HgCl<sub>2</sub> treatment (control, low [0.5 µg L<sup>-1</sup>], and high [2.0 µg L<sup>-1</sup>]).

Model: Cumulative number of resting eggs ~ HgCl<sub>2</sub> × Temperature

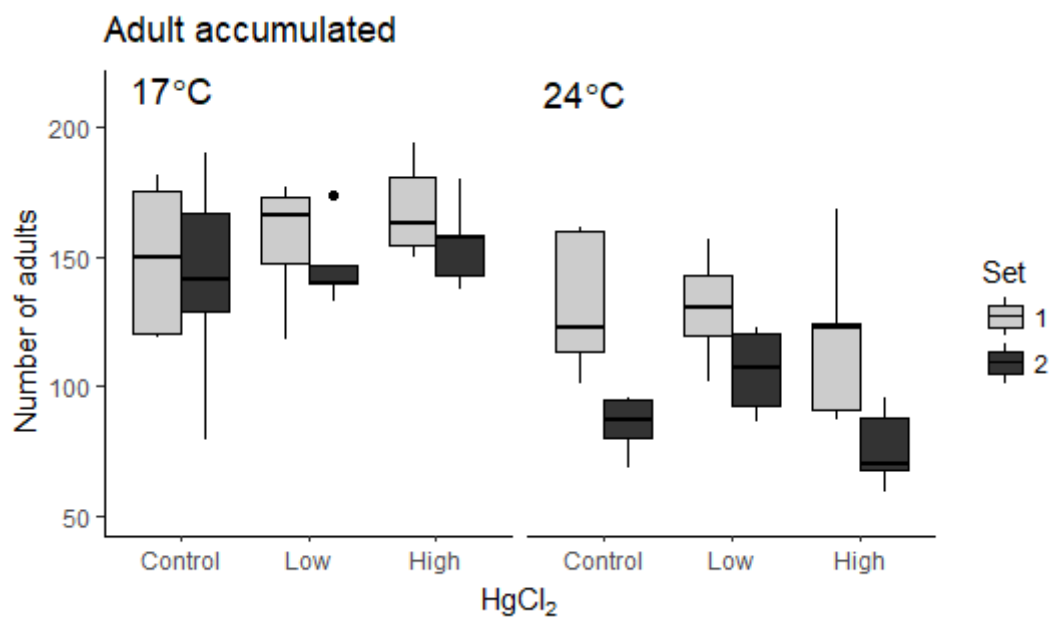
<b>Coefficients</b>	<b>Value</b>	<b>Std. E</b>	<b>t-value</b>	<b><i>p</i>-value</b>
Intercept	29.5	9.2031	3.2055	0.0023
Low HgCl <sub>2</sub>	9.7	13.0151	0.7453	0.4593
High HgCl <sub>2</sub>	36.5	13.0151	2.8044	0.0070
24°C	79.4	13.0151	6.1006	< 0.001
Low HgCl <sub>2</sub> × 24°C	13.7	18.4061	0.7443	0.4599
High HgCl <sub>2</sub> × 24°C	- 44.5	18.4061	- 2.4177	0.0190

**Table B.4** Summary output, including coefficient values, standard error (St. E), t-value, and *p*-value for best model fit by maximum likelihood describing cumulative resting eggs per adult accumulated as a function of temperature (17 and 24°C), HgCl<sub>2</sub> treatment (control, low [0.5 µg L<sup>-1</sup>], and high [2.0 µg L<sup>-1</sup>]), and set (1 and 2).

Model: Resting eggs per adult ~ HgCl<sub>2</sub> + (Temperature × Set)

<b>Coefficients</b>	<b>Value</b>	<b>Std. E</b>	<b>t-value</b>	<b><i>p</i>-value</b>
Intercept	0.2342	0.0513	4.5669	< 0.001
Low HgCl <sub>2</sub>	0.0569	0.0601	0.9474	0.3477
High HgCl <sub>2</sub>	0.1670	0.0601	2.7795	0.0075
24°C	0.5778	0.0958	6.0337	< 0.001
Set 2	- 0.0475	0.0534	- 0.8892	0.3779
24°C × Set 2	0.5159	0.1354	3.8088	0.0004

## Appendix C



**Figure C.1** Total number of *D. magna* adult individuals at 17 and 24°C obtained during nine-week exposure to control, low ( $0.5 \mu\text{g L}^{-1}$ ), and high ( $2.0 \mu\text{g L}^{-1}$ ) concentrations of  $\text{HgCl}_2$  ( $n = 60$ ). The middle, lower, and upper horizontal lines represent median, 25<sup>th</sup>, and 75<sup>th</sup> percentile of the data, respectively. Whiskers extend up to  $1.5 \times$  the interquartile range (IQR) of the sample. The black dot is an outlying value extending beyond  $1.5 \times$  IQR.