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Semona Issa

Combined effects of environmental variation and pollution on zooplankton life history and population dynamics

Doctoral thesis

NTNU
Norwegian University of Science and Technology
Thesis for the Degree of
Philosophiae Doctor
Faculty of Natural Sciences
Department of Biology

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Trondheim, March 2021

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A female *Daphnia* with eggs,

ready to fight any stressor,

like a true superhero.

Preface

In the past four years, I've met some wonderful people, some of which I've had the pleasure to work with, some to celebrate with, others both.

Of these, two have greatly shaped my scientific path: my supervisors, Sigurd and Veerle. Your skills combined have taught me a great deal over the years. Thank you for always being patient and supportive. Tomasz and Marlene, it was a pleasure working with you.

To the members of the *Daphnia* and ENVITOX groups, for the social events, the knowledge exchange and the scientific contributions. To CBD, who I am lucky to be part of, thank you for the rich working environment and the endless supply of wine. To my friends, especially those who I've met during my PhD, I can't thank you enough for making Trondheim a warm home far from home.

Finally, much love and appreciation to my family, for their endless support. Especially mom and Ole, I am forever grateful to you both.

Semona Issa
Trondheim
December 2020

Contents

List of papers

I. **Issa S**, Ciesielski TM, Mikkelsen Ø, Einum S, & Jaspers VLB (2020) Biofilms grown in aquatic microcosms affect mercury and selenium accumulation in *Daphnia*. *Ecotoxicology* 29: 485-492. doi:10.1007/s10646-020-02194-4

II. **Issa S**, Gamelon M, Ciesielski TM, Vike-Jonas K, Asimakopoulos AG, Jaspers VLB, & Einum S (2020) Dopamine mediates life-history responses to food abundance in *Daphnia*. *Proceedings of the Royal Society B: Biological Sciences* 287: 20201069. doi:10.1098/rspb.2020.1069

III. **Issa S**, Simonsen A, Jaspers VLB, & Einum S. (in press). Population dynamics and resting egg production in *Daphnia*: Interactive effects of mercury, population density and temperature. *Science of the Total Environment*

IV. **Issa S**, Chaabani S, Asimakopoulos AG, Jaspers VLB, & Einum S. Maternal dopamine exposure in *Daphnia* boosts the survival of starved offspring. Manuscript.

Author contributions

I: SI conceived the study. SI, SE, VLBJ, TMC and ØM designed the study. SI carried out the laboratory work. SI analysed the data with input from SE and VLBJ. SI wrote the manuscript with contributions and comments from all co-authors.

II: SE conceived the study. SI, SE, VLBJ and TMC designed the study. SI carried out the laboratory work. SI analysed the data with input from SE and MG. AGA and KV-J carried out the UPLC-MS/MS analysis. SI wrote the manuscript with contributions and comments from all co-authors.

III: SI conceived the study. SI, AS, SE and VLBJ designed the study. AS carried out the laboratory work. SI analysed the data with input from SE. SI wrote the manuscript based on the master thesis of AS, with contributions and comments from SE and VLBJ.

IV: SI and SE conceived the study. SI, SE and VLBJ designed the study. SI and SC carried out the laboratory work. SI analysed the data. AGA headed the UPLC-MS/MS analysis. SI drafted the manuscript with contributions and comments from all co-authors.

Introduction

Toxicity and standardization

Toxicity studies on freshwater organisms are commonly conducted under highly standardized conditions that exclude interactions with environmental factors (OECD, 2004). *Daphnia* are keystone zooplankton species in freshwater ecosystems and commonly used as model organisms for ecotoxicological tests (Shaw et al., 2008). Hence, effects of chemical pollutants on *Daphnia* can potentially impact different trophic levels in the aquatic ecosystem. However, aquatic invertebrates like *Daphnia* regularly experience extensive spatial and temporal variation of environmental factors in nature, which may interact with pollutant exposure and effects and as such influence important characteristics of individuals and populations (Kim et al., 2010; Wang, 1987).

Mercury

Metal pollution in aquatic ecosystems is a worldwide concern (Fernández-Luqueño et al., 2013). The heavy metal mercury (Hg) receives a distinctly high attention, because of its long-range transport across the globe and its high toxic properties (Lavoie et al., 2013; UNEP, 2013). Most of the Hg present in the environment comes from anthropogenic sources such as fossil fuel combustion, mining and waste incineration (Eisler, 2006). In turn, the bioavailability and bioaccumulation potential of Hg determines its level of toxicity and varies according to the metal's chemical and physical form. The methylated forms of Hg are the most toxic and bioaccumulative forms. Their high bioaccumulation rate is due to their high lipid solubility (Govind & Madhuri, 2014). The less toxic inorganic Hg, in the form of mercuric ions (Hg^{2+}), is mostly soluble in water. In aquatic environments, organisms can take up Hg^{2+} from water by adsorption onto and absorption through biological membranes, in addition to food ingestion (Boening, 2000). The toxic action of Hg can in turn be counteracted by the essential nutrient selenium (Se). In the biota, Se binding to Hg yields an insoluble and inert Hg-Se complex, which reduces the bioavailability of Hg and potentially mitigates its toxicity (Raymond & Ralston, 2004).

Antidepressants

Pharmaceutical residues are widespread pollutants in aquatic ecosystems. Some of the most frequently found pharmaceutical residues in aquatic ecosystems are antidepressants, which inhibit the reuptake of different neurotransmitters (Fent et al., 2006). Antidepressants enter aquatic ecosystems through multiple pathways, most importantly through wastewater and the household disposal of unused products (Bound & Voulvoulis, 2005). By directly

affecting the nervous systems, exposure to antidepressants can alter the behaviour, development, reproduction and survival of aquatic biota (Brodin et al., 2014; Sehonova et al., 2018).

Environmental variation

Despite organisms in nature commonly experiencing environmental variation, standard ecotoxicological studies generally overlook potential pollutant interactions with environmental factors. For example, trophic interactions can greatly influence the bioavailability of metals and hence their toxicity. Indeed, aquatic biofilms (algae) have been shown to readily accumulate Hg (Dranguet et al., 2017). Because they serve as food for aquatic grazers (Siehoff et al., 2009), biofilms can affect the accumulation of metals in *Daphnia*. Despite the effect that biofilms may have on the bioaccumulation and toxicity of metals, classic toxicity studies prevent their growth in the exposure media. Other environmental factors that may influence the effect of pollutants to *Daphnia* are temperature and population density. At elevated temperatures, a higher metabolic rate and cell membrane permeability can e.g. increase metal uptake from food and the aqueous environment (Sokolova & Lannig, 2008). In turn, high population density and associated high intraspecific competition, can reduce the per capita amount of resources available for allocation to detoxification and repair processes (Heugens et al., 2001). Resource availability and abundance is an additionally important factor in pharmaceutical pollution in aquatic ecosystems. Indeed, interactive effects between antidepressant disruption of neurotransmitter systems and resource abundance have been previously documented in aquatic biota (Campos et al., 2012), but remain understudied.

Plasticity, life history and food

Phenotypic plasticity is an environmentally induced phenomenon, whereby a given genotype displays different phenotypes in response to a changing environment (West-Eberhard, 2003). The slope and elevation of the relationship between environment and phenotype can be described as a reaction norm (Ghalambor et al., 2007). Under natural selection, adaptive phenotypic plasticity evolves such that the resulting reaction norm maximizes fitness across the changing environment (Ghalambor et al., 2007; Lande, 2009). Phenotypic plasticity can also be transferred from mothers to offspring, with effects to offspring fitness (Marshall, 2008). Therefore, expressing adaptive reaction norms is important for maintaining high fitness and population viability in environments that vary across space and in time. This is especially true for reaction norms in response to food abundance, as changes in these can have

strong effects on different components of life history, such as reproduction, growth and somatic maintenance (Boggs, 2009). Indeed, trade-offs between life-history traits are common, due to the need to allocate a limited amount of resources to one life-history trait versus the other (Ricklefs & Wikelski, 2002). In turn, food abundance can alter how individuals allocate their resources to different traits, thereby affecting these trade-offs (Kaiser et al., 2015).

Dopamine and food

Dopamine is a key neurotransmitter in crustaceans and other biota. It is involved in many different metabolic pathways, with widespread effects in the nervous, cardiovascular, and endocrine systems, as well as potentially involved in modulating adaptive behaviour (Tierney et al., 2003). Dopamine has been shown to play an important role in regulating behavioural and morphological responses to food availability (See Barron et al. (2010) for a review on dopamine-mediated behavioural and morphological responses to food across taxa). Hence, the dopamine system may also be important in regulating the responses of life-history traits to food availability and abundance. If this is the case, chemically induced changes to dopamine levels, through for example the action of antidepressants that modulate the reuptake of dopamine, can be expected to alter these life-history responses.

Aims

Individual and population-level responses to long-term chemical exposures are typically understudied. Moreover, most of the existing data have been collected from laboratory experiments conducted under highly standardized conditions that exclude interactions with environmental factors. This leads me to the first aim of this thesis: to extend the knowledge on both individual and population-level effects of chemical pollutants in aquatic ecosystems, with focus on Hg and antidepressants, under different environmental scenarios. The second aim of this thesis is to answer questions about ecology and evolution from an ecotoxicological perspective, using pollutant exposure studies. *Daphnia magna* was used as a focal species to address these aims.

Specific questions

1. Is metal toxicity a function of the environment and population density **(papers I and III)**?

2. Is dopamine involved in regulating life-history responses to food and how do changes in dopamine affect fitness **(papers II and IV)**?

3. Which additional fitness measures that are typically overlooked in pollutant exposure studies are important to consider **(papers II, III and IV)**?

General methods

Daphnia as a model organism

Daphnia are short-lived, with a relatively short generation time, freshwater keystone zooplankton (Decaestecker et al., 2009). They are highly suitable for and widely used as model organisms in toxicological and ecological studies (Lampert, 2011; Shaw et al., 2008), and have also been used in studies of the dopamine signalling system (Bownik et al., 2018; McCoole et al., 2012). *Daphnia* are keystone consumers of phytoplankton, bacteria and fungi, as well as a key food source for fish and larger invertebrates (Miner et al., 2012). In addition to their key ecological role in freshwater ecosystems, *Daphnia* display a worldwide distribution (Adamowicz et al., 2009). These characteristics are only a few of the reasons why *Daphnia*, specifically *D. magna*, were chosen as a study species in this thesis.

D. magna, including most *Daphnia* species, are facultative parthenogenetic organisms with an asexual (parthenogenetic) phase and a sexual phase (Fig. 1). During the asexual phase, the mature female produces diploid eggs that develop directly into daughters that are genetically identically to their mother. This is the primary mode of reproduction in *D. magna* (Ebert, 2005), and it allows for clones (genetically identical individuals) to be maintained asexually for many generations. *Daphnia* clones are in turn a useful tool for studying phenotypic plasticity. On one hand, using individuals of the same clone allows for the observed phenotypic variation in response to environmental change between individuals, to be attributed directly to phenotypic plasticity rather than genetic variation amongst these individuals. On the other hand, different clones of *Daphnia* can be used to study the genotype-by-environment interaction (i.e. the genetic variation of reaction norms; Barata & Baird, 1998; Gianoli & Valladares, 2012).

The production of resting eggs, through sexual reproduction, is another characteristic of *Daphnia* that makes them particularly relevant to this thesis. The sexual cycle in *Daphnia* is initiated when environmental conditions deteriorate, starting by the asexual production of diploid males. Upon maturation, the males can fertilize haploid eggs produced by other females. This cycle ends with haploid eggs developing into resting eggs, which are enclosed in a protective membranous shell called an ephippium. The ephippium, typically containing two resting eggs, is released from the female's brood chamber through moulting (Ebert, 2005). The resting eggs within the ephippium can survive unfavourable environmental conditions for years and are thus a crucial component of the long-term fitness of *Daphnia* (Hebert, 1978).

Individuals that hatch from the resting eggs are genetically different from their mother (Ebert, 2005). This maintains genetic diversity within *Daphnia* populations and hence their potential for adaptation to new environmental conditions.

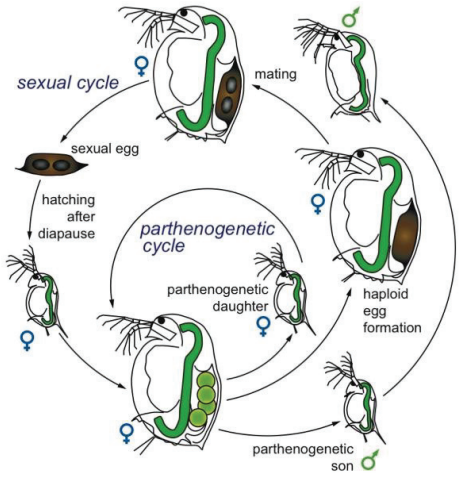

Fig. 1. *Life cycle of the facultative parthenogenetic D. magna. Artwork: Ebert (2005).*

Three different clones from a single wild population of *D. magna* were studied in this thesis. *D. magna* is one of the largest daphnid species in Norway, reaching a body length of 6 mm, which facilitates handling and observing experimental individuals. Ephippia containing resting eggs resulting from sexual reproduction of *D. magna* were collected in November 2014, in a pond at Værøy Island (1.0 ha, 67.687°N 12.672°E), northern Norway. Ephippial eggs were hatched in the laboratory and propagated clonally to obtain the three clones used in this thesis. The culture medium (and simultaneously the exposure medium) in which the daphnids are grown is a modified "Aachener Daphnien Medium" (ADaM), prepared according to the recipe of Klüttgen et al. (1994) but with a 50% reduction in the concentration of selenium dioxide (SeO_2).

Exposure compounds

Mercury (II) chloride ($HgCl_2$)

Mercury (II) chloride is a highly toxic water-soluble compound. When it dissolves, $HgCl_2$ releases mercuric ions (Hg^{2+}), which can be found in natural waters (Chau & Saitoh, 1970). In **papers I and III**, *D. magna* were exposed to non-lethal and environmentally relevant concentrations of Hg using 99.5% pure mercury (II) chloride.

Bupropion hydrochloride ($C_{13}H_{19}Cl_2NO$)

Bupropion was first introduced in the United States of America (US) in 1989 as an antidepressant for treatment of patients with major depressive disorder (Fava et al., 2005). Bupropion inhibits the neuronal reuptake of norepinephrine and dopamine, increasing their concentration in the synaptic cleft (Fig. 2; Richmond & Zwar, 2003; Stahl et al., 2004). It is also used as treatment for smoking cessation (Richmond & Zwar, 2003). In **paper II**, *D. magna* mothers were exposed to environmentally relevant concentrations of bupropion using bupropion hydrochloride.

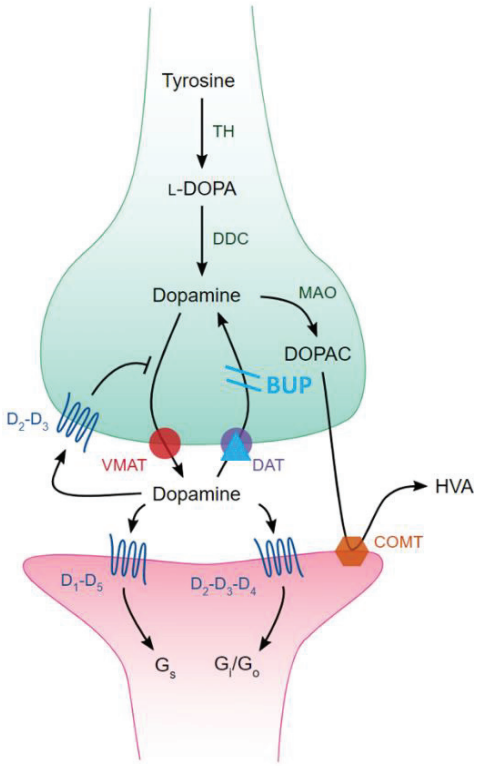

Fig. 2. *Bupropion (BUP) inhibits the reuptake of dopamine into the presynaptic neuron by blocking the dopamine reuptake pump (DAT). This causes an increase in the synaptic levels of dopamine. Original artwork: Smedlib (based on original work by Pancrat). The original artwork has been modified for use in this thesis.*

Dopamine hydrochloride ($C_8H_{12}ClNO_2$)

Dopamine hydrochloride, the hydrochloride salt form of dopamine, was first introduced as a drug in the US in 1982 for treatment of low blood pressure, low cardiac output and low blood flow to the kidneys (National Center for Biotechnology Information, 2020). In **papers II and IV**, *D. magna* mothers were exposed to dopamine hydrochloride at a concentration chosen for successfully inducing changes in their growth rate, based on previous work by Weiss et al. (2015). This was done to directly manipulate dopamine concentrations in the experimental organisms.

Chemical analysis

Analyses of Hg and Se (an ingredient of ADaM), in the daphnid tissue samples and exposure media, were performed at the department of chemistry at NTNU, using high resolution inductively coupled plasma mass spectrometry. This was done to detect changes in overall Hg and Se levels in the presence of biofilm. A detailed description of the chemical analyses can be found in **paper I**.

Analyses of dopamine and bupropion in the exposure media, were performed at the Faculty of Natural Sciences at NTNU, using ultra-performance liquid chromatography coupled to a triple quadrupole mass analyser. This was done to detect overall levels of dopamine and bupropion in the media. A detailed description of the chemical analysis can be found in **paper II**.

Demographic and life-history data

In **paper III**, the number of resting eggs produced by *D. magna* during the sexual phase was counted on a weekly basis. In addition, estimates on the number of live daphnid individuals and their sizes were obtained from weekly video recordings that

were analysed using the R package *trackdem*. From these estimates, weekly rates of biomass growth were derived. The data collected allowed us to study effects of Hg on the sexual and asexual phases of *D. magna* under varying environmental conditions.

In **papers II and IV**, a range of life-history traits were recorded in *D. magna*. These included age at maturation and age at second reproduction (defined as the time when eggs were first visible in the brood chamber), body length at maturation (measured from the upper margin of the eye to the junction of the carapace and spine), clutch size (defined as the number of live progeny released at a given reproductive event), body length of offspring, maternal longevity and offspring longevity under starvation. Rates of somatic growth and intrinsic population growth (*r*) were also calculated (See **paper II** for a detailed description of the equations used). Using this data, we investigated changes to life-history reaction norms in response to food abundance from maternal dopamine exposure, as well as associated fitness costs to offspring.

Main results and discussion

Metal toxicity as a function of the environment and population density

In **paper I**, we examined whether environmental variation in the form of biofilm affected metal bioavailability to individuals of *D. magna*. Specifically, we investigated how trophic interactions between *Daphnia* and biofilm growing in the exposure media altered Hg and Se accumulation in the animals. An important finding was the negative effect of biofilm on Hg content in *Daphnia*. Active uptake of Hg by biofilm can reduce Hg uptake by animals through multiple mechanisms. One mechanism is by reducing the amount of aqueous Hg available for uptake by the animals (Tsui & Wang, 2004). The other mechanism is by diluting the concentration of metals per algal cell, either through bloom dilution, as algal biomass increases (Chen & Folt, 2005), or through growth biodilution, specific to fast growing plankton (Hill & Larsen, 2005). Bloom dilution and growth biodilution lead to lower Hg exposure concentrations from diet than from the aqueous phase. However, since our findings did not show significant uptake of aqueous Hg by biofilm, the exact driver of the lower tissue Hg content in the presence of biofilm could not be determined. Nonetheless, there was successful uptake of aqueous Se by biofilm, which then acted as a dietary source of Se to the animals. Indeed, dietary uptake may be the predominant exposure route of Se to aquatic biota (Stewart et al., 2004). In turn, higher tissue Se content in the presence of biofilm boosted Se/Hg molar ratios in the animals, providing potential protection against Hg toxicity. These results overall show that biofilms can affect the transfer of metals across the aquatic food web. Hence, including interactions with biofilms in pollutant exposure studies may produce more realistic estimates on metal toxicity in aquatic ecosystems.

An additional biotic interaction that can influence effects of metals to focal organisms is intraspecific competition for food. In **paper III**, we examined how this interaction altered effects of Hg to *Daphnia* populations through density dependence at different temperatures. Biomass growth rate, which was a result of individual somatic growth, asexual reproduction and mortality, was surprisingly unaffected by Hg when population density and temperature were included. Generally, metal stress can affect biomass growth rate through changes in body size and population size. For example, energy expenditure associated with detoxification costs can lower survival and fecundity (Muyssen et al., 2006). Moreover, metal stress can impair feeding activity and thereby reduce the mean body size in the population (Enserink et al., 1995), or

contrastingly, eliminate smaller individuals from the population and increase mean body size (Bianchini et al., 2002). Previous studies have also shown that temperature and intraspecific competition can interact to enhance metal toxicity to *Daphnia*. Specifically, a higher metal uptake with increasing temperature, coupled with intraspecific competition and the metabolic costs of metal detoxification, can lower the amount of resources available for allocation to somatic growth and/or reproduction (Heugens et al., 2006). This was however not observed in our study, as population density interacted with temperature alone to influence biomass growth rate. Specifically, the density dependence of biomass growth rate increased with temperature, thereby reducing carrying capacity (i.e. the maximum population size sustainable by the environment over time) at high temperature. This two-way interaction was likely due to high metabolic rates at high temperature worsening food conditions for competing individuals (Savage et al., 2004).

Dopamine effects on life-history responses to food and fitness

Food abundance is one environmental factor that varies extensively in space and time. Expressing adaptive reaction norms in response to food abundance is important for individual fitness and population viability. At the molecular level, dopamine plays an important role in modulating behavioural and morphological responses to food (Adams et al., 2011; Wright et al., 2010). Through pollutant exposure studies **II and IV**, we showed that dopamine was also important in regulating life-history reaction norms to food abundance. Specifically, in the controls, low food abundance slowed somatic growth, thereby delaying reproduction and increasing size at maturation. Additionally, we observed a trade-off between offspring size and offspring number in **paper II**, with offspring size increasing at low food abundance at the expense of clutch size (same response in offspring size to food abundance in **paper IV**). Resource allocation theory predicts increased investment in adult size at the expense of early reproduction at low food levels (Ernande et al., 2004). In *Daphnia*, this is caused by an increase in the filtering rate and hence feeding rate with increasing body size (Porter et al., 1983), which is metabolically advantageous when food is scarce. This and the fact that mothers are energetically constrained, drives investment towards offspring size at the expense of clutch size at low food levels (Ebert, 1993). Hence, in both **papers II and IV**, controls responded adaptively to changes in food abundance.

Maternal dopamine exposure accelerated somatic growth and reproduction at the expense of adult size and offspring size at low

food abundance (**papers II and IV**). This resulted in higher population growth rates (*r*) at low food, compared to controls, without any costs to maternal longevity (**paper II**). Hence, by showing that changes to dopamine levels induced changes to life-history reaction norms in response to food abundance, we demonstrated for the first time that dopamine was a key regulator of life-history responses to food. However, the observed boost in fitness under dopamine exposure (boost in *r*), was unexpected, given that controls were supposed to have the highest fitness from having evolved adaptive reaction norms in response to food abundance. Moreover, the boost in *r* did not come at any observed costs to the fitness of mothers, raising the question of why *D. magna* do not evolve towards higher endogenous dopamine levels. We therefore suspect there may be proximate and ultimate constraints on why individuals do not produce higher endogenous dopamine. One proximate constraint may be that increased dopamine production potentially promotes dopamine oxidation and the production of reactive oxygen species (Blesa et al., 2015). This may in turn lead to oxidative stress and ultimately increased mortality risk, in the case of limited investment in energetically costly antioxidant defences (Alonso-Alvarez et al., 2006). On the other hand, a higher investment in antioxidant defences from increased dopamine oxidation may reduce investment in immune defences (Takahashi et al., 2017), making animals more vulnerable to parasites and diseases, as well as reduce investment in reproduction (Speakman & Garratt, 2013). Moreover, a faster growth from enhanced dopamine levels (**papers II and IV**), may increase predation risk from increased feeding in the presence of predators (Urban, 2007). Hence, investigating these potential fitness costs of enhanced dopamine levels may help explain the evolution of the dopamine system.

Our findings emphasize the role of dopamine as regulator of life-history responses to food abundance, as well as demonstrate that pharmaceutical pollution can strongly affect the life history of aquatic species such as *D. magna*.

Overlooked fitness measures in toxicity studies

Offspring survival and fitness

In **papers II and IV**, we found that maternal exposure to dopamine accelerated the life cycle of *D. magna* at the expense of offspring size. A smaller offspring size may have detrimental effects on offspring survival under low food levels because of lower feeding efficiency (Gliwicz & Guisande, 1992). Offspring survival and fitness is an important component of maternal fitness (Wolf & Wade, 2001), yet toxicity studies typically ignore maternal effects on offspring fitness (González-Pérez et al., 2018; Guo et

al., 2012). In **paper IV**, we investigated the potential costs to offspring survival under starvation from enhanced maternal dopamine levels. Mothers at low food abundance produced larger offspring than at high food abundance in both control and dopamine treatments. The larger offspring in turn survived longer under starvation. Mothers that experience low food abundance tend to produce offspring with a larger maternal lipid reserve, which can increase offspring starvation resistance (Tessier et al., 1983). Nonetheless, maternal dopamine exposure reduced offspring size at low food compared to controls. This however did not negatively affect offspring longevity. Indeed, offspring from mothers with enhanced dopamine levels survived longer than controls across food levels. Hence, we did not detect any costs to offspring survival and fitness from enhanced maternal dopamine levels. Further research on the ultimate and proximate costs of higher dopamine production is needed to better understand the evolution of the dopamine system.

Resting egg production

An additional fitness measure that is commonly overlooked in toxicity studies is one specific to *Daphnia*: resting egg production. Resting eggs are produced via sexual reproduction and can survive unfavourable environmental condition for a long period of time, making them crucial for long-term fitness in *Daphnia* (Hebert, 1978; Pijanowska & Stolpe, 1996). In **paper III**, we investigated for the first time how Hg affects sexual reproduction and the associated production of resting eggs in *D. magna,* under varying temperature and population density. We found that resting egg production was density dependent as well as overall higher at high temperature. This was expected, given that low resource availability and high encounter rates between females at high population density, in addition to heat stress, induce the production of resting eggs (Carvalho & Hughes, 1983; Holm et al., 2018). Temperature rise combined with high population density, can in turn lead to resource limitation, with negative effects on sexual reproduction under Hg stress. Indeed, Hg stress under high resource limitation can negatively affect resting egg production, if detoxification and repair processes are prioritized over reproduction (Heugens et al., 2001; Sokolova & Lannig, 2008). However, Hg interacted solely with temperature to affect resting egg production in this study. Specifically, resting egg production increased with increasing Hg levels at low temperature. Thermal stress at high temperature may have been more important at inducing resting egg production than Hg stress. Nonetheless, our findings support previous studies that have shown an increased production of resting eggs in response to metal exposure, suggested as an adaptive strategy against

adverse environmental conditions (Aránguiz-Acuña & Serra, 2016). The overall findings of **paper III** show that rates of sexual reproduction in *D. magna* can respond to metal exposure and that this depends on environmental conditions. Hence, resting egg production should be considered in toxicity studies using *Daphnia*, especially given its importance for individual fitness and population viability.

Synthesis and perspectives

Classic toxicity tests usually apply high levels of standardization. Hence, results from these can be difficult to extrapolate to natural environments considering that natural populations are constantly subjected to spatial and temporal environmental variation. With focus on metal and pharmaceutical pollution, we showed that effects of chemicals on individuals and populations vary with environmental conditions. By measuring effects of chemicals at environmentally relevant concentrations and under more natural conditions, we can obtain more realistic estimates of toxicity. In the case of metals, we found that the bioaccumulation of Hg and Se in zooplankton individuals depended on trophic interactions with biofilm components. Although biofilms are an important dietary source to aquatic grazers, classic toxicity tests hinder their growth in exposure media (OECD, 1992, 2012), likely because they vary highly in their composition across space and time. Nonetheless, we provided evidence that they are an important dietary source of Se to zooplankton, potentially providing additional protection against Hg toxicity. Since biofilm grazers can also act as conduits of metals to higher trophic levels (Cardoso et al., 2013), this may change the toxicity of Hg across the food web. Hence, excluding biofilm from metal exposure studies may lead to inaccurate predictions on metal toxicity to individuals and the aquatic ecosystem.

Temperature and population density are other environmental factors that were included in our focus on Hg toxicity in aquatic ecosystems. Indeed, a higher metabolic rate with increasing temperature, combined with an increase in intraspecific competition as the population grows, should potentiate resource depletion. This would then lower the amount of resources available for allocation to metal detoxification (Heugens et al., 2001). Hence, accurately predicting effects of Hg to populations under a scenario of climate change requires accounting for density dependence. Our results indeed showed that Hg-induced changes in *Daphnia* population dynamics depended on population density and temperature. Specifically, when temperature and density dependence were accounted for, Hg effects on population growth became relatively unimportant.

Not only did we show the importance of including environmental variation when looking at effects of metals, we also highlighted the importance of including resting egg production, an often-ignored life cycle component of *Daphnia*, despite its high relevance to long-term fitness. This is to our knowledge, the first study to look at effects of Hg on sexual reproduction in *Daphnia*. We

found that contrary to population growth, resting egg production responded to environmentally relevant concentrations of Hg, but only under specific temperature conditions. Hence, our findings build a solid case for including this aspect of *Daphnia* life cycle and biology, in addition to environmental variation, in toxicity studies.

Resource availability does not only affect metal toxicity to species, it can also largely alter their life history. Indeed, life-history traits can respond plastically to changes in food availability, with important implications for individual fitness (Ernande et al., 2004). Hence, expressing appropriate reaction norms in response to food is crucial. We investigated life-history responses to food abundance and the molecular pathways underlying these, by chemically enhancing dopamine levels in *Daphnia*. We found that life-history reaction norms to food abundance changed under dopamine and antidepressant exposure. This is the first study to ever show a role for dopamine in regulating life-history responses to food in species, as previous studies focused mainly on morphological and behavioural responses (Barron et al., 2010). Moreover, with specific focus on pharmaceutical pollution, we provided novel results on the effects of bupropion as a pollutant in aquatic ecosystems, while demonstrating that low but environmentally relevant levels of antidepressants can alter the life history of zooplankton species. Our results were nonetheless surprising, given that controls were expected to express adaptive reaction norms that procure the highest fitness. Indeed, life-history changes from dopamine and antidepressant exposure resulted in an accelerated life cycle and overall higher population growth rates, when food was restricted. This apparent boost in fitness without any detected maintenance costs raised the question of why *Daphnia* do not evolve towards higher endogenous dopamine levels. In order to try and answer this, we investigated potential costs to offspring survival from enhanced maternal dopamine levels under varying food abundance.

The negative maternal effects on offspring size from dopamine exposure did not lower offspring survival under starvation. This finding did not support a positive relationship between offspring size and starvation resistance in *Daphnia* (Gliwicz, 1990; Tessier & Consolatti, 1989). Contrastingly, maternal dopamine exposure boosted *Daphnia* offspring survival across food levels. By showing that maternally induced changes in offspring phenotype can affect offspring fitness, we highlight the importance of including maternal effects in chemical exposure studies. Moreover, we demonstrate the potential for using chemical exposures in evolutionary research. Indeed, our work opens for new questions regarding the evolution of the dopamine system.

Further understanding of the evolution of this system will require looking at other factors than offspring survival under starvation. These may include more proximate factors such as the energetically costly process of dopamine oxidation, which may ultimately lower survival through oxidative stress, or lower immunity and reproduction through increased investment in antioxidant defences. Other ultimate factors include increased predation risk from faster growth, expressed under enhanced dopamine levels. Hence, further understanding of the evolution of the dopamine signalling system may require a combined investigation of ultimate and proximate causes.

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Paper I

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Biofilms grown in aquatic microcosms affect mercury and selenium accumulation in *Daphnia*

Semona Issa[1] · Tomasz Maciej Ciesielski[2] · Øyvind Mikkelsen[3] · Sigurd Einum[1] · Veerle L. B. Jaspers[2]

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Abstract

Experiments examining mercury (Hg) toxicity in *Daphnia* are usually conducted in highly standardized conditions that prevent the formation of biofilm. Although such standardization has many advantages, extrapolation of results to natural conditions and inference of ecological effects is challenging. This is especially true since biofilms can accumulate metals/metalloids and play a key role in their transfer to higher trophic level organisms. In this study, we experimentally tested the effects of spontaneously appearing biofilm in *Daphnia* cultures on accumulation of Hg and its natural antagonist selenium (Se) in *Daphnia magna*. We added Hg (in the form of mercury (II) chloride) at two concentrations (0.2 µg/L and 2 µg/L) to experimental microcosms and measured the uptake of Hg and Se by *D. magna* in the presence and absence of biofilm. To test for consistent and replicable results, we ran two identical experimental sets one week apart. Biofilm presence significantly reduced the accumulation of Hg, while increasing the tissue Se content in *D. magna*, and these findings were reproducible across experimental sets. These findings indicate that highly standardized tests may not be adequate to predict the bioaccumulation and potential toxicity of metals/metalloids under natural conditions.

Keywords Ecotoxicity testing · Trophic transfer · Metal exposure · Biotic interaction

Introduction

Lab-based aquatic toxicity tests are usually conducted under highly standardized conditions (OECD 1992, 2004), which allows for comparisons of toxicity of different compounds from experiments run in different laboratories and during different times. Yet, extrapolating results of such studies to natural populations is challenging, as they overlook the potential for interactions with other components in the biotic community to influence the effects of toxins on focal organisms (Holmstrup et al. 2010; Bone et al. 2012). *Daphnia*, freshwater zooplankton, are highly suitable for and widely used as model organisms for standardized tests to infer toxicity thresholds of aquatic organisms (Shaw et al. 2008). They are keystone grazers of phytoplankton as well as known consumers of bacteria and fungi (Kagami et al. 2004; Eckert and Pernthaler 2014). Understanding how toxic compounds affect the fitness of *Daphnia* is essential to understand the ecological consequences of pollution in freshwater ecosystems.

The inclusion of biotic interactions between *Daphnia* and biofilm in toxicity tests could provide a more ecologically realistic approach. Biofilms are aggregates of microorganisms (algae, cyanobacteria, bacteria, fungi, and protozoa) growing on surfaces and embedded in a matrix of extracellular polymeric substances (EPS; Decho 2000). In aquatic environments, biofilms are involved in organic matter cycling, primary production and respiration (Wetzel 1993; Kühl et al. 1996; Decho 2000). They serve as food to higher trophic levels through grazing (Huws et al. 2005; Siehoff et al. 2009), and their algal components exude organic carbon to be taken up by bacteria (Søndergaard et al. 1995; Goto et al. 2001).

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✉ Semona Issa
semona.issa@ntnu.no

✉ Veerle L. B. Jaspers
veerle.jaspers@ntnu.no

[1] Department of Biology, Centre for Biodiversity Dynamics (CBD), Norwegian University of Science and Technology, Høgskoleringen 5, 7491 Trondheim, Norway

[2] Department of Biology, Norwegian University of Science and Technology, Høgskoleringen 5, 7491 Trondheim, Norway

[3] Department of Chemistry, Norwegian University of Science and Technology, Høgskoleringen 5, 7491 Trondheim, Norway

Despite the role played by biofilms in the aquatic food web, classic toxicity tests apply standardized methods that hinder their growth in exposure media (OECD 2012).

One aspect of biofilms that makes them potentially relevant for toxicity studies is the fact that they efficiently accumulate metals. This makes biofilms useful for monitoring metal contamination in aquatic ecosystems (Leguay et al. 2016), but may also influence the exposure experienced by higher trophic levels (Stewart et al. 2004; Cardoso et al. 2013). For example, aquatic biofilms can readily accumulate mercury (Hg) (Dranguet et al. 2017), as well as its natural antagonist selenium (Se) (Janz et al. 2014). In media and living organisms, selenide ions (Se^{2-}) can bind to mercuric ions (Hg^{2+}) to form mercuric selenide (HgSe), a stable and biologically inert complex, thereby reducing the risk of Hg toxic effects. Se/Hg molar ratios in organisms that approach or exceed one indicate that toxic effects of Hg are likely counteracted by Se (Yang et al. 2008). Successful accumulation of Hg and Se in biofilms may in turn affect their accumulation in *Daphnia*.

In this study, we experimentally tested for the effect of biofilm presence on Hg and Se uptake by *Daphnia magna*. Biofilm is an important food source for *Daphnia* (Siehoff et al. 2009) and can provide aquatic animals with an additional source of both Hg and Se through grazing. Because diet is the main source of Se accumulation in aquatic animals (Sandholm et al. 1973), biofilm is expected to have a positive effect on Se bioaccumulation. On the other hand, direct uptake of Hg by biofilm could reduce the amount of aqueous Hg available for uptake by animals (Ayangbenro and Babalola 2017), but may increase their dietary uptake of Hg. Hence, biofilms may play a central role in the transfer of both Hg and Se to higher trophic levels, possibly altering the effects of Hg pollution in aquatic ecosystems. Given the high international concern for Hg, due to its long-range transport across the globe and its various toxic properties (UNEP 2013), knowledge about how ecological interactions between *Daphnia* and biofilms may influence the relative uptake of Hg and Se and hence influence toxicity levels, seems crucial, but is currently lacking.

Materials and methods

Study organisms

Ephippia containing resting eggs resulting from sexual reproduction of *D. magna* were collected in November 2014, in a pond at Værøy Island (1·0 ha, 67·687°N 12·672° E), northern Norway. Ephippial eggs were hatched in the laboratory and propagated clonally. For this experiment, juveniles of a single clone (clone 47) of *D. magna* were asexually propagated for eight successive generations prior to use. *D. magna* were cultured in 2.5 L aquaria at 20 °C in a modified "Aachener Daphnien Medium" (ADaM) (Klüttgen et al. 1994, SeO_2 concentration reduced by 50%), under long photoperiods (16 h L: 8 h D) using white fluorescent lamps. The medium was exchanged weekly and the animals were fed three times a week with Shellfish Diet 1800® (Reed mariculture Inc.; Rikard and Walton 2012) at a final concentration of 3.2×10^5 algal cells/mL.

Experimental design

To test for consistent and replicable results, we ran two identical experimental sets (Fig. 1), one week apart, where we allowed for the spontaneous growth of biofilm in the *Daphnia* culture medium, which may have resulted in differences in biofilm composition between the experimental sets.

For each experimental set, a full factorial design with two different starting concentrations of Hg (0.2 µg/L and 2 µg/L) and two biofilm treatments (present or absent) was applied, with five replicate beakers for each of the four combinations. In addition, two blanks containing only ADaM medium were used parallel to each experimental set and were treated to the same conditions as the exposure beakers. The two exposure concentrations (0.2 µg/L and 2 µg/L) were selected for being non-lethal and environmentally relevant concentrations of Hg based on literature research (Table A.1). Hg stock solutions (0.0016 g/L) were prepared at the onset of each experimental set, by dissolving 99.5% pure mercury (II) chloride ($HgCl_2$) (Fluka, Switzerland) in Milli-Q water (18.2 MΩ cm) (Milli-Q Plus, Millipore Corp.). The exposure glass beakers and equipment used for making Hg stock solutions were acid-washed overnight before use with 1 M HNO_3 suprapure quality prepared with a sub-boiling distillation system (Milestone, SubPUR) and subsequently washed with Milli-Q water. The stock solutions were added to ADaM to create the desired Hg exposure concentrations. For the biofilm absence treatment, glass beakers were used immediately after the cleaning procedure was completed. For the biofilm presence treatment, glass beakers were allowed to develop biofilm on their walls, in the presence of ten juveniles of a single clone of *D. magna*

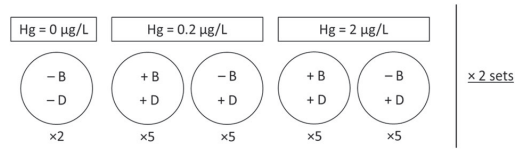

Fig. 1 Schematic diagram of the experimental design. ×2 sets: two identical experimental sets were run one week apart. Two replicates per set for the control (×2), 5 replicates per treatment (×5). Treatments are defined by the Hg concentration (0 µg/L Hg versus 0.2 µg/L Hg and 2 µg/L Hg), presence of biofilm (absent (−B) versus present (+B)) and presence of *Daphnia* (absent (−D) versus present (+D))

(clone 47), during two weeks prior to the experiment. Moreover, a total of eight controls containing only ADaM were added parallel to the beakers with biofilm and were subjected to identical biofilm growth conditions.

In each experimental replicate beaker (600 mL non-aerated borosilicate beakers, Fisherbrand), 30 adults of the same clone were kept in 400 mL medium at 20 °C for a period of 96 h. The ADaM medium was not replaced during the experimental period, and the animals were maintained under long photoperiods (16 h L: 8 h D) and fed with Shellfish Diet 1800® on days 1 and 3 of each experimental set at a final concentration of 3.2×10^5 algal cells/mL.

Sampling procedure

On the third day of the biofilm growth period, we sampled the medium in the biofilm-containing beakers and their associated blanks ($n = 28$) for Hg and Se analysis. On the first and last experimental days, we sampled 100 mL of medium for measurements of pH (WTW pH 340i), conductivity (WTW LF 330 conductivity meter), and dissolved oxygen (WTW Multi 3410 multiprobe meter), and took 5 mL of medium for measurements of Hg, Se, chloride (Cl), calcium (Ca) and magnesium (Mg) ($n = 88$; experimental beakers and their associated blanks). In addition, ADaM used for Hg dilution and exposure was sampled ($n = 4$) on the first experimental days for testing of Hg and Se levels. Immediately after collection, all medium samples ($N = 120$) were filtered through 25 mm diameter polyethersulphone membrane (0.45 µm) disposable syringe filters (VWR International) and acidified to 0.1 M HNO_3 (Milestone, SubPUR). Prior to sample withdrawal, the syringe filters were flushed with a few mL of clean ADaM and subsequently with the medium. Ca and Mg concentrations were used to calculate Ca hardness as follows: $CaCO_3$ (mg/L) = 2.5 Ca (mg/L) + 4.1 Mg (mg/L).

To obtain tissue blanks for Hg and Se analysis, animals were collected from the *Daphnia* cultures (clone 47) prior to the start of each experimental set. For analysis of final tissue Hg and Se content, animals were sampled on the last experimental day, and then washed in a new culture medium for 5 min to remove Hg from the carapace fluid. Finally, all tissue samples ($N = 44$) were freeze-dried and stored at room temperature until further analysis (for more details on quality control check Table A.2).

Chemical analysis

The medium samples were analyzed using high resolution inductively coupled plasma mass spectrometry (HR-ICP-MS, Element 2, Thermo-Fisher Scientific). The freeze-dried tissue samples (6.30 ± 0.12 mg dry mass per replicate beaker, mean ± SE) were first acid digested using 3 mL 50% HNO_3 per tissue sample (Milestone, SubPUR) in a high-pressure microwave system (Milestone UltraClave, EMLS, Leutkirch Germany) according to a temperature profile that increases gradually from room temperature up to 250 °C within 1 h, followed by a cooling step that allows temperature to return back to its initial value within ca. 1 h. After cooling to room temperature, the digested samples were diluted with Milli-Q water (18.2 MΩ cm) (Milli-Q Plus, Millipore Corp.) in polypropylene vials to achieve a final HNO_3 concentration of 0.6 M. Finally, samples were analyzed with HR-ICP-MS and the tissue Hg and Se content was calculated on a dry-mass basis. Seven blank samples containing Milli-Q water and HNO_3 (0.6 M in final solution) were run parallel to the digestion of the tissue samples. Results were corrected for reagent blank values. Certified reference material Polish Virginia Tobacco Leaves (INCT-PVTL-6) (Samczyński et al. 2012) was used to verify the accuracy of the Hg and Se analysis. The mean concentrations found (0.0264 ± 0.0006 µg/g dry weight) were in good agreement with the certified values (0.0232 ± 0.0016 µg/g dry weight).

Statistical analysis

All statistical analyses and graphic illustrations were performed in R v. 3.4.3. (R Development Core Team 2016). For exposure and water quality variables (Hg, Se, Cl, calcium hardness, dissolved oxygen, conductivity and pH), summary statistics (mean ± standard error) were calculated and full models were fitted using Hg concentration, biofilm and experimental set (hereafter "set") as fixed predictor variables and replicate beaker as a random predictor variable. For tissue Hg and Se content and Se/Hg molar ratios in *Daphnia*, full models with fixed predictor variables being Hg concentration, biofilm and set were fitted.

The models were implemented using the *lme* and *gls* functions in the package *nlme* (Pinheiro et al. 2018). Model selection followed a backwards selection procedure, where variables were removed sequentially, starting with random effects, using likelihood ratio tests (Zuur et al. 2009). Model residuals were checked for homogeneous variance and for normal distribution. The VarIdent command from the *nlme* package was used to allow residual variance to differ among Hg concentrations, biofilm levels and sets. Tukey's multiple comparison test was implemented where groups were significantly different. We used a significance level $\alpha = 0.05$ for hypothesis testing.

Results

No mortality occurred during the exposure. The Hg concentration was significantly higher in media without biofilm compared to media with biofilm that were exposed to 2 µg/L

Hg in set 1 only (Table 1). Se in the medium, pH and conductivity significantly decreased in the presence of biofilm (Table 1). Furthermore, Cl, hardness and conductivity were significantly higher in set 1 compared to set 2 (Table 1). The opposite was true for pH and dissolved oxygen (Table 1). In addition, dissolved oxygen significantly increased in the presence of biofilm in set 1 only (Table 1). Nevertheless, despite significant effects of treatments and/or sets on conductivity, dissolved oxygen, pH, Cl and hardness, the magnitude of change in these measures was relatively small. Indeed, pH, conductivity and Cl varied by a maximum of 5%, hardness by 8% and dissolved oxygen by 11% (Table 1).

Tissue Hg content was significantly higher in daphnids exposed to 2 µg/L Hg compared to 0.2 µg/L Hg (6.1 ± 0.5 µg/g dry mass versus 0.8 ± 0.07 µg/g dry mass, $P < 0.001$) and approximately twofold higher in set 2 compared to set 1 (4.6 ± 0.8 µg/g dry mass versus 2.4 ± 0.4 µg/g dry mass, $P < 0.001$) (Fig. 2). In addition, biofilm presence significantly reduced the overall *Daphnia* tissue Hg content from 0.9 ± 0.1 to 0.7 ± 0.1 µg/g dry mass at 0.2 µg/L Hg, and from 6.4 ± 0.7 to 5.8 ± 0.7 µg/g dry mass at 2 µg/L Hg ($P < 0.001$) (Fig. 2). On the other hand, the overall *Daphnia* tissue Se content significantly increased in the presence of biofilm from 3.4 ± 0.2 µg/g dry mass to 5.1 ± 0.1 µg/g dry mass ($P < 0.001$) (Fig. 3). Consequently, Se/Hg molar ratios significantly increased in the presence of biofilm in set 1 from 11 ± 0.9 (no biofilm) to 31 ± 2.1 (with biofilm; $P < 0.001$) under exposure to 0.2 µg/L Hg and from 1.8 ± 0.1 (no biofilm) to 3.5 ± 0.1 (with biofilm; $P < 0.001$) under exposure to 2 µg/L Hg. However, biofilm presence did not significantly affect molar ratios in set 2 (12 ± 0.7 versus 8.8 ± 1.1 at 0.2 µg/L Hg, ns, and 1.6 ± 0.1 versus 1.1 ± 0.1 at 2 µg/L Hg, ns) (Fig. 4). More detailed results can be found in the Supplementary Material Tables A.3 and A.4.

Discussion

In this study, we examined whether biofilm could affect Hg and Se accumulation in *Daphnia magna* through aqueous and dietary uptake pathways. The Hg exposure concentrations used were lower than the acute LC_{50} of 2.2 µg/L Hg in cladocerans (Nichols et al. 1997), although exceeding mean total concentrations of 0.006 µg/L typically found in the aquatic environment (Chen et al. 2000). Conductivity, dissolved oxygen and pH were also within the recommended range for testing metals in OECD test protocols for this species (OECD 2004). While hardness exceeded the recommended range, hardness has a negligible effect on Hg toxicity, in contrast to other heavy metals (Rathore and Khangarot 2003). Overall, there was no observed *Daphnia* mortality from either Hg exposure or changes in water quality.

There was a clear difference in Hg bioaccumulation between experimental sets, with higher tissue Hg content in set 2 compared to set 1. This was not due to differences in medium Hg concentrations but was possibly related to the higher medium Cl concentrations in set 1. Increased Cl has been shown to reduce the bioavailability of inorganic Hg through speciation (Wang and Wang 2010). In addition, dissolved organic carbon (DOC) could differ between sets, although this was not assessed in the current study. It is therefore important to always record and consider the potential influence of water quality variables when interpreting results from experimental exposure studies in aquatic microcosms. Moreover, differences between sets

Table 1 Exposure and water quality variable averages (averaged over the first and last experimental days) are compared across all sets and treatment combinations

	Treatment							
	Biofilm present 0.2 Hg (µg/L)		Biofilm absent 0.2 Hg (µg/L)		Biofilm present 2 Hg (µg/L)		Biofilm absent 2 Hg (µg/L)	
Set	1	2	1	2	1	2	1	2
Hg^{2+} (µg/L)	0.024 ± 0.003^{a}	0.026 ± 0.003^{a}	0.050 ± 0.01^{a}	0.027 ± 0.01^{a}	0.12 ± 0.01^{a}	0.15 ± 0.02^{a}	0.51 ± 0.1^{b}	0.14 ± 0.03^{a}
Se^{2-} (µg/L)	5.8 ± 0.05^{b}	5.7 ± 0.1^{b}	5.9 ± 0.05^{a}	5.9 ± 0.09^{a}	5.7 ± 0.06^{b}	5.7 ± 0.07^{b}	5.9 ± 0.04^{a}	5.7 ± 0.04^{a}
Conductivity (mS/cm)	2.2 ± 0.004^{bc}	2.1 ± 0.01^{bd}	2.2 ± 0.01^{ac}	2.2 ± 0.01^{ad}	2.2 ± 0.004^{bc}	2.1 ± 0.01^{bd}	2.3 ± 0.002^{ac}	2.1 ± 0.01^{ad}
Dissolved oxygen (mg/L)	7.9 ± 0.05^{bc}	8.1 ± 0.1^{bd}	7.5 ± 0.08^{a}	8.2 ± 0.06^{bd}	7.8 ± 0.1^{bc}	8.2 ± 0.1^{bd}	7.6 ± 0.02^{a}	8.3 ± 0.1^{bd}
pH	7.7 ± 0.02^{bc}	7.8 ± 0.04^{bd}	7.8 ± 0.04^{ac}	7.9 ± 0.02^{ad}	7.7 ± 0.02^{bc}	7.9 ± 0.05^{bd}	7.8 ± 0.05^{ac}	8.0 ± 0.08^{ad}
Ca hardness (mg/L)	350 ± 3^{a}	320 ± 10^{b}	340 ± 5^{a}	340 ± 4^{b}	340 ± 5^{a}	320 ± 8^{b}	340 ± 3^{a}	330 ± 8^{b}
Cl^{-} (mg/L)	630 ± 4^{a}	605 ± 9^{b}	630 ± 6^{a}	610 ± 8^{b}	630 ± 3^{a}	602 ± 6^{b}	630 ± 3^{a}	610 ± 6^{b}

Values are given as mean ± SE. Means with the same letter are not significantly different from each other

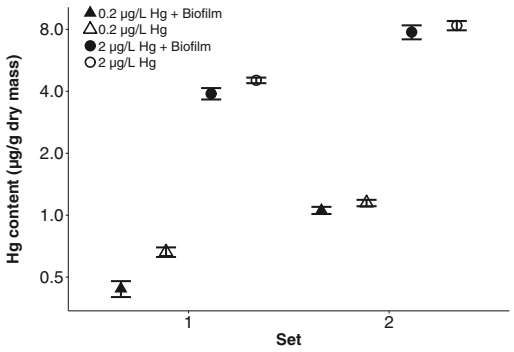

Fig. 2 Tissue Hg content (µg/g dry mass) in *Daphnia* in response to growth medium Hg concentrations, biofilm presence versus absence and set (mean ± SE). The y-axis is on a logarithmic scale with base 2

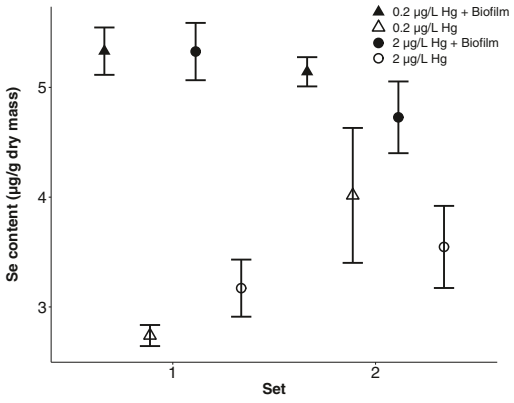

Fig. 3 Tissue Se content (µg/g dry mass) in *Daphnia* in response to growth medium Hg concentrations, biofilm presence versus absence and set (mean ± SE). The y-axis is on a linear scale

could be due to a potential difference in *Daphnia* age, albeit unlikely, and evidence to support this is currently lacking.

Daphnia tissue analysis showed that the increase in Hg content with increasing medium Hg concentrations was less significant in the presence of biofilm. Furthermore, biofilm presence increased *Daphnia* tissue Se content. These findings were replicable across experimental sets, which is important to highlight, as consistent and replicable effects are critical for improving risk analysis approaches (National Research Council 2009).

Previous research has provided evidence of aquatic biofilms accumulating heavy metals (Hill and Larsen 2005; Kohušová et al. 2011), through rapid absorption and removal from the aqueous solution (Chang et al. 2006; Ancion et al. 2010). This is because biofilms have a high capacity to absorb metals, which makes them a useful bioremediation tool (Dixit et al. 2015). Therefore,

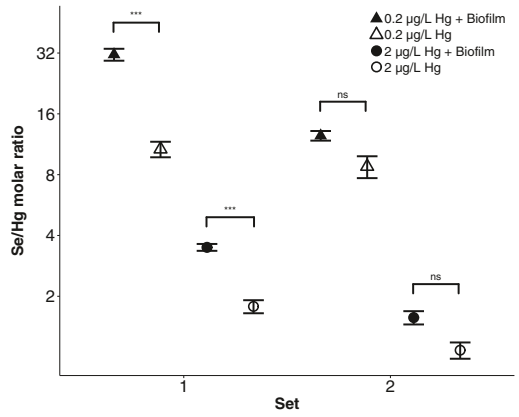

Fig. 4 Se/Hg molar ratios in *Daphnia* in response to growth medium Hg concentrations, biofilm presence versus absence and set (mean ± SE). Significance was evaluated using the Tukey's HSD post hoc test. Significant differences were observed between the two biofilm treatments for set 1 only (***$P < 0.001$; ns not significant). The y-axis is on a logarithmic scale with base 2

the biofilm in our study may potentially have acted as an available dietary source of Hg to the *Daphnia*, which could however not be confirmed as we were unable to analyze the Hg and Se content in the whole biofilm in this study. To determine the total mass balance of Hg and Se (total mass of biofilm and its Hg and Se content) in the exposure beakers, we would need to sample the entirety of the biofilm from the beakers. This was unfortunately not possible as it adhered to the walls and could not be entirely scraped off. Future studies should consider the possibility of growing the biofilm in filters placed inside the beakers at the start of the experiment for collection later. However, Tsui and Wang (2004) showed that *D. magna* accumulated Hg(II) mainly from the aqueous phase, through absorption, rather than from the ingestion of Hg enriched food. In the current study, uptake of aqueous Hg by daphnids may have been more important than dietary uptake as the presence of biofilm decreased the accumulation of Hg in the *Daphnia*. Indeed, biofilm may have indirectly reduced Hg accumulation in the animals, by reducing the amount of bioavailable aqueous Hg. However, this is not supported by our measurements in the medium, as observed changes in medium Hg concentrations (Table 1) do not suggest extensive uptake of the metal by biofilm.

Alternatively, biofilms can further reduce metal accumulation at higher trophic levels through "bloom dilution", whereby increased phytoplankton biomass reduces the concentration of metal per cell available to grazers (Pickhardt et al. 2002). Another phenomenon is "growth biodilution", observed in rapidly growing phytoplankton,

where the concentration of metals within cells is diluted by growth during the day (measured as photosynthetically fixed carbon) (Hill and Larsen 2005; Poste et al. 2015). Hill and Larsen (2005) showed how this phenomenon substantially decreased metal concentrations in biofilm within a short period of 4 days, equal to the duration of the current experiment. Thus, if biofilm was an important dietary source of Hg to the *Daphnia* in the current study, the animals would have been exposed to lower Hg concentrations from diet than from the aqueous phase because of "growth biodilution". However, it was not possible to determine unambiguously the main source of Hg to the daphnids in our study, as we were unable to determine the total mass balance of Hg (total mass of biofilm and its Hg content) in the exposure beakers.

Although the exact mode of action of biofilm presence on Hg accumulation in *Daphnia* is unknown, the presence of biofilm significantly reduced tissue Hg content and increased tissue Se content in *Daphnia*. The latter suggests dietary uptake of Se from biofilm. Lower Se concentrations measured in media with biofilm imply the metalloid's uptake by biofilm microorganisms. Indeed, biofilm components are known to readily absorb Se under both laboratory and field conditions, rendering it bioavailable to higher trophic levels through diet (Fan et al. 2002; Ranjard et al. 2003; Tuzen and Sarı 2010). In agreement with the majority of studies that have investigated Se transfer in aquatic ecosystems (Stewart et al. 2004; Conley et al. 2009), our findings support that diet may be the predominant route of Se exposure for organisms in aquatic food webs. Nevertheless, at high aqueous Se concentrations, the proportion of dietary to direct Se uptake may decrease. This was previously detected in daphnids exposed to 31.6 µg/L Se (Guan and Wang 2004), a concentration that is however far above those observed in the current experiment.

Biofilm-induced changes in tissue Se and Hg content led to an increase in Se/Hg molar ratios in the animals, which remained above one in all treatments. This suggests that tissue Se content was probably high enough to counteract toxic effects of Hg in the *Daphnia* in the current experiment (Peterson et al. 2009).

In summary, the presence of biofilm reduced Hg accumulation in *Daphnia*. This reduction was probably not due to a reduction in dissolved Hg available to animals or to "growth biodilution" in the biofilm, as these processes would require significant uptake of Hg by the biofilm, which was not supported by observed changes in medium Hg concentrations. Therefore, the exact driver of the lower tissue Hg content in the presence of biofilm remains unknown and future analysis of biofilm content and DOC is strongly recommended. On the other hand, the presence of biofilm increased the accumulation of Se in the animals. Thus, biofilms could play a central role in the transfer of Se through the freshwater food web, subsequently providing potential protection against Hg toxicity in the animals. Thus, aquatic biofilms can affect the transfer of Hg and Se to grazing zooplankton, which then act as conduits of Hg and Se to subsequent higher trophic levels of the food web. Our findings support the observation that including natural variability in toxicity studies and allowing for food web interactions may be important for more realistic environmental exposure assessments (De Laender et al. 2008; Viaene et al. 2015). Therefore, in order to obtain ecologically relevant results, we recommend that aquatic toxicity studies on metals/metalloids should include interactions with biofilm components. However, further research is necessary to conclude on the main mechanism of Hg and Se accumulation in *Daphnia* in the presence of biofilm.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. This study does not contain any studies with human participants performed by any of the authors.

Informed Consent This study does not contain any studies with human participants performed by any of the authors.

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Supplementary material of the paper

Biofilms grown in aquatic microcosms affect mercury and selenium accumulation in *Daphnia*

Semona Issa, Tomasz Maciej Ciesielski, Øyvind Mikkelsen, Sigurd Einum, Veerle L. B. Jaspers

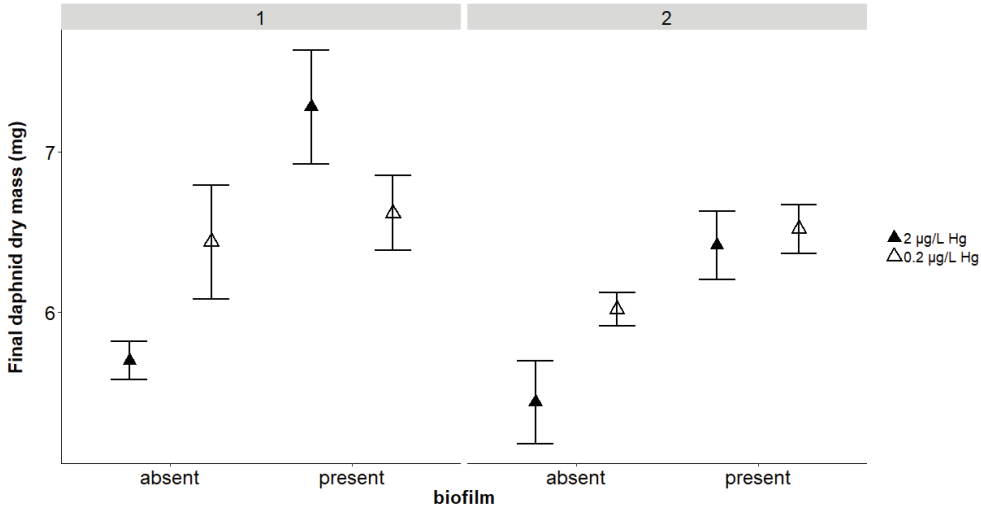

Fig A1. Final dry mass of *Daphnia* (mg) in response to growth medium Hg concentrations, biofilm presence versus absence and set (mean ± SE). The y-axis is on a linear scale.

Table A.1. Studies examining Hg toxicity to *Daphnia* species.

Study	Test type	Exposure duration	Hg^{2+} exposure concentration	Life stage	Sample size	Endpoints
Tsui, M. T. K., & Wang, W.-X. (2005). Multigenerational acclimation of *Daphnia magna* to mercury: Relationships between biokinetics and toxicity. *Environ. Toxicol. Chem., 24*(11), 2927-2933. doi:10.1897/05-085R.1	Static nonrenewal acute	48 h	Control; 20; 40; 60; 80 and 105 µg/L	Juveniles (4 day old) and adults (21 day old)	5 individuals/replicate 4 replicates/concentration level	LC_{50} Mortality
	Static renewal chronic	21 days	Control and 3.8 ± 0.7 µg/L	Juveniles (4 day old)	100 individuals/replicate 3 replicates/concentration level	Biokinetics
Khangarot, B. S., & Das, S. (2009). Toxicity of mercury on in vitro development of parthenogenetic eggs of a freshwater cladoceran	Static nonrenewal chronic and acute	24-72 h	Control; 0.1; 0.32; 1; 3.2; 10 and 32 µg/L	Daphnid eggs (2-6 h old)	5 replicates/concentration level	Mortality Development time EC_{50}

Daphnia carinata. *J. Hazard. Mater., 161*(1), 68-73. doi:10.1016/j.jhazmat.2008.03.068						Abnormalities in development
Biesinger, K. E., Anderson, L. E., & Eaton, J. G. (1982). Chronic effects of inorganic and organic mercury on *Daphnia magna*: Toxicity, accumulation, and loss. *Arch. Environ. Contam. Toxicol., 11*(6), 769-774. doi:10.1007/BF01059166	Static renewal chronic	21 days	Control; 0.85; 1.7; 3.4; 6.8 and 13.6 µg/L	Neonates (12 ± 12 h old)	5 individuals/replicate 4 replicates/concentration level	Survival Reproduction
Tsui, M. T. K., & Wang, W.-X. (2006). Acute toxicity of mercury to *Daphnia magna* under different conditions. *Environ. Sci. Technol.,*	Static nonrenewal acute	24 h	Control plus 5 to 8 concentrations	Juveniles (4 day old)	10-15 individuals/replicate 3 replicates/concentration level	LC_{50} Survival

40(12), 4025-4030. doi:10.1021/es052377g			ranging from 5 to 105 µg/L			
Rodrigues, A. C. M., Jesus, F. T., Fernandes, M. A. F., Morgado, F., Soares, A. M. V. M., & Abreu, S. N. (2013). Mercury toxicity to freshwater organisms: Extrapolation using species sensitivity distribution. *Bull. Environ. Contam. Toxicol., 91*(2), 191-196. doi:10.1007/s00128-013-1029-0	Static nonrenewal acute	24-48 h	<u>*D. magna*</u>: control; 0.5; 1; 2; 4; 6; 8 and 16 µg/L <u>*D. longispina*</u>: control; 0.5; 1; 3; 6; 9; 18 and 36 µg/L	Neonates (<24 h old)	5 individuals/replicate 5 replicates/concentration level	24 h EC_{50} 48 h EC_{50} EC50 measured as immobilization

De Coen, W. M., Janssen, C. R., & 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. *Ecotoxicol. Environ. Saf., 48*(3), 223-234. doi:10.1006/eesa.2000.2009	Static renewal	96 h	Control; 1.8; 3.2; 5.6; 10; 13 and 18 µg/L	Juveniles (<24 h)	100 individuals/replicate 3 replicates/concentration level	Survival at 48 and 96 h Enzymatic activity
De Coen, W. M., & Janssen, C. R. (1997). The use of biomarkers in *Daphnia magna* toxicity testing II. Digestive enzyme activity in	Static renewal chronic	48-96 h	Control; 1.8; 3.2; 5.6; 10 and 30 µg/L	Juveniles (<24 h)	3 replicates/concentration level	Enzymatic activity

Daphnia magna exposed to sublethal concentrations of cadmium, chromium and mercury. *Chemosphere, 35*(5), 1053-1067. doi:10.1016/S0045-6535(97)00172-0						
De Coen, W. M., & Janssen, C. R. (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. *J. Aquat. Ecosyst. Stress Recovery, 6*(1), 43-	Static renewal acute	96 h	Control; 1.8; 3.2; 5.6; 10; 18; 24 and 32 µg/L	Juveniles (<24 h)	11 individuals/replicate 3 replicates/concentration level	Energy budget Growth (carapace length)
	Static renewal chronic	21 days	Control; 1.8; 3.2; 5.6; 10; 18; 24 and 32 µg/L	Juveniles (<24 h)	10 individuals/replicate 4 replicates/concentration level	r_m Offspring number

55. doi:10.1023/A:1008228517955						Age-specific survival and reproduction
Meng, Q., Li, X., Feng, Q., & Cao, Z. (2008). *The acute and chronic toxicity of five heavy metals on the Daphnia magna*. Paper presented at the 2008 2nd International Conference on Bioinformatics and Biomedical Engineering, Shanghai, China.	Static renewal chronic	14 days	Control and 0.5 µg/L	Neonates (6-12 h old)	10 individuals/replicate 3 replicates/concentration level	Adult survival rate Time to first reproduction Number of juveniles per brood

Table A.2. Summary of method blanks. Conductivity, dissolved oxygen, pH, hardness and Hg, Se and Cl ion concentrations are compared across all sets (1 versus 2). In the case of experimental blanks, these variables are averaged over the first and last experimental days for all replicates per set. Values are given as mean ± SE. n is the number of replicates.

	Method blank type										
	Biofilm growth				ADaM used for dilution and exposure (µg/L)		Experimental blanks (µg/L)		Tissue blanks (µg/g)		Shellfish Diet 1800® (µg/g)
	Beakers with animals (µg/L)		Beakers without animals (µg/L)								
Set	1	2	1	2	1	2	1	2	1	2	-
Hg^{2+}	0.00 ± 0.00 (n = 10)	0.00 ± 0.00 (n = 10)	0.01 ± 0.00 (n = 4)	0.00 ± 0.00 (n = 4)	0.00 ± 0.00 (n = 2)	0.02 ± 0.00 (n = 2)	0.01 ± 0.00 (n = 4)	0.00 ± 0.00 (n = 4)	0.00 ± 0.00 (n = 2)	0.00 ± 0.00 (n = 2)	0.00 ± 0.00 (n = 1)
Se^{2-}	5.77 ± 0.06 (n = 10)	5.77 ± 0.05 (n = 10)	5.76 ± 0.01 (n = 4)	5.72 ± 0.12 (n = 4)	5.47 ± 0.15 (n = 2)	5.65 ± 0.04 (n = 2)	5.95 ± 0.11 (n = 4)	6.09 ± 0.10 (n = 4)	3.90 ± 0.24 (n = 2)	3.95 ± 1.18 (n = 2)	-
Conductivity (mS/cm)	-	-	-	-	-	-	2.25 ± 0.00 (n = 2)	2.14 ± 0.01 (n = 4)	-	-	-

Dissolved oxygen (mg/L)	-	-	-	-	-	-	8.75 ± 0.00 (n = 2)	8.92 ± 0.04 (n = 4)	-	-	-
pH	-	-	-	-	-	-	7.98 ± 0.04 (n = 4)	8.12 ± 0.10 (n = 4)	-	-	-
Ca hardness (mg/L)	-	-	-	-	-	-	347 ± 5.88 (n = 4)	346 ± 8.16 (n = 4)	-	-	-
Cl^- (mg/L)	-	-	-	-	-	-	648 ± 7.34 (n = 4)	617 ± 12.0 (n = 4)	-	-	-

Table A.3. Model selection using AICc of candidate models for testing effects of Hg concentration (0.2 µg/L Hg(II) versus 2 µg/L Hg(II)), Biofilm (absent versus present) and Set (1 versus 2) on Hg and Se concentrations in the medium and their content in the animals; Se/Hg molar ratios in the animals; Cl, calcium hardness, pH, conductivity and dissolved oxygen in the medium; and final dry mass of *Daphnia* (mg). Models were sorted by ΔAICc. The best random effect structure was first determined with REML on models that included all listed fixed effects. Fixed effects were then compared with ML using the best random effect structure. K is the number of parameters estimated. The least complex model within 2 ΔAICc is bolded. vI refers to the varIdent function.

Response variable	Model	K	AICc	ΔAICc	wAICc
Hg in medium (µg/L)					
Fixed effects	**Hg_{medium} ~ Biofilm:Set:Hg**	10	-200.20	0.00	0.94
	Hg_{medium} ~ Biofilm:Set + Biofilm:Hg + Hg:Set	9	-193.20	6.98	0.03
	Hg_{medium} ~ Biofilm:Hg + Hg:Set	8	-190.50	9.65	0.01
	Hg_{medium} ~ Biofilm:Set + Biofilm:Hg	8	-190.40	9.72	0.01
	Hg_{medium} ~ Biofilm:Set + Hg:Set	8	-189.20	11.01	0.00
Random effects	**vI (Hg)**	10	-151.80	0.00	0.79
	vI (Hg) + (1 | Beaker)	11	-149.10	2.69	0.21
	vI (Biofilm)	10	-109.70	42.14	0.00
	vI (Biofilm) + (1 | Beaker)	11	-107.00	44.83	0.00

	vI (Set)	10	-74.00	77.78	0.00

Se in medium (µg/L)		**K**	**AICc**	**ΔAICc**	**wAICc**
Fixed effects	**Se_{medium} ~ Biofilm**	4	-12.10	0.00	0.33
	Se_{medium} ~ Biofilm + Set	5	-11.00	1.12	0.19
	Se_{medium} ~ Biofilm + Hg	5	-10.40	1.76	0.14
	Se_{medium} ~ Biofilm + Set + Hg	6	-9.20	2.93	0.08
	Se ~ Biofilm:Set	6	-8.80	3.30	0.06
Random effects	**vI (Set)**	10	25.20	0.00	0.69
	vI (Set) + (1 | Beaker)	11	27.90	2.69	0.18
	vI (Hg)	10	29.70	4.52	0.07
	vI (Biofilm)	10	32.00	6.87	0.02
	vI (Hg) + (1| Beaker)	11	32.40	7.22	0.02

Cl in medium (mg/L)		**K**	**AICc**	**ΔAICc**	**wAICc**
Fixed effects	**Cl ~ Set**	4	1794.20	0.00	0.44
	Cl ~ Biofilm + Set	5	1796.30	2.04	0.16
	Cl ~ Set + Hg	5	1796.40	2.20	0.14
	Cl ~ Biofilm:Set	6	1797.60	3.43	0.08
	Cl ~ Biofilm + Set + Hg	6	1798.50	4.30	0.05
	vI (Set)	10	1654.90	0.00	0.72

Random effects	vI (Set) + (1 | Beaker)	11	1657.60	2.69	0.19
	vI (Hg)	10	1659.50	4.64	0.07
	vI (Hg) + (1 | Beaker)	11	1662.20	7.33	0.02
	vI (Biofilm)	10	1665.20	10.30	0.00

Calcium hardness (mg/L)		**K**	**AICc**	**ΔAICc**	**wAICc**
Fixed effects	**Hardness ~ Set**	4	1816.40	0.00	0.30
	Hardness ~ Set + Hg	5	1817.30	0.93	0.19
	Hardness ~ Biofilm + Set	5	1818.70	2.27	0.10
	Hardness ~ Biofilm:Set	6	1819.00	2.64	0.08
	Hardness ~ Hg:Set	6	1819.60	3.24	0.06
Random effects	**vI (Set)**	10	1671.10	0.00	0.79
	vI (Set) + (1 | Beaker)	11	1673.80	2.69	0.21
	vI (Biofilm)	10	1685.00	13.90	0.00
	vI (Biofilm) + (1 | Beaker)	11	1686.30	15.11	0.00
	vI (Hg)	10	1690.70	19.54	0.00

pH		**K**	**AICc**	**ΔAICc**	**wAICc**
Fixed effects	**pH ~ Biofilm + Set**	5	-91.40	0.00	0.32
	pH ~ Biofilm + Set + Hg	6	-90.30	1.11	0.18

	pH ~ Biofilm:Set	6	-89.40	2.01	0.12
	pH ~ Biofilm:Hg + Set	7	-89.00	2.48	0.09
	pH ~ Hg:Set + Biofilm	7	-88.90	2.56	0.09
Random effects	**vI (Hg)**	10	-46.40	0.00	0.61
	vI (Hg) + (1 | Beaker)	11	-43.70	2.69	0.16
	vI (Set)	10	-42.50	3.91	0.09
	vI (Biofilm)	10	-42.50	3.94	0.08
	vI (Set) + (1 | Beaker)	11	-39.80	6.60	0.02

Conductivity (mS/cm)		**K**	**AICc**	**ΔAICc**	**wAICc**
Fixed effects	**Conductivity ~ Biofilm + Set**	5	-263.90	0.00	0.25
	Conductivity ~ Biofilm:Set	6	-263.70	0.15	0.23
	Conductivity ~ Biofilm + Set + Hg	6	-262.70	1.14	0.14
	Conductivity ~ Biofilm:Set + Hg	7	-262.50	1.39	0.13
	Conductivity ~ Hg:Set + Biofilm	7	-261.00	2.92	0.06
Random effects	**vI (Set)**	10	-192.10	0.00	0.82
	vI (Set) + (1 | Beaker)	11	-189.00	3.01	0.18
	vI (Hg)	10	-167.50	24.54	0.00
	vI (Biofilm)	10	-166.70	25.38	0.00
	(1 | Beaker)	10	-166.70	25.38	0.00

Dissolved oxygen (mg/L)		**K**	**AICc**	**ΔAICc**	**wAICc**
Fixed effects	**Dissolved oxygen ~ Biofilm:Set**	6	35.40	0.00	0.50
	Dissolved oxygen ~ Biofilm:Set + Hg	7	38.00	2.59	0.14
	Dissolved oxygen ~ Biofilm:Set + Hg:Set	8	39.10	3.72	0.08
	Dissolved oxygen ~ Set	4	39.50	4.04	0.07
	Dissolved oxygen ~ Biofilm:Set + Biofilm:Hg	8	39.50	4.11	0.06
Random effects	**vI (Set)**	10	64.70	0.00	0.67
	vI (Set) + (1 | Beaker)	11	67.80	3.08	0.14
	vI (Hg)	10	69.30	4.55	0.07
	vI (Biofilm)	10	69.80	5.11	0.05
	(1 | Beaker)	10	70.90	6.16	0.03

Hg in animals (µg/g)		**K**	**AICc**	**ΔAICc**	**wAICc**
Fixed effects	$Hg_{animals}$ ~ Biofilm:Set + Hg:Set	8	25.10	0.00	0.35
	$Hg_{animals}$ ~ Hg:Set + Biofilm	7	25.10	0.06	0.34
	$Hg_{animals}$ ~ Biofilm:Hg + Hg:Set	8	26.80	1.69	0.15
	$Hg_{animals}$ ~ Biofilm:Set + Biofilm:Hg + Hg:Set	9	26.90	1.85	0.14
	$Hg_{animals}$ ~ Biofilm:Set:Hg	10	30.5	5.42	0.02
	vI (Hg)	10	49.90	0.00	1.00

Random effects	vI (Set)	10	89.50	39.58	0.00
	vI (Biofilm)	10	100.00	50.08	0.00

Se in animals (µg/g)		**K**	**AICc**	**ΔAICc**	**wAICc**
Fixed effects	**$Se_{animals}$ ~ Biofilm:Set**	5	93.60	0.00	0.48
	$Se_{animals}$ ~ Biofilm:Set + Hg	6	96.10	2.49	0.14
	$Se_{animals}$ ~ Biofilm:Set + Hg:Set	7	96.60	2.98	0.11
	$Se_{animals}$ ~ Biofilm	3	96.80	3.22	0.10
	$Se_{animals}$ ~ Biofilm + Set	4	98.40	4.84	0.04

Se/Hg molar ratio in animals		**K**	**AICc**	**ΔAICc**	**wAICc**
Fixed effects	**$Se/Hg_{animals}$ ~ Biofilm:Set:Hg**	10	124.10	0.00	1.00
	$Se/Hg_{animals}$ ~ Biofilm:Set+ Biofilm:Hg + Hg:Set	9	143.80	19.67	0.00
	$Se/Hg_{animals}$ ~ Biofilm:Set+ Biofilm:Hg	8	153.50	29.34	0.00
	$Se/Hg_{animals}$ ~ Biofilm:Set+ Hg:Set	8	157.60	33.50	0.00
	$Se/Hg_{animals}$ ~ Biofilm:Set+ Hg	7	161.10	36.92	0.00
Random effects	**vI (Hg)**	10	124.80	0.00	1.00
	vI (Set)	10	173.90	49.09	0.00
	vI (Biofilm)	10	176.30	51.43	0.00

Final *Daphnia* dry mass (mg)					
Fixed effects	**Dry mass ~ Biofilm:Hg + Set**	6	73.3	0.00	0.43
	Dry mass ~ Biofilm:Hg + Hg:Set	7	75.3	2.06	0.15
	Dry mass ~ Biofilm:Set + Biofilm:Hg	7	76.0	2.76	0.11
	Dry mass ~ Biofilm:Hg	5	76.6	3.34	0.08
	Dry mass ~ Biofilm + Set	4	76.9	3.61	0.07

Table A.4. Summary statistics of fitted final models.

Response variable	Final model	Parameter	Estimate ± SE
Hg in animals (µg/g)	$Hg_{animals}$ ~ Hg:Set + Biofilm + vI (Hg)	Intercept	4.29 ± 0.28
		Biofilm presence	-0.16 ± 0.04
		Set 2	3.84 ± 0.39
		0.2 µg/L Hg	-3.66 ± 0.28
		0.2 µg/L Hg:Set 2	-3.29 ± 0.39
Se in animals (µg/g)	$Se_{animals}$ ~ Biofilm:Set	Intercept	2.95 ± 0.22
		Biofilm presence	2.37 ± 0.32
		Set 2	0.82 ± 0.32
		Biofilm presence:Set 2	-1.22 ± 0.45
Se/Hg molar ratio in animals	$Se/Hg_{animals}$ ~ Biofilm:Set:Hg + vI (Hg)	Intercept	1.78 ± 0.12
		Biofilm presence	1.71 ± 0.17
		Set 2	-0.70 ± 0.17
		0.2 µg/L Hg	8.92 ± 1.34
		0.2 µg/L Hg:Biofilm presence	19.05 ± 1.89
		0.2 µg/L Hg:Set 2	-1.22 ± 1.89
		Biofilm presence:Set 2	-1.23 ± 0.24

		0.2 µg/L Hg:Biofilm presence:Set 2	-15.84 ± 2.68
Cl in medium (mg/L)	Cl ~ Set + vI (Set)	Intercept	633.31 ± 2.05
		Set 2	-25.88 ± 4.16
Calcium hardness (mg/L)	Hardness ~ Set + vI (Set)	Intercept	344.92 ± 2.08
		Set 2	-14.16 ± 5.14
Hg in medium (µg/L)	Hg_{medium} ~ Biofilm:Set:Hg + vI (Hg)	Intercept	0.51 ± 0.06
		Biofilm presence	-0.40 ± 0.09
		Set 2	-0.37 ± 0.09
		0.2 µg/L Hg	-0.46 ± 0.06
		Biofilm presence:Set 2	0.41 ± 0.12
		0.2 µg/L Hg:Set 2	0.35 ± 0.09
		0.2 µg/L Hg:Biofilm presence	0.37 ± 0.09
		0.2 µg/L Hg:Biofilm presence:Set 2	-0.39 ± 0.12
Se in medium (µg/L)	Se_{medium} ~ Biofilm + vI (Set)	Intercept	5.89 ± 0.03
		Biofilm presence	-0.14 ± 0.04

pH	pH ~ Biofilm + Set + vI (Hg)	Intercept	7.8 ± 0.02
		Biofilm presence	-0.1 ± 0.03
		Set 2	0.14 ± 0.03
Conductivity (mS/cm)	Conductivity ~ Biofilm + Set + vI (Set)	Intercept	2.26 ± 0.003
		Biofilm presence	-0.02 ± 0.004
		Set 2	-0.1 ± 0.007
Dissolved oxygen (mg/L)	Dissolved oxygen ~ Biofilm:Set + vI (Set)	Intercept	7.56 ± 0.07
		Biofilm presence	0.28 ± 0.1
		Set 2	0.74 ± 0.11
		Biofilm presence:Set 2	-0.4 ± 0.15
Final *Daphnia* dry mass (mg)	Dry mass ~ Biofilm:Hg + Set	Intercept	5.77 ± 0.19
		Set 2	-0.41 ± 0.17
		Biofilm presence	1.28 ± 0.24
		0.2 µg/L Hg	0.66 ± 0.24
		0.2 µg/L Hg:Biofilm presence	-0.94 ± 0.34

Table A.5. Exposure variable averages on the first and last experimental days are compared across all sets and treatment combinations. Values are given as mean ± SE.

		Treatment							
		Biofilm present 0.2 Hg (µg/L)		Biofilm absent 0.2 Hg (µg/L)		Biofilm present 2 Hg (µg/L)		Biofilm absent 2 Hg (µg/L)	
		Day 1	Day 3	Day 1	Day 3	Day 1	Day 3	Day 1	Day 3
	Set								
Hg^{2+} (µg/L)	1	0,026 ± 0,003	0,021 ± 0,005	0,076 ± 0,007	0,023 ± 0,01	0,15 ± 0,01	0,080 ± 0,01	0,84 ± 0,02	0,19 ± 0,08
	2	0,027 ± 0,004	0,025 ± 0,005	0,028 ± 0,009	0,026 ± 0,01	0,11 ± 0,01	0,20 ± 0,006	0,20 ± 0,05	0,080 ± 0,03
Se^{2-} (µg/L)	1	5,8 ± 0,09	5,7 ± 0,04	5,8 ± 0,04	6,0 ± 0,06	5,7 ± 0,1	5,7 ± 0,06	5,9 ± 0,04	6,0 ± 0,08
	2	5,9 ± 0,05	5,5 ± 0,2	5,7 ± 0,06	6,2 ± 0,1	5,8 ± 0,06	5,6 ± 0,1	5,7 ± 0,07	5,7 ± 0,06

Paper II

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Author for correspondence:
Semona Issa
e-mail: semona.issa@ntnu.no

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Dopamine mediates life-history responses to food abundance in *Daphnia*

Semona Issa[1], Marlène Gamelon[1], Tomasz Maciej Ciesielski[2], Kristine Vike-Jonas[3], Alexandros G. Asimakopoulos[3], Veerle L. B. Jaspers[2] and Sigurd Einum[1]

[1]Centre for Biodiversity Dynamics (CBD), Department of Biology, [2]Department of Biology, and [3]Department of Chemistry, Norwegian University of Science and Technology, Høgskoleringen 5, 7491 Trondheim, Norway

SI, 0000-0002-5628-2516; MG, 0000-0002-9433-2369; SE, 0000-0002-3788-7800

Expression of adaptive reaction norms of life-history traits to spatio-temporal variation in food availability is crucial for individual fitness. Yet little is known about the neural signalling mechanisms underlying these reaction norms. Previous studies suggest a role for the dopamine system in regulating behavioural and morphological responses to food across a wide range of taxa. We tested whether this neural signalling system also regulates life-history reaction norms by exposing the zooplankton *Daphnia magna* to both dopamine and the dopamine reuptake inhibitor bupropion, an antidepressant that enters aquatic environments via various pathways. We recorded a range of life-history traits across two food levels. Both treatments induced changes to the life-history reaction norm slopes. These were due to the effects of the treatments being more pronounced at restricted food ration, where controls had lower somatic growth rates, higher age and larger size at maturation. This translated into a higher population growth rate (r) of dopamine and bupropion treatments when food was restricted. Our findings show that the dopamine system is an important regulatory mechanism underlying life-history trait responses to food abundance and that bupropion can strongly influence the life history of aquatic species such as *D. magna*. We discuss why *D. magna* do not evolve towards higher endogenous dopamine levels despite the apparent fitness benefits.

1. Introduction

Phenotypic plasticity is the propensity of a genotype to produce different phenotypes across environments [1,2]. Under natural selection, the slope and elevation of the relationship between environment and phenotype (i.e. the reaction norm) can evolve such that it approaches optimality with respect to fitness [3]. In this case, the reaction norm is adaptive since it gives higher fitness in each environment than any alternative reaction norm [4–6]. Expression of adaptive reaction norms is therefore crucial to maintain high fitness in environments that vary across space and time. One of the environmental factors that shows extensive spatial and temporal variation is food availability. Expression of reaction norms to food availability includes allocation patterns to different components of the life history such as reproduction, growth and somatic maintenance [7–9]. For example, resource allocation to somatic maintenance (survival) increases at the cost of growth and reproduction under food limitation in short-lived species [7,10–12]. Thus, expressing appropriate reaction norms for different life-history traits in response to food availability has important fitness consequences.

At the molecular level, the responses to environmental stimuli that produce these reaction norms are mediated by neural signalling mechanisms. Thus, knowledge about these mechanisms is important to understand how organisms adjust their phenotypes under environmental change (see [13] for a review on

neuronal pathways involved in phenotypic plasticity). For the specific case of food abundance, the neurotransmitter dopamine, which is synthesized by most animals, has been shown to play an important role in modulating behavioural and morphological responses. In the nematode *C. elegans*, dopamine is released from dopaminergic neurons when food is present [14], causing a reduction in the animal's rate of locomotion [15] and possibly regulating their lifespan [14]. In mammals, obese individuals release more dopamine upon food consumption and hence experience a higher reward sensation from food intake compared with lean individuals [16]. In honeybees (*Apis mellifera carnica*) and *Drosophila* larvae, dopamine is involved in learning to associate food odour with aversiveness of taste, and thereby mediates an avoidance behaviour towards toxic and/or unpalatable food [17,18]. In sea urchin larvae (*Strongylocentrotus purpuratus*), dopamine reduces food acquisition through a shortening in arm length when food is abundant, which preserves energy that can be allocated to other functions [19]. Hence, the dopamine system appears to be tightly linked to the regulation of food responses and may therefore be a likely candidate neural signalling system regulating the life-history reaction norms. If so, chemically induced changes to dopamine levels are predicted to change the slopes of these reaction norms.

Insights into the mediation of reaction norms by neurotransmitters are also of potential value for environmental risk assessment of pharmaceutical products. Specifically, in aquatic biota, neurotransmitter systems can be directly altered by anthropogenic activity through environmental release of antidepressants. Following administration to humans, antidepressants can be eliminated unmetabolized or as active metabolites and enter the aquatic environment through wastewater [20]. Another path by which pharmaceuticals can enter the aquatic environment is by the disposal of unused products [21]. Exposure to released pharmaceuticals can influence the behaviour, development, reproduction and survival of fish, invertebrates and amphibians [22,23]. Hence, more research on ecological effects of antidepressants in aquatic habitats is needed, as these can impact individual fitness and population viability [23]. Furthermore, interactive effects between antidepressant disruption of neurotransmitter systems and environmental variables such as food abundance can be expected. Of particular interest in the case of the dopamine system is bupropion, which is used both as an antidepressant and as treatment for smoking cessation [24]. Bupropion inhibits the neuronal reuptake of norepinephrine and dopamine, increasing their concentration in the synaptic cleft [25]. Bupropion has previously been detected in natural surface water, stream sediments as well as in fish brain tissue [26,27], and has been shown to affect the physiology, morphology and behaviour of aquatic animals. For example, bupropion can alter the morphology and predator avoidance behaviour of fathead minnows (*Pimephales promelas*), as well as directly affect their survival [28,29]. Hence, if dopamine is indeed involved in regulating life-history reaction norms in response to food abundance, then disruption of the dopamine system by bupropion is expected to lead to changes in the slopes of these reaction norms.

In this study, we experimentally tested for the effects of dopamine and bupropion exposure on the reaction norms of life-history traits of *Daphnia magna* in response to high versus restricted food ration. *Daphnia* are keystone zooplankton in freshwater ecosystems and model organisms for studying anthropogenic and natural stressors in these ecosystems [30]. They have also been used in studies of the dopamine signalling system [31,32]. We hypothesize that *D. magna* with natural dopamine levels will have life-history reaction norms that approach optimality with respect to fitness in response to food abundance, and that disruption of these levels will lead to a change in the response to food abundance and hence the slopes of these reaction norms. Furthermore, bupropion administration causes an increase in extracellular dopamine in the brain [33]. Thus, if this is the dominating effect of this treatment, we expect dopamine and bupropion exposure to induce similar changes to the slopes of these reaction norms relative to the control treatment.

2. Material and methods

(a) Study organisms

Ephippia containing resting eggs resulting from sexual reproduction of *D. magna* were collected in November 2014, in a pond at Værøy Island (1.0 ha, 67.687°N 12.672°E), northern Norway. Ephippial eggs were hatched in the laboratory and propagated clonally. For this experiment, juveniles of a single clone (clone 47) of *D. magna* were asexually propagated for four successive generations prior to use. A maximum of 30 individuals of *D. magna* were cultured in 2.5 l aquaria at 20°C in a modified Aachener Daphnien Medium (ADaM) [34] (SeO_2 concentration reduced by 50%), under long photoperiods (16 h L : 8 h D) using white fluorescent lamps. The medium was exchanged weekly and the animals were fed three times a week with Shellfish Diet 1800 (Reed Mariculture Inc.) at a final concentration of 3.2×10^5 cells ml^{-1}.

(b) Experimental design

A full factorial design with control, dopamine, bupropion and two food rations (high versus restricted) was used, with thirty 50 ml replicate tubes for each of the six combinations (electronic supplementary material, figure A1). Aqueous exposure to dopamine allows us to directly manipulate this compound in the experimental organisms. The exposure concentration of dopamine (2.3 mg l^{-1}) was chosen for successfully inducing changes in *D. magna* growth based on a study by Weiss *et al*. [35], and that of bupropion (1 µg l^{-1}) was selected for being an environmentally relevant concentration that can be expected to influence life-history traits based on a pilot study we conducted prior to this experiment (see [36] and electronic supplementary material, figures A2 and A3). Bupropion stock solutions (0.0016 g l^{-1}) were prepared by dissolving bupropion hydrochloride (Sigma-Aldrich, St Louis, MO, USA) in ultrapure water (18.2 MΩ cm; Milli-Q Plus, Millipore Corp.). The stock solutions were then added to ADaM to create the desired bupropion exposure concentration. For the dopamine treatment, dopamine hydrochloride (Sigma-Aldrich, St Louis, MO, USA) was first dissolved in 100 ml ultrapure water before dilution in ADaM to the desired exposure concentration. Controls containing only ADaM medium were performed parallel to the exposure replicates.

For each replicate tube, a single female neonate (less than 24 h old) was introduced and kept at 20°C under long photoperiods (16 h L: 8 h D) until death. The medium was renewed in all replicates ($n = 180$) three times a week, and the animals were fed at each renewal event with Shellfish Diet 1800 at a final concentration of 2.88×10^5 cells ml^{-1} (*ad lib* at 20°C) for the high food ration and 8.6×10^4 cells ml^{-1} (30% *ad lib* at 20°C) for the restricted food ration. Day 0 marks the start of the experiment, which was completed when the last individual died. Male individuals ($n = 9$) and individuals that died from pipetting ($n = 2$) were removed and not replaced.

(c) Sampling procedure and measurements of life-history traits

Conductivity (WTW LF 330 conductivity metre), pH (WTW pH 340i) and dissolved oxygen (WTW Multi 3410 multiprobe metre) were measured throughout the experiment, after medium renewal, in the new exposure solutions and ADaM medium used for the controls ($n = 27$; nine samples collected in total from each of the dopamine, bupropion and control treatments). Simultaneously, the new exposure solutions and ADaM medium were sampled for bupropion and dopamine analysis ($n = 21$; seven samples collected in total from each of the dopamine, bupropion and control treatments).

The samples were stored at −20°C for a maximum of four months after collection, prior to analysis. Two complementary sample preparation protocols were employed to cover all concentration ranges: (i) dilute-and-shoot and (ii) liquid–liquid extraction. Subsequent analysis was performed by ultra-performance liquid chromatography coupled to a triple quadrupole mass analyser (UPLC-MS/MS). Further details on the method are provided in electronic supplementary material. Over the course of the experiment, pH, conductivity and dissolved oxygen were within the recommended range for testing of chemicals in *D. magna*, according to OECD guidelines [37]. The conductivity remained at 1.1 mS/cm, mean dissolved oxygen at 9.0 mg l^{-1} and pH at 8.3 across treatments, whereas measured average concentrations of dopamine and bupropion were within 13% and 10% of their nominal concentrations, respectively (electronic supplementary material, table A2). Lower than expected concentrations of these compounds may have been caused by degradation during storage.

Immediately prior to exposure on day 0, neonates were photographed for body length measurements (BL, mm, measured from the upper margin of the eye to the junction of the carapace and spine) using ImageJ v. 1.52a (National Institutes of Health, Bethesda, MD). BL measurements were then transformed to dry mass (DM, mg) using the equation by Yashchenko *et al*. [38]: $DM = 0.00535 \times BL^{2.72}$. Thereafter, individual replicates were checked daily to record age at maturation and age at second reproduction, defined as the time when eggs were first visible in the brood chamber. Body length at first reproduction was also measured using ImageJ as described above. Live progeny released were collected and counted to yield first and second clutch size. For each replicate, we measured the BL of three offspring that were randomly sampled from each of the first and second clutch. As a derived parameter, we calculated the first clutch biomass as the product of clutch size and average offspring DM for that clutch. Offspring from all subsequent clutches were removed at each medium change, and the longevity of the mothers was recorded.

The somatic growth rate (SGR) of each replicate was calculated using the equation

$$SGR = \frac{\ln(DM_{end}) - \ln(DM_{start})}{duration}, \tag{2.1}$$

where DM_{start} is the dry mass (in mg) of the replicate at the neonatal stage, DM_{end} is the dry mass (in mg) of the replicate at maturation and duration is the number of days between the two stages.

The intrinsic population growth rate (r) was calculated based on the two first reproductive events from the Euler–Lotka equation,

$$\sum_{x=0}^{\infty} l_x m_x e^{-rx} - 1 = 0, \tag{2.2}$$

where age x can be either age at maturation or age at second reproduction, l_x is the probability of survival to age x and m_x is the average number of offspring produced by an individual of age x.

(d) Statistical analyses

All statistical analyses and graphic illustrations were performed in R v. 3.5.2. [39]. We first tested whether the slopes of the reaction norms of the measured life-history traits in response to food abundance differed among treatments. To assess this for DM at maturation and SGR, we used generalized least-squares regression (GLS) models including the effects of the two categorical predictors, treatment and food (high versus restricted) and their interaction. For offspring DM (first and second clutch analysed separately), linear mixed effects (LME) models were fitted with treatment, food and their interaction as fixed predictor variables and replicate as a random predictor variable. We also tested the effects of treatment, food and their interaction on clutch size, age at maturation, age at second reproduction and longevity, using Poisson generalized linear models (Poisson GLMs).

Model selection followed a backwards selection procedure, where variables were removed sequentially, starting with random effects, using likelihood ratio tests [40]. For GLS and LME models, residuals were checked for homogeneous variance and for normal distribution. The VarIdent command from the *nlme* package was used to allow residual variance to differ among treatments and food (see [41] for an example using a variance function [42]). Poisson GLM models were tested for overdispersion and their Pearson and deviance residuals were checked for patterns and lack of fit. To deal with overdispersion for models for age at maturation and longevity, we used a quasi-Poisson GLM instead of a Poisson GLM. Tukey's multiple comparison test was implemented where groups were significantly different. For intrinsic population growth rates (r), bootstrapped sample means were used to compute r values for which 95% confidence intervals were derived using the percentile method. Between-group differences in r were considered statistically significant in the case where 95% confidence intervals did not overlap. The models were implemented using the *lme* and *gls* functions in the package *nlme* [43] and the *glm* function in the package *stats*.

To determine the causal pathways from food ration to first clutch biomass through age and DM at maturation, we used confirmatory path analyses [44,45]. Because we expected the causal path model to be the same for the three treatments (dopamine, bupropion and control) but the relationships between life-history traits to differ in terms of strength and/or direction among treatments, we fitted a model of hypothesized paths between traits, which we applied separately for each of the control, bupropion and dopamine datasets. This path model consisted of a sequence of linear regressions where food ration was used as a main effect explaining the variation in the different traits. Note, however, that an interaction between food ration and age at maturation was added in the path model for the bupropion treatment (see results). For each linear regression, we recovered both standardized and unstandardized regression coefficients and their SE. The overall goodness-of-fit of the models was assessed using Shipley's test of directional separation which yields a chi-squared distributed Fisher's C statistic. A $p > 0.05$ indicates that no significant paths are missing from the model and that it fits the dataset well [44]. The paths models were implemented using the *piecewiseSEM* package [46].

3. Results

(a) Reaction norms in response to food ration

For all traits except longevity, the reaction norm slopes in response to food ration were of the same sign for the dopamine, bupropion and control treatments. This indicates that life-history traits responded in the same direction to a change in food ration, irrespective of the treatment. For all treatments, SGR, first and second clutch size increased with higher food ration ($p < 0.001$), whereas age at maturation, DM at

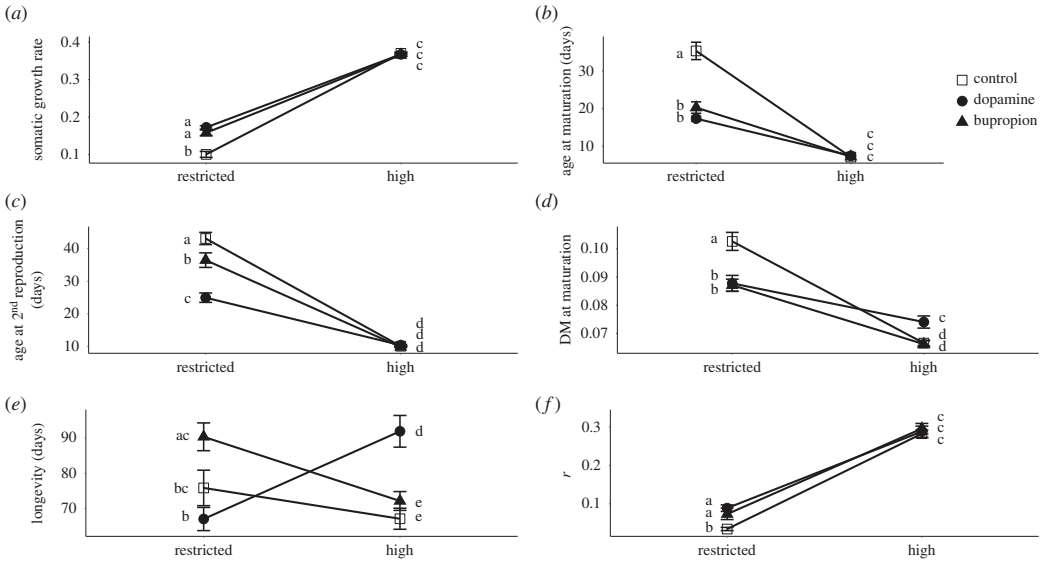

Figure 1. Effect of food ration on maternal traits in *D. magna* in the dopamine, bupropion and control treatments. (*a*) Somatic growth rate, (*b*) age at maturation (days), (*c*) age at second reproduction (days), (*d*) dry mass at maturation (mg), (*e*) longevity (days) and (*f*) intrinsic population growth rate (*r*). Error bars give 1 s.e. for (*a–e*) and 95% CI for (*f*). Means with the same letter are not significantly different from each other.

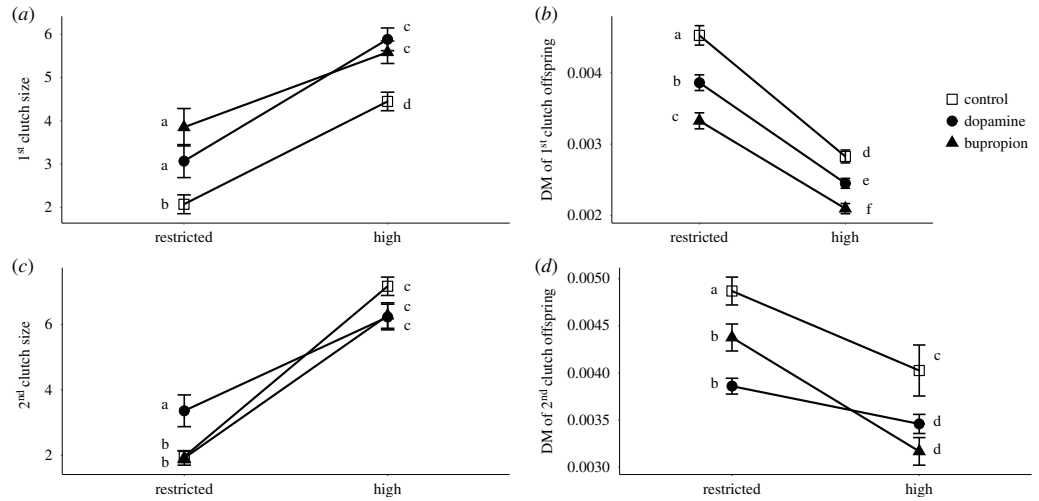

Figure 2. Effect of food ration on offspring production traits in *D. magna* in the dopamine, bupropion and control treatments. (*a*) First clutch size, (*b*) dry mass of first clutch offspring (mg), (*c*) second clutch size and (*d*) dry mass of second clutch offspring (mg). Error bars give 1 s.e. Means with the same letter are not significantly different from each other.

maturation, DM of first and second clutch and age at second reproduction decreased when the resources became sufficient ($p < 0.01$) (figures 1 and 2). For longevity, food restriction tended to increase it in both control and bupropion treatments (ns for control treatment, $p < 0.05$ for bupropion treatment), whereas the opposite pattern was observed in the dopamine treatment ($p < 0.001$). Although the sign of the reaction norm (i.e. positive versus negative slope) did not depend on the exposure treatment for most traits, their steepness did (for model selection results see electronic supplementary material, table A3). This was generally due to a more pronounced effect of dopamine and bupropion under restricted than under high food regimes (figures 1 and 2).

Specifically, at high food ration, treatment had no effect for SGR, age at maturation and age at second reproduction (ns), whereas a strong effect of dopamine treatment was observed for DM at first reproduction ($p < 0.01$). By contrast, at restricted food ration, the differences between control on one hand and dopamine and bupropion treatments on the other hand, became more pronounced ($p < 0.01$) (figure 1*a–d*; electronic supplementary material, table A5). Moreover, exposure to dopamine and bupropion induced lower DM for first and second clutch compared to controls, independent of food level ($p < 0.01$) (figure 2; electronic supplementary material, table A5). Finally, whereas the effects of food ration on life-history traits described above translated into an expected

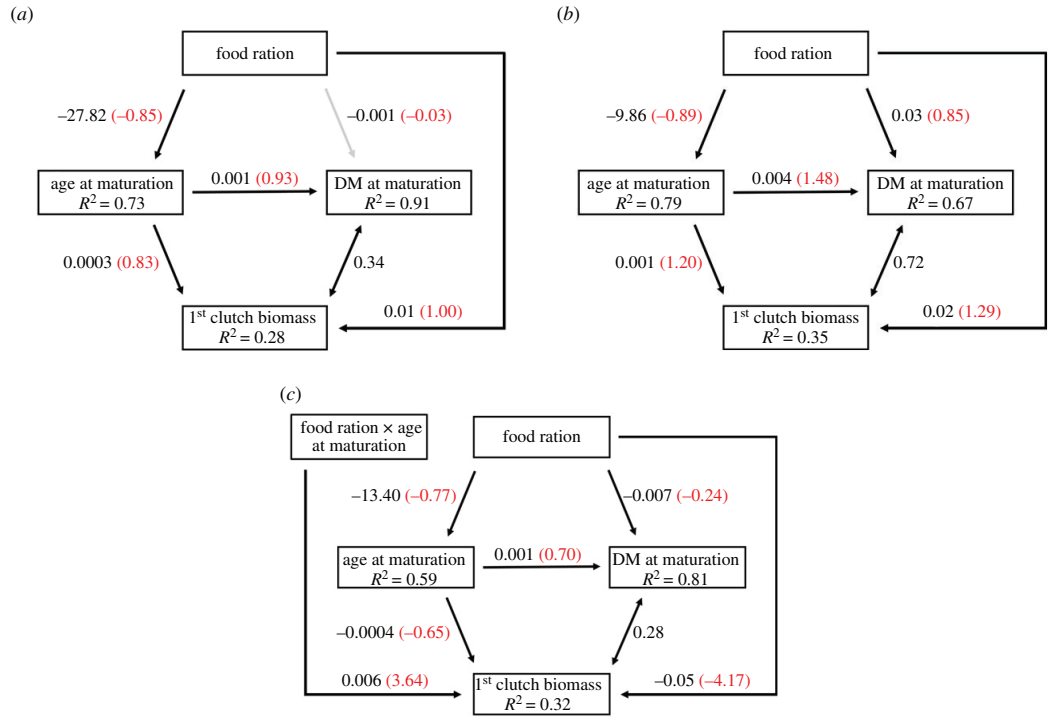

Figure 3. Structural equation models (SEM) exploring the effects of food ration on age at maturation, DM at maturation and first clutch biomass and the relationships between these across the (*a*) control, (*b*) dopamine and (*c*) bupropion treatments. Single-headed arrows represent unidirectional relationships among variables while double-headed arrows represent correlated errors between two dependent variables. Arrow for non-significant path ($p \geq 0.05$) is shown in grey. R^2 for component models are given in the boxes of response variables. Standardized coefficients, obtained by scaling the coefficients β by the ratio of the standard deviation of x over the standard deviation of y, are given in red (in parentheses) and unstandardized coefficients are given in black. (Online version in colour.)

strong decline in population growth rate (r) at restricted food, this effect was steeper for the control than for the dopamine and bupropion treatments ($p < 0.05$). Under restricted food ration, r was 159% and 114% higher in the dopamine and bupropion treatments than in the control, respectively (figure 1*f*; electronic supplementary material, table A5).

(b) Relationships among life-history traits at different food rations

The path models for the exposure treatments and the control fit the datasets very well ($p = 1$ for all groups). A high food ration favoured earlier maturation ($p < 0.05$). The direct effect of food on age was however much smaller in magnitude in the dopamine and bupropion treatments compared to the control ($\beta_{control} = -27.82 \pm 2.3$, $\beta_{dopamine} = -9.86 \pm 0.7$ and $\beta_{bupropion} = -13.40 \pm 1.6$, unstandardized coefficients; figure 3). In turn, age at maturation was positively associated with DM at maturation that was itself positively correlated with first clutch biomass ($p < 0.05$) (figure 3). DM at maturation was also affected by food ration directly (ns for control treatment, $p < 0.05$ for dopamine and bupropion treatments), but this effect was of smaller magnitude ($\beta_{control} = -0.03$, $\beta_{dopamine} = 0.85$ and $\beta_{bupropion} = -0.24$, standardized coefficients) than its indirect effect (through age at maturation), which is obtained by multiplying the path coefficients ($\beta_{control} = -0.85 \times 0.93 = -0.79$, $\beta_{dopamine} = -1.32$ and $\beta_{bupropion} = -0.54$, standardized coefficients; figure 3).

Direct effects of age at maturation and food ration on first clutch biomass were observed in addition to the positive correlation with DM at maturation ($p < 0.05$). In the control and dopamine treatments, the direct effect of age at maturation on biomass was positive, whereas it was negative in the bupropion treatment (figure 3). This negative effect was nonetheless weaker under high food ration ($\beta_{food\ ration \times age\ at\ maturation} > 0$, $p < 0.001$; figure 3*c*; electronic supplementary material, figure A4). Furthermore, the direct effect of food on biomass was larger in magnitude than its indirect effect ($\beta_{direct} = 1.00$ versus $\beta_{indirect} = -0.71$ in control; $\beta_{direct} = 1.29$ versus $\beta_{indirect} = -1.07$ in dopamine; $\beta_{direct} = -4.17$ versus $\beta_{indirect} = 1.82$ in bupropion, standardized coefficients; figure 3).

4. Discussion

In this study, we examined how dopamine mediates the responses of life-history traits to food abundance in *D. magna*, through aqueous exposure to dopamine and the antidepressant bupropion, a dopamine reuptake inhibitor. As hypothesized based on previous studies documenting behavioural and morphological effects of dopamine, dopamine and bupropion treatments significantly changed the slopes of life-history reaction norms to food abundance. The changes in slopes were due to effects of the treatments being more pronounced at a restricted food ration.

Life-history reaction norms to food abundance in the controls were consistent with previous empirical and theoretical

studies. Specifically, somatic growth rate decreased at restricted food ration thereby delaying maturation [47,48]. In turn, delayed maturation resulted in an increase in adult size (measured as DM at maturation). A larger size at maturity is believed to be metabolically advantageous, as it lowers the threshold food concentration at which assimilation equals respiration, making larger individuals able to grow and reproduce at lower food levels compared to smaller individuals [47]. This is caused by larger individuals having higher filtering rates than smaller individuals [49] and consequently higher feeding rates [50], which increases food uptake at low food concentrations. Once the threshold size is reached, energy can be allocated to reproduction [51,52]. Therefore, at restricted food ration, a higher somatic investment (adult size) at the expense of early life reproduction is likely to be adaptive, in line with resource allocation theory [12,53]. A similar argument can be made for an adaptive role of the observed reaction norm in terms of offspring size. At restricted food ration, offspring size increased whereas offspring number decreased. This trade-off between offspring size and number is due to energy limitations [54,55]. The optimal solution to this trade-off depends in turn on the food abundance [56]. Since the ability to support metabolic requirements at low food concentrations increases with body size in *Daphnia*, larger offspring have higher chances of surviving starvation [57]. Thus, mothers allocate their energy towards few but large offspring at low food conditions [58,59].

Although growth, somatic investment and reproduction responded qualitatively in the same way to food ration across treatments, quantitative differences were observed. This supports the view expressed above that these reaction norms to food abundance are under active physiological control and hence can respond to selection in an adaptive way, rather than being passive outcomes of energy availability. If observed differences between high and restricted food rations were solely based on the amount of energy available at each food ration, there would be no difference observed between the treatments at a given food ration.

At restricted food, under dopamine and bupropion exposure, resource allocation to maturation increased, leading to accelerated somatic growth rates, smaller adults, earlier ages at maturity and eventually shorter generation times (i.e. mean age of mothers) compared to the control. A positive effect of dopamine upregulation on somatic growth rate was also seen in Weiss *et al*. [35], who suspected it could be due to an effect of dopamine on cell proliferation and/or cell volume. In addition to accelerating growth, dopamine upregulation can stress organisms by exacerbating dopamine autoxidation, which produces reactive oxygen species and neurotoxins that damage dopaminergic neurons and cause oxidative stress [60,61]. Evidence for this may lie in the observed shorter generation times in the exposure treatments compared to the control. Indeed, several empirical studies have shown that fast species might exhibit accelerated life histories in response to stressful environmental conditions by reproducing earlier and accelerating their turnover [62,63]. Accordingly, we found that *D. magna*, a fast species, exhibits a faster pace of life under dopamine and bupropion exposure at the expense of adult size and offspring size. The smaller mothers in the exposure treatments produced smaller offspring, as can be expected from the known positive correlation between offspring size and mother size [64,65].

Despite the similar effects of bupropion and dopamine treatments on life-history reaction norms to food abundance, path analyses identified differences in their resource allocation responses. Specifically, the relative importance of direct and indirect resource allocation (through age at maturation) to reproduction (first clutch biomass) changed according to food abundance across treatments. In the control and dopamine treatments, indirect resource allocation to reproduction increased at restricted food ration while direct allocation decreased ($\beta_{direct} > 0$ and $\beta_{indirect} < 0$ for both treatments). The opposite was true in the bupropion treatment ($\beta_{direct} < 0$ and $\beta_{indirect} > 0$). Moreover, direct allocation at restricted food ration was, given its magnitude, sufficient to offset the negative effect of delayed maturation on clutch biomass seen in the bupropion treatment. The negative effect of delayed maturation on clutch biomass in the bupropion treatment was unexpected, given the positive association between adult age, adult size and ultimately offspring size, and it could be due to physiological disruptions specific to bupropion's mode of action. Previous studies on aquatic animals have reported a variety of negative effects of bupropion exposure on reproductive physiology and development. One study showed bupropion negatively affecting the testicular morphology and reproductive physiology of adult male fathead minnows [29]. Another study reported disruption of zebrafish (*Danio rerio*) development, as well as a disruption of enzymatic activity related to energy production, movement and detoxification [66]. Finding differences in the resource allocation strategies of aqueous dopamine and bupropion was surprising, given that they were expected to have similar effects on the dopamine system and hence produce comparable physiological changes. However, aqueous dopamine and bupropion may be differently metabolized upon uptake and thus differ in their mechanisms of action and effects.

Regardless of their mode of action, aqueous dopamine and bupropion induced similar changes with respect to population growth rates (r). At restricted food ration, both treatments caused an increase in population growth rate (r). Individuals in these treatments allocated more resources to maturation and reproduction, advancing the timing of reproduction, which resulted in faster rates of population growth compared to the control. This boost in fitness did not induce any apparent long-term costs as longevity did not differ significantly across treatments at restricted food. This is an important finding as both the principle of allocation [9] and the disposable soma theories [67] predict reduced longevity as a consequence of a greater allocation to reproduction and/or growth early in life. Thus, one question arising from the present study is why *D. magna* do not evolve towards higher endogenous dopamine levels. One potential explanation for this may be that population growth rate estimates based on the timing and fecundity of the first two clutches is not always an appropriate fitness measure [68]. For example, this measure does not consider offspring survival and reproduction, which is an additional component of maternal fitness. Elevated dopamine levels caused reduced offspring size, and this may have negative fitness effects at low food abundance due to the relatively lower feeding efficiency of small individuals (see above). Alternatively, there may be ecological costs of expressing high dopamine levels and hence rapid growth, due to biotic interactions that were not quantified in this study. Rapid growth can increase predation costs through higher risk-taking behaviour from increased feeding in the presence of predators [69,70], as well as increased parasitism costs due to fewer resources being allocated to disease resistance [71,72]. Thus,

future studies should evaluate to what extent such selective factors can shape the evolution of the dopamine signalling system.

In summary, we found that the sign of the reaction norm in response to food abundance did not depend on the exposure treatment for most traits. Indeed, we showed an increase in adult size at the expense of growth and reproduction at restricted food ration for all treatments. Despite this general trend, the slopes of the reaction norms depended on the exposure treatment, as resource allocation to maturation and reproduction increased under dopamine and bupropion exposure when food rations were restricted, resulting in the advanced timing of reproduction at the expense of adult size and offspring size. Accelerated life cycles in the dopamine and bupropion treatments in turn resulted in higher population growth rates compared with the control, without any costs to longevity. This boost in fitness from dopamine upregulation contradicts our prediction that controls would have the highest fitness from having evolved adaptive reaction norms to food abundance. Further understanding of the evolution of the dopamine signalling system may require alternative measures of fitness that incorporate any effects on offspring survival and reproduction, as well as evaluating the potential for interactive effects between dopamine and ecological factors (predation, parasitism) on fitness. Nonetheless, our findings emphasize the role of the dopamine system as regulator of trait responses to food abundance and demonstrate that low but environmentally relevant concentrations of bupropion can alter the life history of *D. magna*, with possible consequences to individual fitness.

Ethics. All applicable international, national and/or institutional guidelines for the care and use of animals were followed. This study does not contain any studies with human participants performed by any of the authors.

Data accessibility. The data supporting this paper are available in the electronic supplementary material.

Authors' contributions. S.I. participated in the design of the study, carried out the laboratory work, carried out the statistical analysis and drafted the manuscript; S.E. conceived the study, participated in the design of the study, participated in data analysis and critically revised the manuscript; V.L.B.J. and T.M.C. participated in the design of the study and critically revised the manuscript; M.G. participated in data analysis and critically revised the manuscript; A.G.A. and K.V.-J. carried out the UPLC-MS/MS analysis and participated in the drafting of the manuscript. All authors gave final approval for publication and agree to be held accountable for the work performed therein.

Competing interests. The authors declare that they have no competing interests.

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Supplementary material of the paper

Dopamine mediates life history responses to food abundance in *Daphnia*

Semona Issa, Marlène Gamelon, Tomasz Maciej Ciesielski, Kristine Vike-Jonas, Alexandros G. Asimakopoulos, Veerle L. B. Jaspers, Sigurd Einum

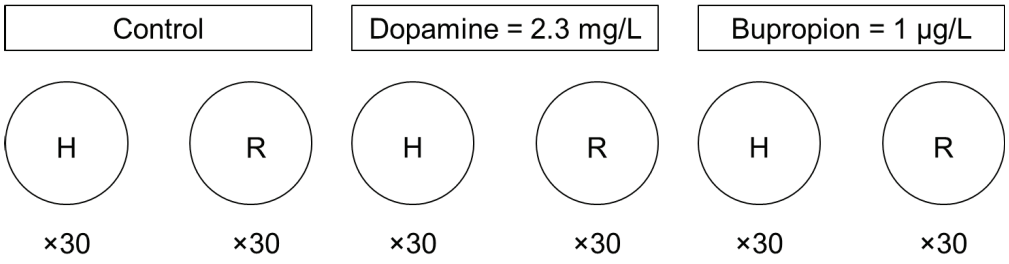

Fig. A1. Schematic diagram of the experimental design. Thirty replicates per food ration for the control, dopamine and bupropion treatments (x30). "H" and "R" refer to high and restricted food ration, respectively.

Bupropion pilot study

A pilot study was performed to determine appropriate sublethal bupropion test concentrations for *Daphnia magna*. Three bupropion (bupropion hydrochloride (Sigma-Aldrich, St. Louis, MO, USA)) concentrations (1 µg/L, 10 µg/L and 100 µg/L) and a control (0 µg/L bupropion) were applied, with 30 replicates for each of the four treatments. For each treatment, 30 juvenile females of clone 47 were kept individually in 15 mL glass tubes at 20 °C in a modified "Aachener Daphnien Medium" (ADaM). The medium was renewed three times a week during the experimental period, and the animals were maintained until maturity under long photoperiods (16h L: 8h D) and fed with Shellfish Diet 1800® three times a week at a final concentration of 2×10^5 cells/mL. Age and dry mass at maturation were measured at the end of the experiment. In addition, the mortality in each treatment was recorded. Based on our findings (Figures A2 and A3), we chose to use a bupropion concentration of 1

µg/L as this concentration induced significant changes in dry mass and age at maturation without having mortality effects.

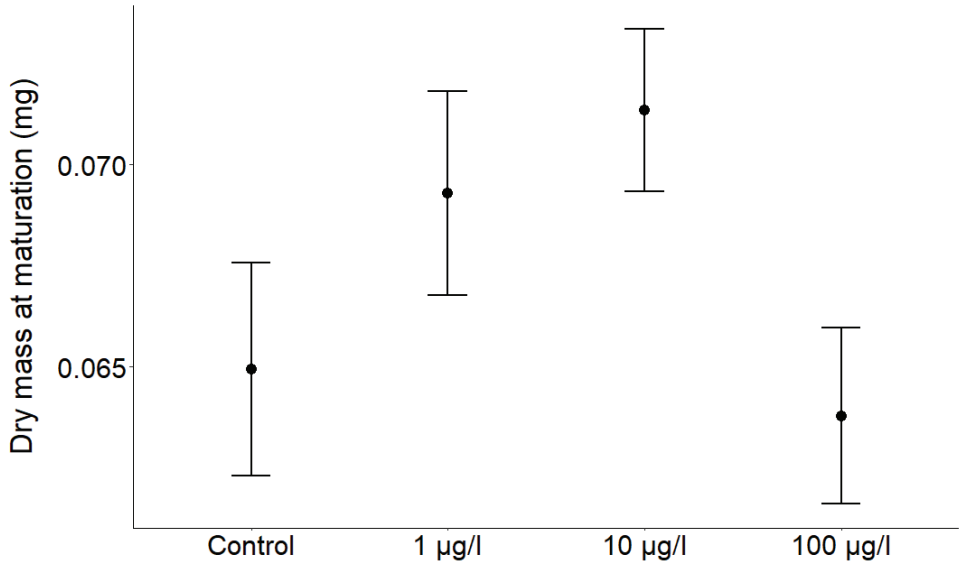

Fig. A2. *Daphnia* dry mass at maturation (mg) in response to growth medium bupropion concentrations (mean ± SE).

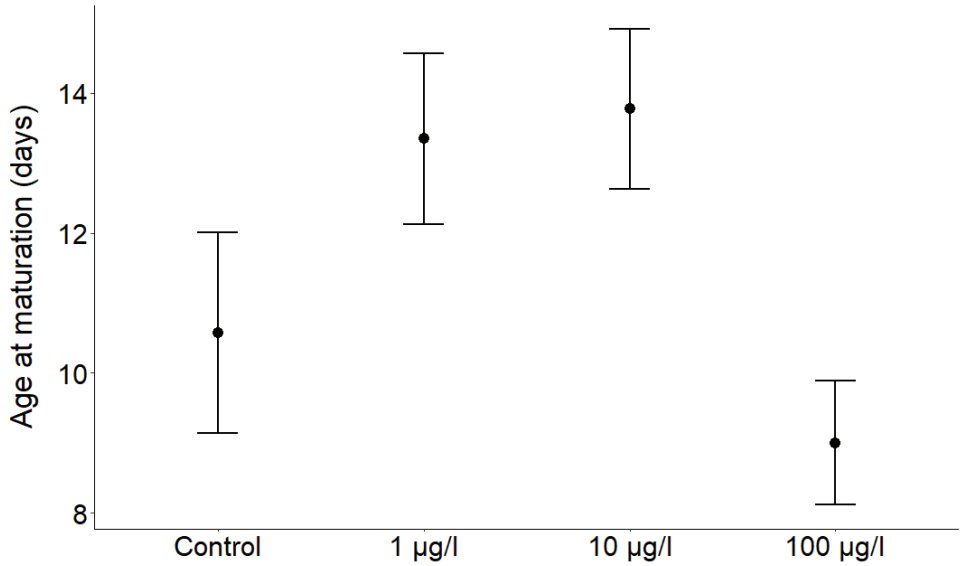

Fig. A3. *Daphnia* age at maturation (days) in response to growth medium bupropion concentrations (mean ± SE).

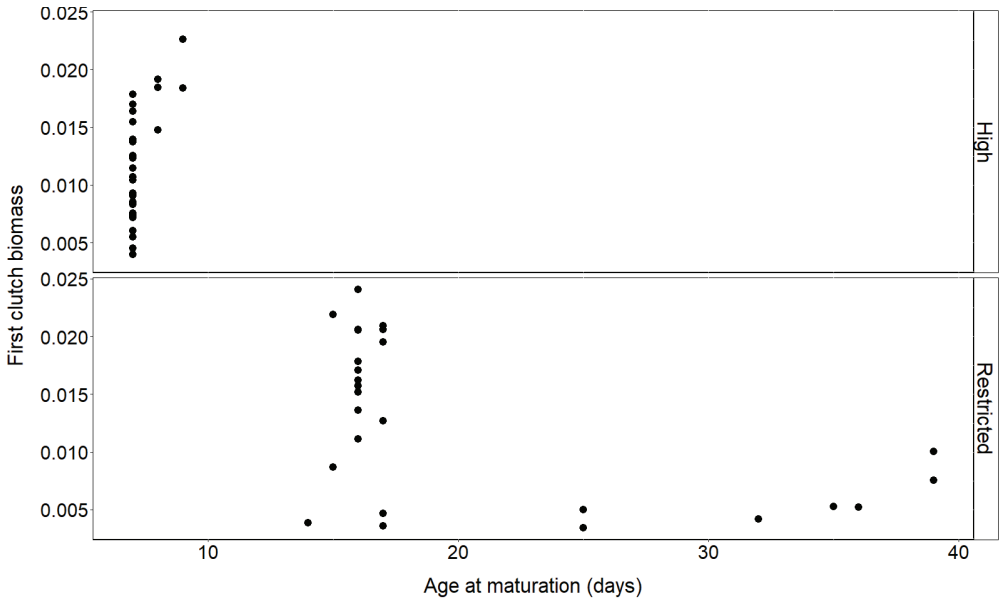

Fig. A4. First clutch biomass in response to *Daphnia* age at maturation (days) at restricted and high food rations in the bupropion treatment. The negative effect of age at maturation on biomass is significantly reduced at high food ration.

UPLC-MS/MS determination of bupropion and dopamine

The analysis of the samples was performed with two complementary sample preparation protocols to cover all concentration ranges: (1) dilute-and-shoot (Method A); and (2) liquid-liquid extraction (LLE; Method B). In both protocols, all reagent blanks and samples were spiked with a known amount of internal standard (10 ng benzotriazole; IS) prior to initiation of sample preparation. Benzotriazole (≥98%) was purchased from Sigma-Aldrich (Steinheim, Germany). All samples were analysed first with the dilute-and-shoot method, where samples were diluted by a factor of 2, and thereafter analysed by the LLE method, where samples were preconcentrated by a factor of 2. The detect values with method A were further confirmed with method B, while the non-detect values with method A were either detected with method B (due to the preconcentration) or remained as non-detects.

In the dilute-and-shoot method, a volume of 500 µL was transferred in an auto-sampler vial (for UPLC–MS/MS analysis), spiked with IS, and 500 µL of methanol were added reaching a total sample volume of 1 mL. Thereafter, the sample was directly injected to the UPLC–MS/MS system. In the LLE method, a volume of 1 mL of sample was transferred into a 15 mL polypropylene (PP) tube, spiked with IS, 300 µL of 1.0 M ammonium acetate$_{(aq.)}$ were added in the samples, and thereafter, the samples were extracted 3 times with 3 mL of ethyl acetate each time (3 × 3). For each successive extraction, the mixture was shaken in an oscillator shaker for 30 min and then centrifuged. The supernatants were combined, and 2 mL of ultrapure water were added. The mixture was centrifuged again, and the supernatant was transferred into a PP tube and concentrated to near-dryness under a gentle nitrogen stream. Finally, 500 µL of MeOH: ultrapure water (1:1 v/v) was added, vortex mixed and transferred into an auto-sampler vial for UPLC–MS/MS analysis.

The chromatographic separation was carried out using an Acquity UPLC I-Class system (Waters, Milford, U.S.) coupled to a triple quadrupole mass analyser (QqQ; Xevo TQ-S) with a ZSpray ESI ion source (Waters, Milford, U.S.). The used LC column was an Atlantis T3 (150 × 2.1 mm, 3 µm) connected to a Phenomenex C18 guard column (2.1 × 2.0 mm). The injection volume was 2 µL and the column temperature was set at 30 °C. The chromatographic separation was carried out using a gradient elution program with an aquatic (ultrapure water with 0.1% v/v formic acid; A) and an organic phase (methanol with 0.1% v/v formic acid; B) as

binary mobile phase at a flow rate of 0.3 mL/min. The gradient elution started at 50% (v/v) A, decreased to 0% A within 3.0 min (3.0rd min), and then reverted to 50% A that was held until the 5.0th min, for a total run time of 5.0 min. The retention times were 1.2 and 2.0 min for dopamine and bupropion, respectively. The electrospray ionisation (ESI) was applied at a potential of +2.5 kV. The cone and source offset voltages were set at 20 and 45V, respectively. The desolvation and cone gas flow rates were set at 800 and 150 L/hr, respectively. The collision gas flow was set at 0.15 mL/min, while the nebuliser gas pressure was set at 87 psi. The source and desolvation temperatures were set at 150 and 350 °C, respectively. The precursor–product ions (transitions), the collision energies and the cone voltage values that were set in the ESI method are presented in Table A1. The instrumental limits of detection (LODs) were calculated for each target analyte as 3 times the signal from the baseline noise (S/N ratio) and were 0.01 and 0.1 ng/mL for bupropion and dopamine, respectively. Quantification of the target drugs was accomplished based on the internal standard method and with matrix-matched standard addition calibration standards prepared by spiking target analytes into the specified matrices prior to extraction (Asimakopoulos et al., 2017).

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Table A1. SRM transitions, collision energies and cone voltage values for UPLC-MS/MS analysis.

Derivative	Transition 1 (T1; Quantitation ion)	Transition 2 (T2; confirmation ion)	Collision energies (V) T1; T2	Cone voltage values (V)
Dopamine	154 > 137	154 > 91	10; 20	16
Bupropion	240 > 184	240 > 131	12; 26	4
Benzotriazole (IS)	120 > 65	120 > 92	16; 14	28

Table A2. Exposure and water quality variables averaged over the entire experiment for all replicates. Conductivity, dissolved oxygen, pH and dopamine and bupropion concentrations are summarized by factors treatment (control versus dopamine and bupropion). Values are given as mean ± SE. Means with the same letter are not significantly different from each other (based on Tukey's post hoc test using an alpha value of 0.05).

	Treatment		
	Control	Dopamine	Bupropion
Dopamine (mg/L)	0.0 ± 0.0	0.29 ± 0.2	0.0 ± 0.0
Bupropion (µg/L)	0.0 ± 0.0	0.0 ± 0.0	0.096 ± 0.04
Conductivity (mS/cm)	1.1 ± 0.3 a	1.1 ± 0.3 a	1.1 ± 0.3 a
Dissolved oxygen (mg/L)	9.0 ± 0.05 a	9.0 ± 0.05 a	9.0 ± 0.05 a
pH	8.3 ± 0.1 a	8.2 ± 0.09 a	8.3 ± 0.1 a

Table A3. Model selection using AICc and quasi-AICc (QAICc) of candidate models for testing effects of treatment (control versus dopamine and bupropion) and food ration (high versus restricted) on somatic growth rate (SGR), age at maturation, age at second reproduction, dry mass (DM) at maturation, 1st and 2nd clutch size, 1st and 2nd clutch offspring DM, longevity; and pH, conductivity and dissolved oxygen in the medium. Models were sorted by ΔAICc and ΔQAICc. The best random effect structure was first determined with REML on models that included all listed fixed effects. Fixed effects were then compared with ML using the best random effect structure. K is the number of parameters estimated. The least complex model within 2 ΔAICc (and ΔQAICc) is bolded. vI refers to the varIdent function.

Response variable	**Model**	**K**	**AICc**	**ΔAICc**	**wAICc**
SGR					
Fixed effects	**SGR ~ Food: Treatment**	8	-735.80	0.00	1.00
	SGR ~ Food	4	-695.10	40.71	0.00
	SGR ~ Food + Treatment	6	-694.60	41.13	0.00
	SGR ~ 1	3	-427.10	308.64	0.00
	SGR ~ Treatment	5	-423.50	312.31	0.00
Random effects	**vI (Food)**	8	-683.40	0.00	1.00
	vI (Treatment)	9	-657.50	25.84	0.00

Age at maturation (days)		**K**	**QAICc**	**ΔQAICc**	**wQAICc**
Fixed effects	**Age $_{maturation}$ ~ Food: Treatment**	6	713.38	0.00	1.00
	Age $_{maturation}$ ~ Food + Treatment	4	745.94	32.56	0.00
	Age $_{maturation}$ ~ Food	2	855.38	142.00	0.00
	Age $_{maturation}$ ~ Treatment	3	1346.85	633.47	0.00
	Age $_{maturation}$ ~ 1	1	1446.15	732.77	0.00

DM at maturation (mg)		**K**	**AICc**	**ΔAICc**	**wAICc**
Fixed effects	**DM $_{maturation}$ ~ Food: Treatment**	8	-1050.80	0.00	1.00
	DM $_{maturation}$ ~ Food + Treatment	6	-1030.10	20.62	0.00

	$DM_{maturation}$ ~ Food	4	-1024.60	26.13	0.00
	$DM_{maturation}$ ~ Treatment	5	-942.00	108.75	0.00
	$DM_{maturation}$ ~ 1	3	-933.50	117.23	0.00
Random effects	**vI (Food)**	8	-987.30	0.00	1.00
	vI (Treatment)	9	-963.00	24.30	0.00

1st clutch size		**K**	**AICc**	**ΔAICc**	**wAICc**
Fixed effects	1st clutch size ~ Food: Treatment	6	654.60	0.00	0.51
	1st clutch size ~ Food + Treatment	4	654.70	0.09	0.49
	1st clutch size ~ Food	2	667.40	12.87	0.001
	1st clutch size ~ Treatment	3	707.20	52.62	0.00
	1st clutch size ~ 1	1	719.70	65.13	0.00

Age at 2nd reproduction (days)		**K**	**AICc**	**ΔAICc**	**wAICc**
Fixed effects	**Age $_{2^{nd}\ reproduction}$ ~ Food: Treatment**	6	943.80	0.00	1.00
	Age $_{2^{nd}\ reproduction}$ ~ Food + Treatment	4	975.40	31.54	0.00
	Age $_{2^{nd}\ reproduction}$ ~ Food	2	1062.40	118.55	0.00
	Age $_{2^{nd}\ reproduction}$ ~ Treatment	3	2137.10	1193.22	0.00
	Age $_{2^{nd}\ reproduction}$ ~ 1	1	2200.90	1257.04	0.00

2nd clutch size		**K**	**AICc**	**ΔAICc**	**wAICc**
Fixed effects	**2nd clutch size ~ Food: Treatment**	6	622.90	0.00	0.95
	2nd clutch size ~ Food	2	629.80	6.86	0.03
	2nd clutch size ~ Food + Treatment	4	631.20	8.25	0.01
	2nd clutch size ~ 1	1	777.40	154.51	0.00
	2nd clutch size ~ Treatment	3	779.70	156.78	0.00

1st clutch offspring DM (mg)		**K**	**AICc**	**ΔAICc**	**wAICc**
Fixed effects	**1st clutch offspring DM ~ Food + Treatment**	7	-4876.30	0.00	0.79
	1st clutch offspring DM ~ Food: Treatment	9	-4873.60	2.73	0.20

	1st clutch offspring DM ~ Food	5	-4848.60	27.76	0.00
	1st clutch offspring DM ~ Treatment	6	-4764.50	111.83	0.00
	1st clutch offspring DM ~ 1	4	-4752.40	123.95	0.00
Random effects	**vI (Food) + (1 | Replicate)**	9	-4778.60	0.00	0.78
	vI (Treatment) + (1 | Replicate)	10	-4775.80	2.77	0.19
	(1 | Replicate)	8	-4771.50	7.02	0.02
	vI (Food)	8	-4568.80	209.73	0.00
	vI (Treatment)	9	-4555.90	222.66	0.00

Offspring DM 2nd clutch (mg)		**K**	**AICc**	**ΔAICc**	**wAICc**
Fixed effects	**2nd clutch offspring DM ~ Food + Treatment**	6	-1919.60	0.00	0.83
	2nd clutch offspring DM ~ Food: Treatment	8	-1915.60	3.97	0.11
	2nd clutch offspring DM ~ Treatment	5	-1912.50	7.10	0.02
	2nd clutch offspring DM ~ Food	4	-1912.40	7.23	0.02
	2nd clutch offspring DM ~ 1	3	-1910.80	8.84	0.01
Random effects	**(1 | Replicate)**	8	-1830.00	0.00	0.59
	vI (Food) + (1 | Replicate)	9	-1828.80	1.24	0.31
	vI (Treatment) + (1 | Replicate)	10	-1826.40	3.61	0.10
	vI (Treatment)	9	-1765.60	64.40	0.00
	vI (Food)	8	-1757.70	72.32	0.00

Longevity (days)		**K**	**QAICc**	**ΔQAICc**	**wQAICc**
Fixed effects	**Longevity ~ Food: Treatment**	6	365.83	0.00	1.00
	Longevity ~ Treatment	3	393.53	27.70	0.00
	Longevity ~ Food + Treatment	4	395.47	29.65	0.00
	Longevity ~ 1	1	396.45	30.63	0.00
	Longevity ~ Food	2	398.36	32.53	0.00

pH		**K**	**AICc**	**ΔAICc**	**wAICc**
Fixed effects	**pH ~ 1**	2	11.00	0.00	0.90
	pH ~ Treatment	4	15.50	4.43	0.10

Conductivity (mS/cm)		K	AICc	ΔAICc	wAICc
Fixed effects	**Conductivity ~ 1**	2	67.60	0.00	0.93
	Conductivity ~ Treatment	4	73.00	5.32	0.07

Dissolved oxygen (mg/L)		K	AICc	ΔAICc	wAICc
Fixed effects	**Dissolved oxygen ~ 1**	2	-25.60	0.00	0.90
	Dissolved oxygen ~ Treatment	4	-21.30	4.32	0.10

Table A4. Summary statistics of fitted final models.

Response variable	Final model	Parameter	Estimate ± SE
SGR	SGR ~ Food: Treatment + vI (Food)	Intercept	0.37 ± 0.004
		Dopamine treatment	-0.003 ± 0.005
		Bupropion treatment	-0.004 ± 0.005
		Restricted food	-0.27 ± 0.008
		Restricted food: Dopamine treatment	0.07 ± 0.01
		Restricted food: Bupropion treatment	0.06 ± 0.01
Age at maturation (days)	$Age_{maturation}$ ~ Food: Treatment	Intercept	1.96 ± 0.08
		Dopamine treatment	0.04 ± 0.12
		Bupropion treatment	0.01 ± 0.11
		Restricted food	1.60 ± 0.09
		Restricted food: Dopamine treatment	-0.76 ± 0.13
		Restricted food: Bupropion treatment	-0.57 ± 0.13
DM at maturation (mg)	$DM_{maturation}$ ~ Food: Treatment+ vI (Food)	Intercept	0.07 ± 0.001
		Dopamine treatment	0.007 ± 0.002
		Bupropion treatment	-0.0004 ± 0.002
		Restricted food	0.036 ± 0.003
		Restricted food: Dopamine treatment	-0.02 ± 0.004
		Restricted food: Bupropion treatment	-0.01 ± 0.004
1^{st} clutch size	1^{st} clutch size ~ Food + Treatment	Intercept	1.43 ± 0.08

		Dopamine treatment	0.31 ± 0.10
		Bupropion treatment	0.36 ± 0.09
		Restricted food	-0.57 ± 0.08
Age at 2nd reproduction (days)	Age $_{2^{nd}\ reproduction}$ ~ Food: Treatment	Intercept	2.31 ± 0.06
		Dopamine treatment	0.03 ± 0.08
		Bupropion treatment	-0.02 ± 0.08
		Restricted food	1.45 ± 0.07
		Restricted food: Dopamine treatment	-0.58 ± 0.10
		Restricted food: Bupropion treatment	-0.14 ± 0.09
2nd clutch size	2nd clutch size ~ Food: Treatment	Intercept	1.97 ± 0.07
		Dopamine treatment	-0.14 ± 0.10
		Bupropion treatment	-0.13 ± 0.10
		Restricted food	-1.30 ± 0.16
		Restricted food: Dopamine treatment	0.68 ± 0.21
		Restricted food: Bupropion treatment	0.11 ± 0.23
Offspring DM 1st clutch (mg)	1st clutch offspring DM ~ Food + Treatment + vI (Food) + (1 | Replicate)	Intercept	0.003 ± 0.0001
		Dopamine treatment	-0.0004 ± 0.0001
		Bupropion treatment	-0.0009 ± 0.0001
		Restricted food	0.001 ± 0.0001
Offspring DM 2nd clutch (mg)	2nd clutch offspring DM ~ Food + Treatment + (1 | Replicate)	Intercept	0.004 ± 0.0003
		Dopamine treatment	-0.0009 ± 0.0003
		Bupropion treatment	-0.0006 ± 0.0003

		Restricted food	0.0009 ± 0.0003
Longevity (days)	Longevity ~ Food: Treatment	Intercept	4.21 ± 0.05
		Dopamine treatment	0.31 ± 0.07
		Bupropion treatment	0.07 ± 0.07
		Restricted food	0.12 ± 0.07
		Restricted food: Dopamine treatment	-0.44 ± 0.10
		Restricted food: Bupropion treatment	0.10 ± 0.10
pH	pH ~ 1	Intercept	8.28 ± 0.05
Conductivity (mS/cm)	Conductivity ~ 1	Intercept	1.06 ± 0.15
Dissolved oxygen (mg/L)	Dissolved oxygen ~ 1	Intercept	8.99 ± 0.03

Table A5. Mean trait responses to high and restricted food rations are compared between the control, and the dopamine and bupropion treatments. Statistically significant differences from the control group are reported as average percentage changes. "+" and "–" indicate an increase and a decrease, respectively, in mean trait response compared to the control. Means that are not significantly different from each other are reported as "ns".

	High food		**Restricted food**	
Trait	Dopamine	Bupropion	Dopamine	Bupropion
Somatic growth rate	ns	ns	+ 72 %	+ 57 %
Age at maturation (days)	ns	ns	– 51 %	– 43 %
Mass at maturation (mg dry mass)	+ 11 %	ns	– 14 %	– 15 %
1st clutch size	+ 32 %	+ 26 %	+ 48 %	+ 86 %
Age at 2nd reproduction (days)	ns	ns	– 42 %	– 15 %
2nd clutch size	ns	ns	+ 72 %	ns
Offspring mass 1st clutch (mg dry mass)	– 13 %	– 26 %	– 15 %	– 26 %
Offspring mass 2nd clutch (mg dry mass)	– 14 %	– 21 %	– 21 %	– 10 %
Longevity (days)	+ 37 %	ns	ns	ns
Intrinsic population growth rate	ns	ns	+ 159 %	+ 114 %

Table A6. Mean trait responses to high and restricted food rations in the dopamine group are compared to those in the bupropion group. Statistically significant differences are reported as average percentage changes in the dopamine group compared to the bupropion group. "+" and "–" indicate an increase and a decrease, respectively, in mean trait response under the effect of dopamine compared to that of bupropion. Means that are not significantly different from each other are reported as "ns".

Trait	High food	Restricted food
	Dopamine vs. Bupropion	Dopamine vs. Bupropion
Somatic growth rate	ns	ns
Age at maturation (days)	ns	ns
Mass at maturation (mg dry mass)	+ 12 %	ns
1st clutch size	ns	ns
Age at 2nd reproduction (days)	ns	– 32 %
2nd clutch size	ns	+ 76 %
Offspring mass 1st clutch (mg dry mass)	+ 17 %	+ 16 %
Offspring mass 2nd clutch (mg dry mass)	ns	ns
Longevity (days)	+ 27 %	– 26 %
Intrinsic population growth rate	ns	ns

Table A7. Coefficient values, standard errors (SE), degrees of freedom (DF), z-scores, P-values (*P*) and standardized path coefficients for each fitted structural equation model.

Path	Estimate	SE	DF	z value	*P*	Standardized estimate
Control						
Food ration → Age at maturation	-27.825	2.3207	54	-11.9899	0	-0.8526
Food ration → DM at maturation	-0.0014	0.0035	53	-0.3885	0.6992	-0.0309
Age at maturation → DM at maturation	0.0012	0.0001	53	11.6448	0	0.9266
Age at maturation → First clutch biomass	0.0003	0.0001	53	3.7021	0.0005	0.8276
Food ration → First clutch biomass	0.0108	0.0024	53	4.4973	0	1.0053
First clutch biomass ~~ DM at maturation	0.3402	NA	56	2.6338	0.0055	0.3402
Dopamine						
Food ration → Age at maturation	-9.8648	0.6987	52	-14.1195	0	-0.8906
Food ration → DM at maturation	0.0256	0.0053	51	4.8230	0	0.8516
Age at maturation → DM at maturation	0.004	0.0005	51	8.3831	0	1.4802
Age at maturation → First clutch biomass	0.0014	0.0003	51	4.8265	0	1.2024
Food ration → First clutch biomass	0.0162	0.0031	51	5.1612	0	1.2857
First clutch biomass ~~ DM at maturation	0.7172	NA	54	7.3493	0	0.7172
Bupropion						
Food ration → Age at maturation	-13.4038	1.5550	52	-8.6198	0	-0.767
Food ration → DM at maturation	-0.0068	0.0027	51	-2.5066	0.0154	-0.2398
Age at maturation → DM at maturation	0.0011	0.0002	51	7.3336	0	0.7016
Age at maturation → First clutch biomass	-0.0004	0.0001	50	-3.5641	0.0008	-0.6487
Food ration → First clutch biomass	-0.0491	0.0123	50	-3.9776	0.0002	-4.1701
Food ration: Age at maturation → First clutch biomass	0.0059	0.0017	50	3.5393	0.0009	3.6415
First clutch biomass ~~ DM at maturation	0.2835	NA	54	2.1114	0.0198	0.2835

Paper III

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

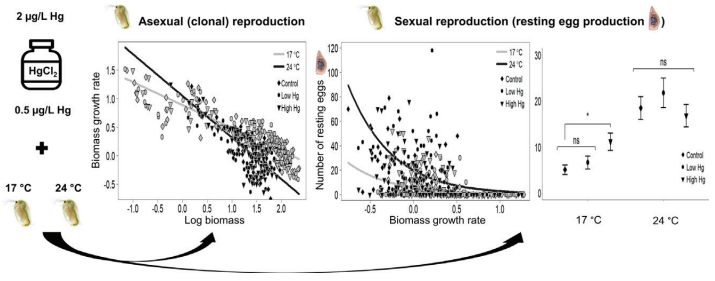
Semona Issa [a,*], Ane Simonsen [b], Veerle L.B. Jaspers [b], Sigurd Einum [a]

[a] Centre for Biodiversity Dynamics (CBD), Department of Biology, Norwegian University of Science and Technology, Høgskoleringen 5, 7491 Trondheim, Norway
[b] Department of Biology, Norwegian University of Science and Technology, Høgskoleringen 5, 7491 Trondheim, Norway

HIGHLIGHTS

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

GRAPHICAL ABSTRACT



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ABSTRACT

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

* Corresponding author.
E-mail address: semona.issa@ntnu.no (S. Issa).

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1. Introduction

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

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

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

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

2. Materials and methods

2.1. Study organisms

Ephippia containing resting eggs resulting from sexual reproduction of *D. magna* were collected in November 2014, in a pond at Værøy Island (1.0 ha, 67.687°N 12.672°E), northern Norway. Ephippial eggs were hatched in the laboratory and propagated clonally. For this experiment, juveniles of a single clone (clone EF7) of *D. magna* were asexually propagated for sixteen successive generations prior to use. During this period, a maximum of 30 *D. magna* individuals were cultured in 3 L aquaria at 17 °C and 24 °C in a modified "Aachener Daphnien Medium" (ADaM) (Klüttgen et al., 1994, SeO_2 concentration reduced by 50%), under long photoperiods (16 h L: 8 h D) using white fluorescent lamps. The medium was exchanged weekly to prevent poor medium quality, and the animals were fed three times a week with Shellfish Diet 1800® (Reed mariculture Inc.; Rikard and Walton, 2012) at a final concentration of 2.4×10^5 algal cells/mL (75% ad lib at 20 °C).

2.2. Experimental design

Replicates were run in two identical experimental sets (Fig. 1) in parallel, which were sampled on different days. For each experimental set, a full factorial design with three different starting concentrations of Hg (0 μg/L, 0.5 μg/L and 2 μg/L) and two temperature treatments (17 °C and 24 °C) was applied, with five replicate beakers for each of the six combinations. The two exposure concentrations (0.5 μg/L and 2 μg/L) were selected for being environmentally relevant concentrations (Berzas Nevado et al., 2003; Gray et al., 2000) that are lower than the acute LC_{50} of 2.2 μg/L Hg in cladocerans (Nichols et al., 1997). Moreover, a pilot study conducted prior to this experiment did not detect any effect of these concentrations on *D. magna* mortality or their capacity to undergo asexual reproduction (see Supporting information). Hg stock solutions (0.0016 g/L) were prepared at the onset of each experimental set, by dissolving 99.5% pure mercury (II) chloride ($HgCl_2$) (Fluka, Switzerland) in Milli-Q water (18.2 MΩ cm) (Milli-Q Plus, Millipore Corp.). The exposure glass beakers (600 mL non-aerated borosilicate beakers, Fisherbrand) and equipment used for making Hg stock solutions were acid-washed overnight before use with 1 M HNO_3 suprapure quality prepared with a sub-boiling distillation system (Milestone, SubPUR) and subsequently washed with Milli-Q water. The stock solutions were added to ADaM to create the desired Hg exposure concentrations while controls (0 μg/L Hg) contained only ADaM.

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

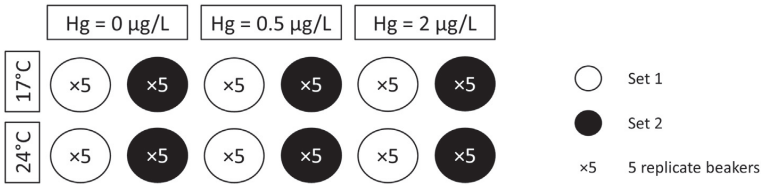

Fig. 1. Schematic diagram of the experimental design.
The experiment was divided into two identical experimental sets run in parallel and sampled weekly on different days. Five replicates per set per treatment (x5). Treatments are defined by the Hg concentration (0 µg/L Hg versus 0.5 µg/L Hg and 2 µg/L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

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

2.3. Sampling procedure

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

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

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

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

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

2.4. Statistical analysis

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

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

3. Results

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

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

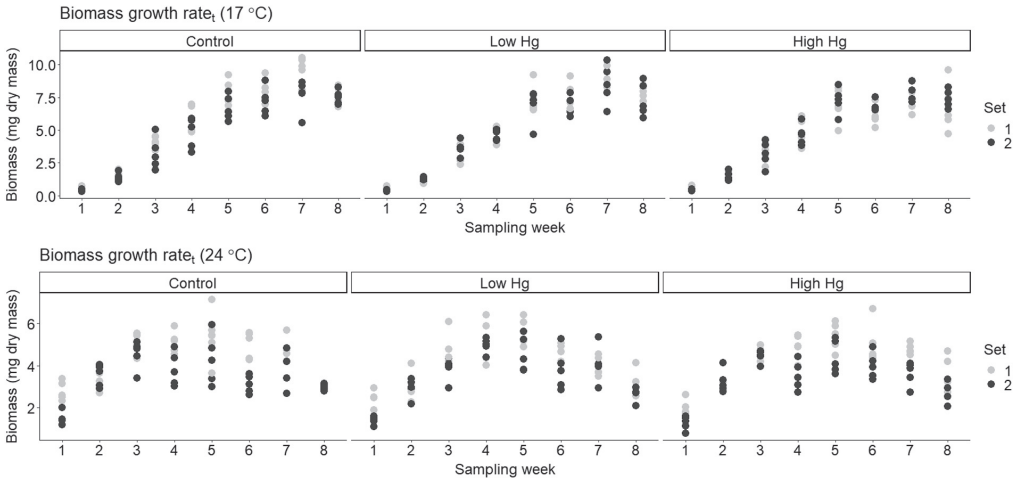

Fig. 2. Biomass growth rate$_t$ at 17 °C and 24 °C across Hg treatments (control versus low Hg and high Hg) and sets (1 versus 2). Scatter points represent measured data.

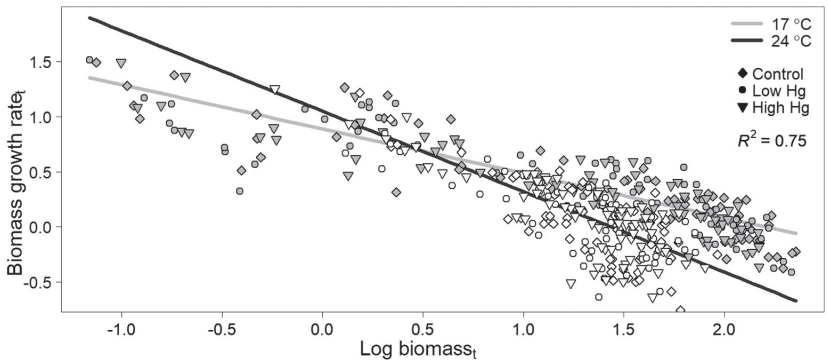

Fig. 3. Modelled effect of log biomass$_t$ (mg) on biomass growth rate$_t$ at 17 °C and 24 °C in the control, low Hg and high Hg treatments. Gray and white scatter points represent measured data at 17 °C and 24 °C, respectively. Efron's R^2, equal to the squared correlation between the predicted values and observed values, was 0.75.

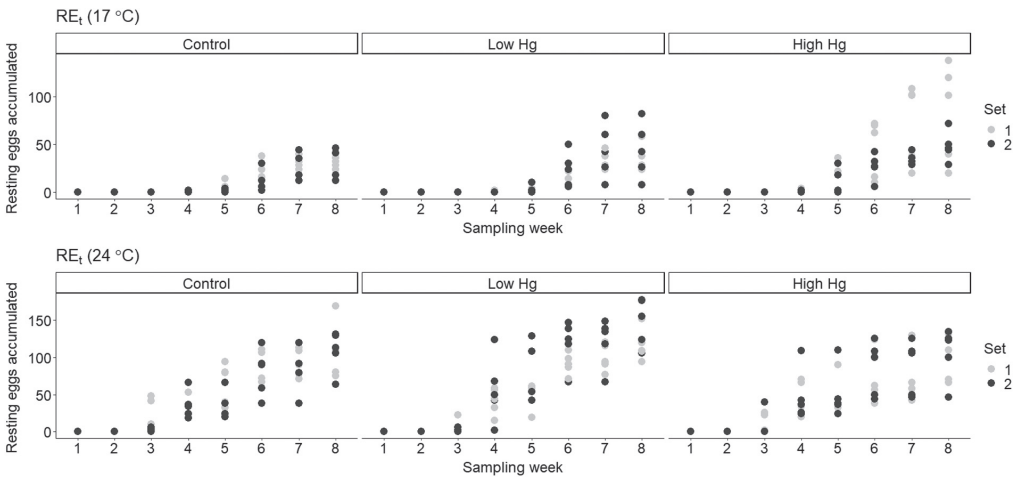

Fig. 4. Number of resting eggs produced per replicate beaker per week (RE$_t$) at 17 °C and 24 °C across Hg treatments (control versus low Hg and high Hg) and sets (1 versus 2). Scatter points represent measured data.

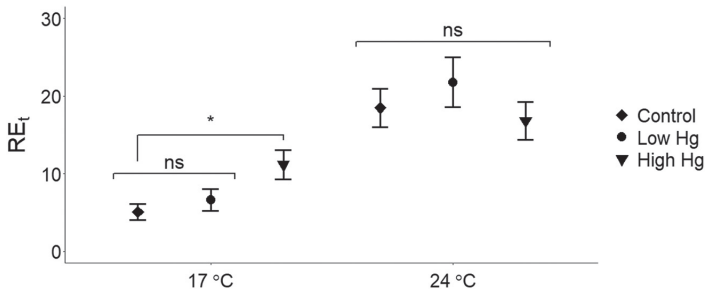

Fig. 5. Effect of temperature on number of resting eggs produced per replicate beaker per week (RE_t) in the control, low Hg and high Hg treatments. Scatter points represent measured data (error bars give 1SE). A significant difference was observed between the high Hg and the control treatment at 17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

4. Discussion

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

The missing response of biomass growth rate to Hg exposure was surprising, given that Hg stress can influence biomass growth through changes in body size and population size. Indeed, animals under metal stress direct more energy towards detoxification and recovery at the expense of other mechanisms such as feeding, somatic growth and reproduction (Muyssen et al., 2006), which can directly decrease population growth rate through lower fecundity and survival rates (Fong et al., 2019; Muyssen et al., 2006). Furthermore, studies show that metal stress can have contrasting effects on mean population body size through different modes of action that depend on the metal and its concentration. On one hand, metal stress can decrease the amount of resources available to *Daphnia* directly, by decreasing their filtration rate (Lopes et al., 2014), and indirectly, by impairing their swimming ability through oxidative stress (Bownik, 2017). A reduction in food uptake can subsequently result in a smaller mean body size for the population (Enserink et al., 1995). On the other hand, metal stress may increase mean population body size as several studies show a negative relationship between body size and metal sensitivity (Alves et al., 2009; Bianchini et al., 2002; Vesela and Vijverberg, 2007), such that smaller individuals are eliminated through mortality. This is explained by smaller individuals having a higher mass-specific metabolic rate that enhances metal uptake (Yu and Wang, 2002). A possible explanation for the overall missing response of biomass growth rate to Hg treatment may be that exposure concentrations were too low to exert a strong effect on body size or population size. Indeed, while the highest exposure concentration used was within the range of concentrations found to significantly lower asexual reproduction in *D. magna* (Biesinger et al., 1982), this was not the case for adult survival and growth (Biesinger et al., 1982; De Coen and Janssen, 1997).

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

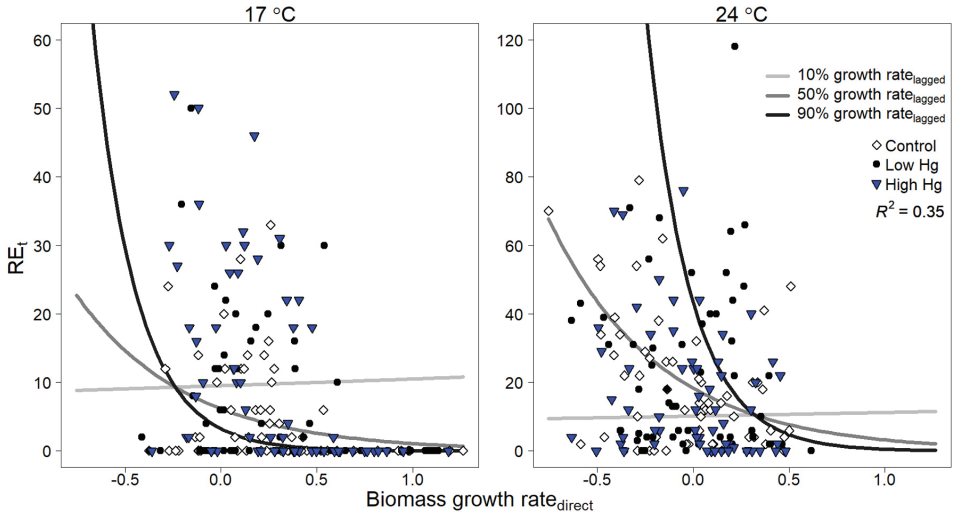

Fig. 6. Modelled direct effect of biomass growth rate (Biomass growth $rate_{direct}$) on number of resting eggs produced per replicate beaker per week (RE_t) at 17 °C and 24 °C. To visualize interactions, cross-sections were taken at the 10th, 50th and 90th percentiles of lagged biomass growth rate (growth $rate_{lagged}$). White, black and blue scatter points represent measured data for each of the control, low Hg and high Hg treatments, respectively. Efron's R^2, equal to the squared correlation between the predicted values and observed values, was 0.35. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

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

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

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

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

CRediT authorship contribution statement

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

Declaration of competing interest

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

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Appendix A. Supplementary data

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

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Supplementary material of the paper

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

Semona Issa, Ane Simonsen, Veerle L. B. Jaspers, Sigurd Einum

Pilot study

A pilot study was performed to determine appropriate sublethal $HgCl_2$ test concentrations for *Daphnia magna* at 17 °C and 24 °C. Two $HgCl_2$ (99.5% pure mercury (II) chloride (Fluka, Switzerland)) concentrations (0.5 µg/L and 1.75 µg/L) and a control (0 µg/L $HgCl_2$) were applied at the two temperatures (17 °C and 24 °C), with 3 replicates for each of the six treatments. For each treatment, 10 adult females of clone EF7 were kept collectively in 600 mL glass beakers in a modified "Aachener Daphnien Medium" (ADaM). The medium was not renewed during the experimental period, and the animals were maintained for two weeks under long photoperiods (16h L: 8h D) and fed with Shellfish Diet 1800® three times a week at a final concentration of 2.4×10^5 cells/mL. The mortality in each treatment was recorded visually. The results from the pilot showed no effect of treatment on *Daphnia* mortality or their capacity to undergo asexual reproduction. Based on these findings, we chose to use $HgCl_2$ exposure concentrations of 0.5 µg/L and 2 µg/L in the current study.

Table A1. Model selection using AICc of candidate models for testing effects of Log $biomass_t$, the direct effect of biomass growth rate during the week resting egg production was quantified (Biomass growth $rate_{direct}$), the lagged effect of biomass growth in the preceding week (Biomass growth $rate_{lagged}$), Hg concentration (control versus low and high), Temperature (24 °C versus 17 °C) and Set (1 versus 2) on Biomass growth $rate_t$ and number of resting eggs produced per replicate beaker per week (RE_t). Models were sorted by ΔAICc. The best random effect structure was first determined on models that included all listed fixed effects. Fixed effects were then compared using the best random effect structure. K is the number of parameters estimated. The least complex model within 2 ΔAICc is bolded.

Response variable	Model	K	AICc	ΔAICc	wAICc
Biomass growth $rate_t$					
Fixed effects	**Biomass growth $rate_t$ ~ Log $biomass_t$:Temperature + Set**	6	-90.30	0.00	0.59
	Biomass growth $rate_t$ ~ Log $biomass_t$:Temperature	5	-88.50	1.85	0.23
	Biomass growth $rate_t$ ~ Log $biomass_t$:Temperature + Hg + Set	8	-86.70	3.58	0.10
	Biomass growth $rate_t$ ~ Log $biomass_t$:Temperature + Hg	7	-84.90	5.46	0.04
	Biomass growth $rate_t$ ~ Log $biomass_t$:Temperature + Log $biomass_t$:Hg + Set	10	-84.00	6.28	0.02
RE_t		**K**	**AICc**	**ΔAICc**	**wAICc**
Fixed effects	**RE_t ~ Biomass growth $rate_{direct}$:Biomass growth $rate_{lagged}$ + Biomass growth $rate_{lagged}$:Temperature + Temperature:Hg + offset (Log $biomass_t$)**	12	2216.40	0.00	0.52
	RE_t ~ Biomass growth $rate_{direct}$:Biomass growth $rate_{lagged}$ + Biomass growth $rate_{lagged}$:Temperature + Temperature:Hg + Set + offset (Log $biomass_t$)	13	2218.50	2.14	0.18
	RE_t ~ Biomass growth $rate_{direct}$:Biomass growth $rate_{lagged}$ + Biomass growth $rate_{direct}$:Temperature + Biomass growth $rate_{lagged}$:Temperature + Temperature:Hg + Set + offset (Log $biomass_t$)	14	2220.20	3.83	0.08

	RE_t ~ Biomass growth $rate_{direct}$:Biomass growth $rate_{lagged}$ + Biomass growth $rate_{lagged}$:Temperature + Biomass growth $rate_{lagged}$:Hg + Temperature:Hg + Set + offset (Log $biomass_t$)	15	2220.50	4.14	0.07
	RE_t ~ Biomass growth $rate_{direct}$:Biomass growth $rate_{lagged}$ + Biomass growth $rate_{lagged}$:Temperature + Hg + Set + offset (Log $biomass_t$)	11	2221.60	5.25	0.04

Table A2. Summary statistics of fitted final models.

Response variable	Final model	Parameter	Estimate ± SE
Biomass growth rate$_t$	Biomass growth rate$_t$ ~ Log biomass$_t$:Temperature + Set	Intercept	0.89 ± 0.03
		Set 2	-0.04 ± 0.02
		24 °C	0.16 ± 0.06
		Log biomass$_t$	-0.40 ± 0.02
		Log biomass$_t$:24 °C	-0.33 ± 0.04
RE$_t$	RE$_t$ ~ Biomass growth rate$_{direct}$:Biomass growth rate$_{lagged}$ + Biomass growth rate$_{lagged}$:Temperature + Temperature:Hg + offset (Log biomass$_t$)	Intercept	0.57 ± 0.22
		Biomass growth rate$_{direct}$	-1.04 ± 0.30
		Biomass growth rate$_{lagged}$	-0.96 ± 0.42
		24 °C	0.71 ± 0.26
		Low Hg	0.22 ± 0.25
		High Hg	0.70 ± 0.24
		Biomass growth rate$_{direct}$:Biomass growth rate$_{lagged}$	-4.03 ± 0.87
		Biomass growth rate$_{lagged}$:24 °C	2.25 ± 0.51

Low Hg:24 °C	0.09 ± 0.32
High Hg:24 °C	-0.68 ± 0.31

Paper IV

Maternal dopamine exposure in *Daphnia* boosts the survival of starved offspring

Semona Issa[1*], Safa Chaabani[1], Alexandros G. Asimakopoulos[3], Veerle L. B. Jaspers[2] and Sigurd Einum[1]

[1]Centre for Biodiversity Dynamics (CBD), Department of Biology, [2]Department of Biology, and [3]Department of Chemistry, Norwegian University of Science and Technology, Høgskoleringen 5, 7491 Trondheim, Norway

*Correspondence: Semona Issa (email: semona.issa@ntnu.no)

Doctoral theses in Biology
Norwegian University of Science and Technology
Department of Biology

Year	Name	Degree	Title
1974	Tor-Henning Iversen	Dr. philos Botany	The roles of statholiths, auxin transport, and auxin metabolism in root gravitropism
1978	Tore Slagsvold	Dr. philos Zoology	Breeding events of birds in relation to spring temperature and environmental phenology
1978	Egil Sakshaug	Dr. philos Botany	The influence of environmental factors on the chemical composition of cultivated and natural populations of marine phytoplankton
1980	Arnfinn Langeland	Dr. philos Zoology	Interaction between fish and zooplankton populations and their effects on the material utilization in a freshwater lake
1980	Helge Reinertsen	Dr. philos Botany	The effect of lake fertilization on the dynamics and stability of a limnetic ecosystem with special reference to the phytoplankton
1982	Gunn Mari Olsen	Dr. scient Botany	Gravitropism in roots of *Pisum sativum* and *Arabidopsis thaliana*
1982	Dag Dolmen	Dr. philos Zoology	Life aspects of two sympartic species of newts (*Triturus, Amphibia*) in Norway, with special emphasis on their ecological niche segregation
1984	Eivin Røskaft	Dr. philos Zoology	Sociobiological studies of the rook *Corvus frugilegus*
1984	Anne Margrethe Cameron	Dr. scient Botany	Effects of alcohol inhalation on levels of circulating testosterone, follicle stimulating hormone and luteinzing hormone in male mature rats
1984	Asbjørn Magne Nilsen	Dr. scient Botany	Alveolar macrophages from expectorates – Biological monitoring of workers exposed to occupational air pollution. An evaluation of the AM-test
1985	Jarle Mork	Dr. philos Zoology	Biochemical genetic studies in fish
1985	John Solem	Dr. philos Zoology	Taxonomy, distribution and ecology of caddisflies (*Trichoptera*) in the Dovrefjell mountains
1985	Randi E. Reinertsen	Dr. philos Zoology	Energy strategies in the cold: Metabolic and thermoregulatory adaptations in small northern birds
1986	Bernt-Erik Sæther	Dr. philos Zoology	Ecological and evolutionary basis for variation in reproductive traits of some vertebrates: A comparative approach
1986	Torleif Holthe	Dr. philos Zoology	Evolution, systematics, nomenclature, and zoogeography in the polychaete orders *Oweniimorpha* and *Terebellomorpha*, with special reference to the Arctic and Scandinavian fauna
1987	Helene Lampe	Dr. scient Zoology	The function of bird song in mate attraction and territorial defence, and the importance of song repertoires
1987	Olav Hogstad	Dr. philos Zoology	Winter survival strategies of the Willow tit *Parus montanus*
1987	Jarle Inge Holten	Dr. philos Botany	Autecological investigations along a coust-inland transect at Nord-Møre, Central Norway

1987	Rita Kumar	Dr. scient Botany	Somaclonal variation in plants regenerated from cell cultures of *Nicotiana sanderae* and *Chrysanthemum morifolium*
1987	Bjørn Åge Tømmerås	Dr. scient Zoology	Olfaction in bark beetle communities: Interspecific interactions in regulation of colonization density, predator - prey relationship and host attraction
1988	Hans Christian Pedersen	Dr. philos Zoology	Reproductive behaviour in willow ptarmigan with special emphasis on territoriality and parental care
1988	Tor G. Heggberget	Dr. philos Zoology	Reproduction in Atlantic Salmon (*Salmo salar*): Aspects of spawning, incubation, early life history and population structure
1988	Marianne V. Nielsen	Dr. scient Zoology	The effects of selected environmental factors on carbon allocation/growth of larval and juvenile mussels (*Mytilus edulis*)
1988	Ole Kristian Berg	Dr. scient Zoology	The formation of landlocked Atlantic salmon (*Salmo salar* L.)
1989	John W. Jensen	Dr. philos Zoology	Crustacean plankton and fish during the first decade of the manmade Nesjø reservoir, with special emphasis on the effects of gill nets and salmonid growth
1989	Helga J. Vivås	Dr. scient Zoology	Theoretical models of activity pattern and optimal foraging: Predictions for the Moose *Alces alces*
1989	Reidar Andersen	Dr. scient Zoology	Interactions between a generalist herbivore, the moose *Alces alces*, and its winter food resources: a study of behavioural variation
1989	Kurt Ingar Draget	Dr. scient Botany	Alginate gel media for plant tissue culture
1990	Bengt Finstad	Dr. scient Zoology	Osmotic and ionic regulation in Atlantic salmon, rainbow trout and Arctic charr: Effect of temperature, salinity and season
1990	Hege Johannesen	Dr. scient Zoology	Respiration and temperature regulation in birds with special emphasis on the oxygen extraction by the lung
1990	Åse Krøkje	Dr. scient Botany	The mutagenic load from air pollution at two work-places with PAH-exposure measured with Ames Salmonella/microsome test
1990	Arne Johan Jensen	Dr. philos Zoology	Effects of water temperature on early life history, juvenile growth and prespawning migrations of Atlantic salmon (*Salmo salar*) and brown trout (*Salmo trutta*): A summary of studies in Norwegian streams
1990	Tor Jørgen Almaas	Dr. scient Zoology	Pheromone reception in moths: Response characteristics of olfactory receptor neurons to intra- and interspecific chemical cues
1990	Magne Husby	Dr. scient Zoology	Breeding strategies in birds: Experiments with the Magpie *Pica pica*
1991	Tor Kvam	Dr. scient Zoology	Population biology of the European lynx (*Lynx lynx*) in Norway
1991	Jan Henning L'Abêe Lund	Dr. philos Zoology	Reproductive biology in freshwater fish, brown trout *Salmo trutta* and roach *Rutilus rutilus* in particular
1991	Asbjørn Moen	Dr. philos Botany	The plant cover of the boreal uplands of Central Norway. I. Vegetation ecology of Sølendet nature reserve; haymaking fens and birch woodlands
1991	Else Marie Løbersli	Dr. scient Botany	Soil acidification and metal uptake in plants
1991	Trond Nordtug	Dr. scient Zoology	Reflectometric studies of photomechanical adaptation in superposition eyes of arthropods
1991	Thyra Solem	Dr. scient Botany	Age, origin and development of blanket mires in Central Norway

1991	Odd Terje Sandlund	Dr. philos Zoology	The dynamics of habitat use in the salmonid genera *Coregonus* and *Salvelinus*: Ontogenic niche shifts and polymorphism
1991	Nina Jonsson	Dr. philos Zoology	Aspects of migration and spawning in salmonids
1991	Atle Bones	Dr. scient Botany	Compartmentation and molecular properties of thioglucoside glucohydrolase (myrosinase)
1992	Torgrim Breiehagen	Dr. scient Zoology	Mating behaviour and evolutionary aspects of the breeding system of two bird species: the Temminck's stint and the Pied flycatcher
1992	Anne Kjersti Bakken	Dr. scient Botany	The influence of photoperiod on nitrate assimilation and nitrogen status in timothy (*Phleum pratense* L.)
1992	Tycho Anker-Nilssen	Dr. scient Zoology	Food supply as a determinant of reproduction and population development in Norwegian Puffins *Fratercula arctica*
1992	Bjørn Munro Jenssen	Dr. philos Zoology	Thermoregulation in aquatic birds in air and water: With special emphasis on the effects of crude oil, chemically treated oil and cleaning on the thermal balance of ducks
1992	Arne Vollan Aarset	Dr. philos Zoology	The ecophysiology of under-ice fauna: Osmotic regulation, low temperature tolerance and metabolism in polar crustaceans.
1993	Geir Slupphaug	Dr. scient Botany	Regulation and expression of uracil-DNA glycosylase and O^6-methylguanine-DNA methyltransferase in mammalian cells
1993	Tor Fredrik Næsje	Dr. scient Zoology	Habitat shifts in coregonids.
1993	Yngvar Asbjørn Olsen	Dr. scient Zoology	Cortisol dynamics in Atlantic salmon, *Salmo salar* L.: Basal and stressor-induced variations in plasma levels and some secondary effects.
1993	Bård Pedersen	Dr. scient Botany	Theoretical studies of life history evolution in modular and clonal organisms
1993	Ole Petter Thangstad	Dr. scient Botany	Molecular studies of myrosinase in Brassicaceae
1993	Thrine L. M. Heggberget	Dr. scient Zoology	Reproductive strategy and feeding ecology of the Eurasian otter *Lutra lutra*.
1993	Kjetil Bevanger	Dr. scient Zoology	Avian interactions with utility structures, a biological approach.
1993	Kåre Haugan	Dr. scient Botany	Mutations in the replication control gene trfA of the broad host-range plasmid RK2
1994	Peder Fiske	Dr. scient Zoology	Sexual selection in the lekking great snipe (*Gallinago media*): Male mating success and female behaviour at the lek
1994	Kjell Inge Reitan	Dr. scient Botany	Nutritional effects of algae in first-feeding of marine fish larvae
1994	Nils Røv	Dr. scient Zoology	Breeding distribution, population status and regulation of breeding numbers in the northeast-Atlantic Great Cormorant *Phalacrocorax carbo carbo*
1994	Annette-Susanne Hoepfner	Dr. scient Botany	Tissue culture techniques in propagation and breeding of Red Raspberry (*Rubus idaeus* L.)
1994	Inga Elise Bruteig	Dr. scient Botany	Distribution, ecology and biomonitoring studies of epiphytic lichens on conifers
1994	Geir Johnsen	Dr. scient Botany	Light harvesting and utilization in marine phytoplankton: Species-specific and photoadaptive responses

1994	Morten Bakken	Dr. scient Zoology	Infanticidal behaviour and reproductive performance in relation to competition capacity among farmed silver fox vixens, *Vulpes vulpes*
1994	Arne Moksnes	Dr. philos Zoology	Host adaptations towards brood parasitism by the Cockoo
1994	Solveig Bakken	Dr. scient Botany	Growth and nitrogen status in the moss *Dicranum majus* Sm. as influenced by nitrogen supply
1994	Torbjørn Forseth	Dr. scient Zoology	Bioenergetics in ecological and life history studies of fishes.
1995	Olav Vadstein	Dr. philos Botany	The role of heterotrophic planktonic bacteria in the cycling of phosphorus in lakes: Phosphorus requirement, competitive ability and food web interactions
1995	Hanne Christensen	Dr. scient Zoology	Determinants of Otter *Lutra lutra* distribution in Norway: Effects of harvest, polychlorinated biphenyls (PCBs), human population density and competition with mink *Mustela vision*
1995	Svein Håkon Lorentsen	Dr. scient Zoology	Reproductive effort in the Antarctic Petrel *Thalassoica antarctica*; the effect of parental body size and condition
1995	Chris Jørgen Jensen	Dr. scient Zoology	The surface electromyographic (EMG) amplitude as an estimate of upper trapezius muscle activity
1995	Martha Kold Bakkevig	Dr. scient Zoology	The impact of clothing textiles and construction in a clothing system on thermoregulatory responses, sweat accumulation and heat transport
1995	Vidar Moen	Dr. scient Zoology	Distribution patterns and adaptations to light in newly introduced populations of *Mysis relicta* and constraints on Cladoceran and Char populations
1995	Hans Haavardsholm Blom	Dr. philos Botany	A revision of the *Schistidium apocarpum* complex in Norway and Sweden
1996	Jorun Skjærmo	Dr. scient Botany	Microbial ecology of early stages of cultivated marine fish; inpact fish-bacterial interactions on growth and survival of larvae
1996	Ola Ugedal	Dr. scient Zoology	Radiocesium turnover in freshwater fishes
1996	Ingibjørg Einarsdottir	Dr. scient Zoology	Production of Atlantic salmon (*Salmo salar*) and Arctic charr (*Salvelinus alpinus*): A study of some physiological and immunological responses to rearing routines
1996	Christina M. S. Pereira	Dr. scient Zoology	Glucose metabolism in salmonids: Dietary effects and hormonal regulation
1996	Jan Fredrik Børseth	Dr. scient Zoology	The sodium energy gradients in muscle cells of *Mytilus edulis* and the effects of organic xenobiotics
1996	Gunnar Henriksen	Dr. scient Zoology	Status of Grey seal *Halichoerus grypus* and Harbour seal *Phoca vitulina* in the Barents sea region
1997	Gunvor Øie	Dr. scient Botany	Eevalution of rotifer *Brachionus plicatilis* quality in early first feeding of turbot *Scophtalmus maximus* L. larvae
1997	Håkon Holien	Dr. scient Botany	Studies of lichens in spruce forest of Central Norway. Diversity, old growth species and the relationship to site and stand parameters
1997	Ole Reitan	Dr. scient Zoology	Responses of birds to habitat disturbance due to damming
1997	Jon Arne Grøttum	Dr. scient Zoology	Physiological effects of reduced water quality on fish in aquaculture

1997	Per Gustav Thingstad	Dr. scient Zoology	Birds as indicators for studying natural and human-induced variations in the environment, with special emphasis on the suitability of the Pied Flycatcher
1997	Torgeir Nygård	Dr. scient Zoology	Temporal and spatial trends of pollutants in birds in Norway: Birds of prey and Willow Grouse used as
1997	Signe Nybø	Dr. scient Zoology	Impacts of long-range transported air pollution on birds with particular reference to the dipper *Cinclus cinclus* in southern Norway
1997	Atle Wibe	Dr. scient Zoology	Identification of conifer volatiles detected by receptor neurons in the pine weevil (*Hylobius abietis*), analysed by gas chromatography linked to electrophysiology and to mass spectrometry
1997	Rolv Lundheim	Dr. scient Zoology	Adaptive and incidental biological ice nucleators
1997	Arild Magne Landa	Dr. scient Zoology	Wolverines in Scandinavia: ecology, sheep depredation and conservation
1997	Kåre Magne Nielsen	Dr. scient Botany	An evolution of possible horizontal gene transfer from plants to sail bacteria by studies of natural transformation in *Acinetobacter calcoacetius*
1997	Jarle Tufto	Dr. scient Zoology	Gene flow and genetic drift in geographically structured populations: Ecological, population genetic, and statistical models
1997	Trygve Hesthagen	Dr. philos Zoology	Population responses of Arctic charr (*Salvelinus alpinus* (L.)) and brown trout (*Salmo trutta* L.) to acidification in Norwegian inland waters
1997	Trygve Sigholt	Dr. philos Zoology	Control of Parr-smolt transformation and seawater tolerance in farmed Atlantic Salmon (*Salmo salar*) Effects of photoperiod, temperature, gradual seawater acclimation, NaCl and betaine in the diet
1997	Jan Østnes	Dr. scient Zoology	Cold sensation in adult and neonate birds
1998	Seethaledsumy Visvalingam	Dr. scient Botany	Influence of environmental factors on myrosinases and myrosinase-binding proteins
1998	Thor Harald Ringsby	Dr. scient Zoology	Variation in space and time: The biology of a House sparrow metapopulation
1998	Erling Johan Solberg	Dr. scient Zoology	Variation in population dynamics and life history in a Norwegian moose (*Alces alces*) population: consequences of harvesting in a variable environment
1998	Sigurd Mjøen Saastad	Dr. scient Botany	Species delimitation and phylogenetic relationships between the Sphagnum recurvum complex (Bryophyta): genetic variation and phenotypic plasticity
1998	Bjarte Mortensen	Dr. scient Botany	Metabolism of volatile organic chemicals (VOCs) in a head liver S9 vial equilibration system in vitro
1998	Gunnar Austrheim	Dr. scient Botany	Plant biodiversity and land use in subalpine grasslands. – A conservation biological approach
1998	Bente Gunnveig Berg	Dr. scient Zoology	Encoding of pheromone information in two related moth species
1999	Kristian Overskaug	Dr. scient Zoology	Behavioural and morphological characteristics in Northern Tawny Owls *Strix aluco*: An intra- and interspecific comparative approach
1999	Hans Kristen Stenøien	Dr. scient Botany	Genetic studies of evolutionary processes in various populations of nonvascular plants (mosses, liverworts and hornworts)
1999	Trond Arnesen	Dr. scient Botany	Vegetation dynamics following trampling and burning in the outlying haylands at Sølendet, Central Norway

1999	Ingvar Stenberg	Dr. scient Zoology	Habitat selection, reproduction and survival in the White-backed Woodpecker *Dendrocopos leucotos*
1999	Stein Olle Johansen	Dr. scient Botany	A study of driftwood dispersal to the Nordic Seas by dendrochronology and wood anatomical analysis
1999	Trina Falck Galloway	Dr. scient Zoology	Muscle development and growth in early life stages of the Atlantic cod (*Gadus morhua* L.) and Halibut (*Hippoglossus hippoglossus* L.)
1999	Marianne Giæver	Dr. scient Zoology	Population genetic studies in three gadoid species: blue whiting (*Micromisistius poutassou*), haddock (*Melanogrammus aeglefinus*) and cod (*Gadus morhua*) in the North-East Atlantic
1999	Hans Martin Hanslin	Dr. scient Botany	The impact of environmental conditions of density dependent performance in the boreal forest bryophytes *Dicranum majus*, *Hylocomium splendens*, *Plagiochila asplenigides*, *Ptilium crista-castrensis* and *Rhytidiadelphus lokeus*
1999	Ingrid Bysveen Mjølnerød	Dr. scient Zoology	Aspects of population genetics, behaviour and performance of wild and farmed Atlantic salmon (*Salmo salar*) revealed by molecular genetic techniques
1999	Else Berit Skagen	Dr. scient Botany	The early regeneration process in protoplasts from *Brassica napus* hypocotyls cultivated under various g-forces
1999	Stein-Are Sæther	Dr. philos Zoology	Mate choice, competition for mates, and conflicts of interest in the Lekking Great Snipe
1999	Katrine Wangen Rustad	Dr. scient Zoology	Modulation of glutamatergic neurotransmission related to cognitive dysfunctions and Alzheimer's disease
1999	Per Terje Smiseth	Dr. scient Zoology	Social evolution in monogamous families:
1999	Gunnbjørn Bremset	Dr. scient Zoology	Young Atlantic salmon (*Salmo salar* L.) and Brown trout (*Salmo trutta* L.) inhabiting the deep pool habitat, with special reference to their habitat use, habitat preferences and competitive interactions
1999	Frode Ødegaard	Dr. scient Zoology	Host specificity as a parameter in estimates of arthropod species richness
1999	Sonja Andersen	Dr. scient Zoology	Expressional and functional analyses of human, secretory phospholipase A2
2000	Ingrid Salvesen	Dr. scient Botany	Microbial ecology in early stages of marine fish: Development and evaluation of methods for microbial management in intensive larviculture
2000	Ingar Jostein Øien	Dr. scient Zoology	The Cuckoo (*Cuculus canorus*) and its host: adaptions and counteradaptions in a coevolutionary arms race
2000	Pavlos Makridis	Dr. scient Botany	Methods for the microbial control of live food used for the rearing of marine fish larvae
2000	Sigbjørn Stokke	Dr. scient Zoology	Sexual segregation in the African elephant (*Loxodonta africana*)
2000	Odd A. Gulseth	Dr. philos Zoology	Seawater tolerance, migratory behaviour and growth of Charr, (*Salvelinus alpinus*), with emphasis on the high Arctic Dieset charr on Spitsbergen, Svalbard
2000	Pål A. Olsvik	Dr. scient Zoology	Biochemical impacts of Cd, Cu and Zn on brown trout (*Salmo trutta*) in two mining-contaminated rivers in Central Norway
2000	Sigurd Einum	Dr. scient Zoology	Maternal effects in fish: Implications for the evolution of breeding time and egg size
2001	Jan Ove Evjemo	Dr. scient Zoology	Production and nutritional adaptation of the brine shrimp *Artemia* sp. as live food organism for larvae of marine cold water fish species

2001	Olga Hilmo	Dr. scient Botany	Lichen response to environmental changes in the managed boreal forest systems
2001	Ingebrigt Uglem	Dr. scient Zoology	Male dimorphism and reproductive biology in corkwing wrasse (*Symphodus melops* L.)
2001	Bård Gunnar Stokke	Dr. scient Zoology	Coevolutionary adaptations in avian brood parasites and their hosts
2002	Ronny Aanes	Dr. scient Zoology	Spatio-temporal dynamics in Svalbard reindeer (*Rangifer tarandus platyrhynchus*)
2002	Mariann Sandsund	Dr. scient Zoology	Exercise- and cold-induced asthma. Respiratory and thermoregulatory responses
2002	Dag-Inge Øien	Dr. scient Botany	Dynamics of plant communities and populations in boreal vegetation influenced by scything at Sølendet, Central Norway
2002	Frank Rosell	Dr. scient Zoology	The function of scent marking in beaver (*Castor fiber*)
2002	Janne Østvang	Dr. scient Botany	The Role and Regulation of Phospholipase A_2 in Monocytes During Atherosclerosis Development
2002	Terje Thun	Dr. philos Biology	Dendrochronological constructions of Norwegian conifer chronologies providing dating of historical material
2002	Birgit Hafjeld Borgen	Dr. scient Biology	Functional analysis of plant idioblasts (Myrosin cells) and their role in defense, development and growth
2002	Bård Øyvind Solberg	Dr. scient Biology	Effects of climatic change on the growth of dominating tree species along major environmental gradients
2002	Per Winge	Dr. scient Biology	The evolution of small GTP binding proteins in cellular organisms. Studies of RAC GTPases in *Arabidopsis thaliana* and the Ral GTPase from *Drosophila melanogaster*
2002	Henrik Jensen	Dr. scient Biology	Causes and consequences of individual variation in fitness-related traits in house sparrows
2003	Jens Rohloff	Dr. philos Biology	Cultivation of herbs and medicinal plants in Norway – Essential oil production and quality control
2003	Åsa Maria O. Espmark Wibe	Dr. scient Biology	Behavioural effects of environmental pollution in threespine stickleback *Gasterosteus aculeatur* L.
2003	Dagmar Hagen	Dr. scient Biology	Assisted recovery of disturbed arctic and alpine vegetation – an integrated approach
2003	Bjørn Dahle	Dr. scient Biology	Reproductive strategies in Scandinavian brown bears
2003	Cyril Lebogang Taolo	Dr. scient Biology	Population ecology, seasonal movement and habitat use of the African buffalo (*Syncerus caffer*) in Chobe National Park, Botswana
2003	Marit Stranden	Dr. scient Biology	Olfactory receptor neurones specified for the same odorants in three related Heliothine species (*Helicoverpa armigera, Helicoverpa assulta* and *Heliothis virescens*)
2003	Kristian Hassel	Dr. scient Biology	Life history characteristics and genetic variation in an expanding species, *Pogonatum dentatum*
2003	David Alexander Rae	Dr. scient Biology	Plant- and invertebrate-community responses to species interaction and microclimatic gradients in alpine and Artic environments
2003	Åsa A Borg	Dr. scient Biology	Sex roles and reproductive behaviour in gobies and guppies: a female perspective
2003	Eldar Åsgard Bendiksen	Dr. scient Biology	Environmental effects on lipid nutrition of farmed Atlantic salmon (*Salmo salar* L.) parr and smolt
2004	Torkild Bakken	Dr. scient Biology	A revision of Nereidinae (Polychaeta, Nereididae)

2004	Ingar Pareliussen	Dr. scient Biology	Natural and Experimental Tree Establishment in a Fragmented Forest, Ambohitantely Forest Reserve, Madagascar
2004	Tore Brembu	Dr. scient Biology	Genetic, molecular and functional studies of RAC GTPases and the WAVE-like regulatory protein complex in *Arabidopsis thaliana*
2004	Liv S. Nilsen	Dr. scient Biology	Coastal heath vegetation on central Norway; recent past, present state and future possibilities
2004	Hanne T. Skiri	Dr. scient Biology	Olfactory coding and olfactory learning of plant odours in heliothine moths. An anatomical, physiological and behavioural study of three related species (*Heliothis virescens, Helicoverpa armigera* and *Helicoverpa assulta*)
2004	Lene Østby	Dr. scient Biology	Cytochrome P4501A (CYP1A) induction and DNA adducts as biomarkers for organic pollution in the natural environment
2004	Emmanuel J. Gerreta	Dr. philos Biology	The Importance of Water Quality and Quantity in the Tropical Ecosystems, Tanzania
2004	Linda Dalen	Dr. scient Biology	Dynamics of Mountain Birch Treelines in the Scandes Mountain Chain, and Effects of Climate Warming
2004	Lisbeth Mehli	Dr. scient Biology	Polygalacturonase-inhibiting protein (PGIP) in cultivated strawberry (*Fragaria* x *ananassa*): characterisation and induction of the gene following fruit infection by *Botrytis cinerea*
2004	Børge Moe	Dr. scient Biology	Energy-Allocation in Avian Nestlings Facing Short-Term Food Shortage
2005	Matilde Skogen Chauton	Dr. scient Biology	Metabolic profiling and species discrimination from High-Resolution Magic Angle Spinning NMR analysis of whole-cell samples
2005	Sten Karlsson	Dr. scient Biology	Dynamics of Genetic Polymorphisms
2005	Terje Bongard	Dr. scient Biology	Life History strategies, mate choice, and parental investment among Norwegians over a 300-year period
2005	Tonette Røstelien	PhD Biology	Functional characterisation of olfactory receptor neurone types in heliothine moths
2005	Erlend Kristiansen	Dr. scient Biology	Studies on antifreeze proteins
2005	Eugen G. Sørmo	Dr. scient Biology	Organochlorine pollutants in grey seal (*Halichoerus grypus*) pups and their impact on plasma thyroid hormone and vitamin A concentrations
2005	Christian Westad	Dr. scient Biology	Motor control of the upper trapezius
2005	Lasse Mork Olsen	PhD Biology	Interactions between marine osmo- and phagotrophs in different physicochemical environments
2005	Åslaug Viken	PhD Biology	Implications of mate choice for the management of small populations
2005	Ariaya Hymete Sahle Dingle	PhD Biology	Investigation of the biological activities and chemical constituents of selected *Echinops* spp. growing in Ethiopia
2005	Anders Gravbrøt Finstad	PhD Biology	Salmonid fishes in a changing climate: The winter challenge
2005	Shimane Washington Makabu	PhD Biology	Interactions between woody plants, elephants and other browsers in the Chobe Riverfront, Botswana
2005	Kjartan Østbye	Dr. scient Biology	The European whitefish *Coregonus lavaretus* (L.) species complex: historical contingency and adaptive radiation

2006	Kari Mette Murvoll	PhD Biology	Levels and effects of persistent organic pollutans (POPs) in seabirds, Retinoids and α-tocopherol – potential biomakers of POPs in birds?
2006	Ivar Herfindal	Dr. scient Biology	Life history consequences of environmental variation along ecological gradients in northern ungulates
2006	Nils Egil Tokle	PhD Biology	Are the ubiquitous marine copepods limited by food or predation? Experimental and field-based studies with main focus on *Calanus finmarchicus*
2006	Jan Ove Gjershaug	Dr. philos Biology	Taxonomy and conservation status of some booted eagles in south-east Asia
2006	Jon Kristian Skei	Dr. scient Biology	Conservation biology and acidification problems in the breeding habitat of amphibians in Norway
2006	Johanna Järnegren	PhD Biology	*Acesta oophaga* and *Acesta excavata* – a study of hidden biodiversity
2006	Bjørn Henrik Hansen	PhD Biology	Metal-mediated oxidative stress responses in brown trout (*Salmo trutta*) from mining contaminated rivers in Central Norway
2006	Vidar Grøtan	PhD Biology	Temporal and spatial effects of climate fluctuations on population dynamics of vertebrates
2006	Jafari R Kideghesho	PhD Biology	Wildlife conservation and local land use conflicts in Western Serengeti Corridor, Tanzania
2006	Anna Maria Billing	PhD Biology	Reproductive decisions in the sex role reversed pipefish *Syngnathus typhle*: when and how to invest in reproduction
2006	Henrik Pärn	PhD Biology	Female ornaments and reproductive biology in the bluethroat
2006	Anders J. Fjellheim	PhD Biology	Selection and administration of probiotic bacteria to marine fish larvae
2006	P. Andreas Svensson	PhD Biology	Female coloration, egg carotenoids and reproductive success: gobies as a model system
2007	Sindre A. Pedersen	PhD Biology	Metal binding proteins and antifreeze proteins in the beetle *Tenebrio molitor* - a study on possible competition for the semi-essential amino acid cysteine
2007	Kasper Hancke	PhD Biology	Photosynthetic responses as a function of light and temperature: Field and laboratory studies on marine microalgae
2007	Tomas Holmern	PhD Biology	Bushmeat hunting in the western Serengeti: Implications for community-based conservation
2007	Kari Jørgensen	PhD Biology	Functional tracing of gustatory receptor neurons in the CNS and chemosensory learning in the moth *Heliothis virescens*
2007	Stig Ulland	PhD Biology	Functional Characterisation of Olfactory Receptor Neurons in the Cabbage Moth, (*Mamestra brassicae* L.) (Lepidoptera, Noctuidae). Gas Chromatography Linked to Single Cell Recordings and Mass Spectrometry
2007	Snorre Henriksen	PhD Biology	Spatial and temporal variation in herbivore resources at northern latitudes
2007	Roelof Frans May	PhD Biology	Spatial Ecology of Wolverines in Scandinavia
2007	Vedasto Gabriel Ndibalema	PhD Biology	Demographic variation, distribution and habitat use between wildebeest sub-populations in the Serengeti National Park, Tanzania
2007	Julius William Nyahongo	PhD Biology	Depredation of Livestock by wild Carnivores and Illegal Utilization of Natural Resources by Humans in the Western Serengeti, Tanzania

2007	Shombe Ntaraluka Hassan	PhD Biology	Effects of fire on large herbivores and their forage resources in Serengeti, Tanzania
2007	Per-Arvid Wold	PhD Biology	Functional development and response to dietary treatment in larval Atlantic cod *(Gadus morhua* L.) Focus on formulated diets and early weaning
2007	Anne Skjetne Mortensen	PhD Biology	Toxicogenomics of Aryl Hydrocarbon- and Estrogen Receptor Interactions in Fish: Mechanisms and Profiling of Gene Expression Patterns in Chemical Mixture Exposure Scenarios
2008	Brage Bremset Hansen	PhD Biology	The Svalbard reindeer (*Rangifer tarandus platyrhynchus*) and its food base: plant-herbivore interactions in a high-arctic ecosystem
2008	Jiska van Dijk	PhD Biology	Wolverine foraging strategies in a multiple-use landscape
2008	Flora John Magige	PhD Biology	The ecology and behaviour of the Masai Ostrich (*Struthio camelus massaicus*) in the Serengeti Ecosystem, Tanzania
2008	Bernt Rønning	PhD Biology	Sources of inter- and intra-individual variation in basal metabolic rate in the zebra finch, *Taeniopygia guttata*
2008	Sølvi Wehn	PhD Biology	Biodiversity dynamics in semi-natural mountain landscapes - A study of consequences of changed agricultural practices in Eastern Jotunheimen
2008	Trond Moxness Kortner	PhD Biology	The Role of Androgens on previtellogenic oocyte growth in Atlantic cod (*Gadus morhua*): Identification and patterns of differentially expressed genes in relation to Stereological Evaluations
2008	Katarina Mariann Jørgensen	Dr. scient Biology	The role of platelet activating factor in activation of growth arrested keratinocytes and re-epithelialisation
2008	Tommy Jørstad	PhD Biology	Statistical Modelling of Gene Expression Data
2008	Anna Kusnierczyk	PhD Biology	*Arabidopsis thaliana* Responses to Aphid Infestation
2008	Jussi Evertsen	PhD Biology	Herbivore sacoglossans with photosynthetic chloroplasts
2008	John Eilif Hermansen	PhD Biology	Mediating ecological interests between locals and globals by means of indicators. A study attributed to the asymmetry between stakeholders of tropical forest at Mt. Kilimanjaro, Tanzania
2008	Ragnhild Lyngved	PhD Biology	Somatic embryogenesis in *Cyclamen persicum.* Biological investigations and educational aspects of cloning
2008	Line Elisabeth Sundt-Hansen	PhD Biology	Cost of rapid growth in salmonid fishes
2008	Line Johansen	PhD Biology	Exploring factors underlying fluctuations in white clover populations – clonal growth, population structure and spatial distribution
2009	Astrid Jullumstrø Feuerherm	PhD Biology	Elucidation of molecular mechanisms for pro-inflammatory phospholipase A2 in chronic disease
2009	Pål Kvello	PhD Biology	Neurons forming the network involved in gustatory coding and learning in the moth *Heliothis virescens:* Physiological and morphological characterisation, and integration into a standard brain atlas
2009	Trygve Devold Kjellsen	PhD Biology	Extreme Frost Tolerance in Boreal Conifers
2009	Johan Reinert Vikan	PhD Biology	Coevolutionary interactions between common cuckoos *Cuculus canorus* and *Fringilla* finches

2009	Zsolt Volent	PhD Biology	Remote sensing of marine environment: Applied surveillance with focus on optical properties of phytoplankton, coloured organic matter and suspended matter
2009	Lester Rocha	PhD Biology	Functional responses of perennial grasses to simulated grazing and resource availability
2009	Dennis Ikanda	PhD Biology	Dimensions of a Human-lion conflict: Ecology of human predation and persecution of African lions (*Panthera leo*) in Tanzania
2010	Huy Quang Nguyen	PhD Biology	Egg characteristics and development of larval digestive function of cobia (*Rachycentron canadum*) in response to dietary treatments - Focus on formulated diets
2010	Eli Kvingedal	PhD Biology	Intraspecific competition in stream salmonids: the impact of environment and phenotype
2010	Sverre Lundemo	PhD Biology	Molecular studies of genetic structuring and demography in *Arabidopsis* from Northern Europe
2010	Iddi Mihijai Mfunda	PhD Biology	Wildlife Conservation and People's livelihoods: Lessons Learnt and Considerations for Improvements. The Case of Serengeti Ecosystem, Tanzania
2010	Anton Tinchov Antonov	PhD Biology	Why do cuckoos lay strong-shelled eggs? Tests of the puncture resistance hypothesis
2010	Anders Lyngstad	PhD Biology	Population Ecology of *Eriophorum latifolium*, a Clonal Species in Rich Fen Vegetation
2010	Hilde Færevik	PhD Biology	Impact of protective clothing on thermal and cognitive responses
2010	Ingerid Brænne Arbo	PhD Medical technology	Nutritional lifestyle changes – effects of dietary carbohydrate restriction in healthy obese and overweight humans
2010	Yngvild Vindenes	PhD Biology	Stochastic modeling of finite populations with individual heterogeneity in vital parameters
2010	Hans-Richard Brattbakk	PhD Medical technology	The effect of macronutrient composition, insulin stimulation, and genetic variation on leukocyte gene expression and possible health benefits
2011	Geir Hysing Bolstad	PhD Biology	Evolution of Signals: Genetic Architecture, Natural Selection and Adaptive Accuracy
2011	Karen de Jong	PhD Biology	Operational sex ratio and reproductive behaviour in the two-spotted goby (*Gobiusculus flavescens*)
2011	Ann-Iren Kittang	PhD Biology	*Arabidopsis thaliana* L. adaptation mechanisms to microgravity through the EMCS MULTIGEN-2 experiment on the ISS: The science of space experiment integration and adaptation to simulated microgravity
2011	Aline Magdalena Lee	PhD Biology	Stochastic modeling of mating systems and their effect on population dynamics and genetics
2011	Christopher Gravningen Sørmo	PhD Biology	Rho GTPases in Plants: Structural analysis of ROP GTPases; genetic and functional studies of MIRO GTPases in Arabidopsis thaliana
2011	Grethe Robertsen	PhD Biology	Relative performance of salmonid phenotypes across environments and competitive intensities
2011	Line-Kristin Larsen	PhD Biology	Life-history trait dynamics in experimental populations of guppy (*Poecilia reticulata*): the role of breeding regime and captive environment
2011	Maxim A. K. Teichert	PhD Biology	Regulation in Atlantic salmon (*Salmo salar*): The interaction between habitat and density

2011	Torunn Beate Hancke	PhD Biology	Use of Pulse Amplitude Modulated (PAM) Fluorescence and Bio-optics for Assessing Microalgal Photosynthesis and Physiology
2011	Sajeda Begum	PhD Biology	Brood Parasitism in Asian Cuckoos: Different Aspects of Interactions between Cuckoos and their Hosts in Bangladesh
2011	Kari J. K. Attramadal	PhD Biology	Water treatment as an approach to increase microbial control in the culture of cold water marine larvae
2011	Camilla Kalvatn Egset	PhD Biology	The Evolvability of Static Allometry: A Case Study
2011	AHM Raihan Sarker	PhD Biology	Conflict over the conservation of the Asian elephant (*Elephas maximus*) in Bangladesh
2011	Gro Dehli Villanger	PhD Biology	Effects of complex organohalogen contaminant mixtures on thyroid hormone homeostasis in selected arctic marine mammals
2011	Kari Bjørneraas	PhD Biology	Spatiotemporal variation in resource utilisation by a large herbivore, the moose
2011	John Odden	PhD Biology	The ecology of a conflict: Eurasian lynx depredation on domestic sheep
2011	Simen Pedersen	PhD Biology	Effects of native and introduced cervids on small mammals and birds
2011	Mohsen Falahati-Anbaran	PhD Biology	Evolutionary consequences of seed banks and seed dispersal in *Arabidopsis*
2012	Jakob Hønborg Hansen	PhD Biology	Shift work in the offshore vessel fleet: circadian rhythms and cognitive performance
2012	Elin Noreen	PhD Biology	Consequences of diet quality and age on life-history traits in a small passerine bird
2012	Irja Ida Ratikainen	PhD Biology	Foraging in a variable world: adaptations to stochasticity
2012	Aleksander Handå	PhD Biology	Cultivation of mussels (*Mytilus edulis):* Feed requirements, storage and integration with salmon (*Salmo salar*) farming
2012	Morten Kraabøl	PhD Biology	Reproductive and migratory challenges inflicted on migrant brown trout (*Salmo trutta* L.) in a heavily modified river
2012	Jisca Huisman	PhD Biology	Gene flow and natural selection in Atlantic salmon
2012	Maria Bergvik	PhD Biology	Lipid and astaxanthin contents and biochemical post-harvest stability in *Calanus finmarchicus*
2012	Bjarte Bye Løfaldli	PhD Biology	Functional and morphological characterization of central olfactory neurons in the model insect *Heliothis virescens*.
2012	Karen Marie Hammer	PhD Biology	Acid-base regulation and metabolite responses in shallow- and deep-living marine invertebrates during environmental hypercapnia
2012	Øystein Nordrum Wiggen	PhD Biology	Optimal performance in the cold
2012	Robert Dominikus Fyumagwa	Dr. Philos Biology	Anthropogenic and natural influence on disease prevalence at the human –livestock-wildlife interface in the Serengeti ecosystem, Tanzania
2012	Jenny Bytingsvik	PhD Biology	Organohalogenated contaminants (OHCs) in polar bear mother-cub pairs from Svalbard, Norway. Maternal transfer, exposure assessment and thyroid hormone disruptive effects in polar bear cubs
2012	Christer Moe Rolandsen	PhD Biology	The ecological significance of space use and movement patterns of moose in a variable environment

2012	Erlend Kjeldsberg Hovland	PhD Biology	Bio-optics and Ecology in *Emiliania huxleyi* Blooms: Field and Remote Sensing Studies in Norwegian Waters
2012	Lise Cats Myhre	PhD Biology	Effects of the social and physical environment on mating behaviour in a marine fish
2012	Tonje Aronsen	PhD Biology	Demographic, environmental and evolutionary aspects of sexual selection
2012	Bin Liu	PhD Biology	Molecular genetic investigation of cell separation and cell death regulation in *Arabidopsis thaliana*
2013	Jørgen Rosvold	PhD Biology	Ungulates in a dynamic and increasingly human dominated landscape – A millennia-scale perspective
2013	Pankaj Barah	PhD Biology	Integrated Systems Approaches to Study Plant Stress Responses
2013	Marit Linnerud	PhD Biology	Patterns in spatial and temporal variation in population abundances of vertebrates
2013	Xinxin Wang	PhD Biology	Integrated multi-trophic aquaculture driven by nutrient wastes released from Atlantic salmon (*Salmo salar*) farming
2013	Ingrid Ertshus Mathisen	PhD Biology	Structure, dynamics, and regeneration capacity at the sub-arctic forest-tundra ecotone of northern Norway and Kola Peninsula, NW Russia
2013	Anders Foldvik	PhD Biology	Spatial distributions and productivity in salmonid populations
2013	Anna Marie Holand	PhD Biology	Statistical methods for estimating intra- and inter-population variation in genetic diversity
2013	Anna Solvang Båtnes	PhD Biology	Light in the dark – the role of irradiance in the high Arctic marine ecosystem during polar night
2013	Sebastian Wacker	PhD Biology	The dynamics of sexual selection: effects of OSR, density and resource competition in a fish
2013	Cecilie Miljeteig	PhD Biology	Phototaxis in *Calanus finmarchicus* – light sensitivity and the influence of energy reserves and oil exposure
2013	Ane Kjersti Vie	PhD Biology	Molecular and functional characterisation of the IDA family of signalling peptides in *Arabidopsis thaliana*
2013	Marianne Nymark	PhD Biology	Light responses in the marine diatom *Phaeodactylum tricornutum*
2014	Jannik Schultner	PhD Biology	Resource Allocation under Stress - Mechanisms and Strategies in a Long-Lived Bird
2014	Craig Ryan Jackson	PhD Biology	Factors influencing African wild dog *(Lycaon pictus*) habitat selection and ranging behaviour: conservation and management implications
2014	Aravind Venkatesan	PhD Biology	Application of Semantic Web Technology to establish knowledge management and discovery in the Life Sciences
2014	Kristin Collier Valle	PhD Biology	Photoacclimation mechanisms and light responses in marine micro- and macroalgae
2014	Michael Puffer	PhD Biology	Effects of rapidly fluctuating water levels on juvenile Atlantic salmon (*Salmo salar* L.)
2014	Gundula S. Bartzke	PhD Biology	Effects of power lines on moose *(Alces alces)* habitat selection, movements and feeding activity
2014	Eirin Marie Bjørkvoll	PhD Biology	Life-history variation and stochastic population dynamics in vertebrates
2014	Håkon Holand	PhD Biology	The parasite *Syngamus trachea* in a metapopulation of house sparrows
2014	Randi Magnus Sommerfelt	PhD Biology	Molecular mechanisms of inflammation – a central role for cytosolic phospholiphase A2

2014	Espen Lie Dahl	PhD Biology	Population demographics in white-tailed eagle at an on-shore wind farm area in coastal Norway
2014	Anders Øverby	PhD Biology	Functional analysis of the action of plant isothiocyanates: cellular mechanisms and in vivo role in plants, and anticancer activity
2014	Kamal Prasad Acharya	PhD Biology	Invasive species: Genetics, characteristics and trait variation along a latitudinal gradient.
2014	Ida Beathe Øverjordet	PhD Biology	Element accumulation and oxidative stress variables in Arctic pelagic food chains: *Calanus*, little auks (*Alle alle*) and black-legged kittiwakes (*Rissa tridactyla*)
2014	Kristin Møller Gabrielsen	PhD Biology	Target tissue toxicity of the thyroid hormone system in two species of arctic mammals carrying high loads of organohalogen contaminants
2015	Gine Roll Skjervø	Dr. philos Biology	Testing behavioral ecology models with historical individual-based human demographic data from Norway
2015	Nils Erik Gustaf Forsberg	PhD Biology	Spatial and Temporal Genetic Structure in Landrace Cereals
2015	Leila Alipanah	PhD Biology	Integrated analyses of nitrogen and phosphorus deprivation in the diatoms *Phaeodactylum tricornutum* and *Seminavis robusta*
2015	Javad Najafi	PhD Biology	Molecular investigation of signaling components in sugar sensing and defense in *Arabidopsis thaliana*
2015	Bjørnar Sporsheim	PhD Biology	Quantitative confocal laser scanning microscopy: optimization of in vivo and in vitro analysis of intracellular transport
2015	Magni Olsen Kyrkjeeide	PhD Biology	Genetic variation and structure in peatmosses (*Sphagnum*)
2015	Keshuai Li	PhD Biology	Phospholipids in Atlantic cod (*Gadus morhua* L.) larvae rearing: Incorporation of DHA in live feed and larval phospholipids and the metabolic capabilities of larvae for the de novo synthesis
2015	Ingvild Fladvad Størdal	PhD Biology	The role of the copepod *Calanus finmarchicus* in affecting the fate of marine oil spills
2016	Thomas Kvalnes	PhD Biology	Evolution by natural selection in age-structured populations in fluctuating environments
2016	Øystein Leiknes	PhD Biology	The effect of nutrition on important life-history traits in the marine copepod *Calanus finmarchicus*
2016	Johan Henrik Hårdensson Berntsen	PhD Biology	Individual variation in survival: The effect of incubation temperature on the rate of physiological ageing in a small passerine bird
2016	Marianne Opsahl Olufsen	PhD Biology	Multiple environmental stressors: Biological interactions between parameters of climate change and perfluorinated alkyl substances in fish
2016	Rebekka Varne	PhD Biology	Tracing the fate of escaped cod (*Gadus morhua* L.) in a Norwegian fjord system
2016	Anette Antonsen Fenstad	PhD Biology	Pollutant Levels, Antioxidants and Potential Genotoxic Effects in Incubating Female Common Eiders (*Somateria mollissima*)
2016	Wilfred Njama Marealle	PhD Biology	Ecology, Behaviour and Conservation Status of Masai Giraffe (*Giraffa camelopardalis tippelskirchi*) in Tanzania
2016	Ingunn Nilssen	PhD Biology	Integrated Enviromental Mapping and Monitoring: A Methodological approach for end users.
2017	Konika Chawla	PhD Biology	Discovering, analysing and taking care of knowledge.

2017	Øystein Hjorthol Opedal	PhD Biology	The Evolution of Herkogamy: Pollinator Reliability, Natural Selection, and Trait Evolvability.
2017	Ane Marlene Myhre	PhD Biology	Effective size of density dependent populations in fluctuating environments
2017	Emmanuel Hosiana Masenga	PhD Biology	Behavioural Ecology of Free-ranging and Reintroduced African Wild Dog (*Lycaon pictus*) Packs in the Serengeti Ecosystem, Tanzania
2017	Xiaolong Lin	PhD Biology	Systematics and evolutionary history of *Tanytarsus* van der Wulp, 1874 (Diptera: Chironomidae)
2017	Emmanuel Clamsen Mmassy	PhD Biology	Ecology and Conservation Challenges of the Kori bustard in the Serengeti National Park
2017	Richard Daniel Lyamuya	PhD Biology	Depredation of Livestock by Wild Carnivores in the Eastern Serengeti Ecosystem, Tanzania
2017	Katrin Hoydal	PhD Biology	Levels and endocrine disruptive effects of legacy POPs and their metabolites in long-finned pilot whales of the Faroe Islands
2017	Berit Glomstad	PhD Biology	Adsorption of phenanthrene to carbon nanotubes and its influence on phenanthrene bioavailability/toxicity in aquatic organism
2017	Øystein Nordeide Kielland	PhD Biology	Sources of variation in metabolism of an aquatic ectotherm
2017	Narjes Yousefi	PhD Biology	Genetic divergence and speciation in northern peatmosses (*Sphagnum*)
2018	Signe Christensen-Dalgaard	PhD Biology	Drivers of seabird spatial ecology - implications for development of offshore wind-power in Norway
2018	Janos Urbancsok	PhD Biology	Endogenous biological effects induced by externally supplemented glucosinolate hydrolysis products (GHPs) on *Arabidopsis thaliana*
2018	Alice Mühlroth	PhD Biology	The influence of phosphate depletion on lipid metabolism of microalgae
2018	Franco Peniel Mbise	PhD Biology	Human-Carnivore Coexistence and Conflict in the Eastern Serengeti, Tanzania
2018	Stine Svalheim Markussen	PhD Biology	Causes and consequences of intersexual life history variation in a harvested herbivore population
2018	Mia Vedel Sørensen	PhD Biology	Carbon budget consequences of deciduous shrub expansion in alpine tundra ecosystems
2018	Hanna Maria Kauko	PhD Biology	Light response and acclimation of microalgae in a changing Arctic
2018	Erlend I. F. Fossen	PhD Biology	Trait evolvability: effects of thermal plasticity and genetic correlations among traits
2019	Peter Sjolte Ranke	PhD Biology	Demographic and genetic and consequences of dispersal in house sparrows
2019	Mathilde Le Moullec	PhD Biology	Spatiotemporal variation in abundance of key tundra species: from local heterogeneity to large-scale synchrony
2019	Endre Grüner Ofstad	PhD Biology	Causes and consequences of variation in resource use and social structure in ungulates
2019	Yang Jin	PhD Biology	Development of lipid metabolism in early life stage of Atlantic salmon (*Salmo salar*)
2019	Elena Albertsen	PhD Biology	Evolution of floral traits: from ecological contex to functional integration
2019	Mominul Islam Nahid	PhD Biology	Interaction between two Asian cuckoos and their hosts in Bangladesh

2019	Knut Jørgen Egelie	Phd Biology	Management of intellectual property in university-industry collaborations – public access to and control of knowledge
2019	Thomas Ray Haaland	Phd Biology	Adaptive responses to enviromental stochasticity on different evolutionary time-scales
2019	Kwaslema Malle Hariohay	Phd Biology	Human wildlife interactions in the Ruaha-Rungwa Ecosystem, Central Tanzania
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