Temperature dependent toxicity of the neonicotinoid imidacloprid on survival and development in an arctic population of the Collembola *Hypogastrura viatica*

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Abstract

Climatic change with rising temperatures might influence how species copes with the effects of exposure to pesticides. A group of insecticides with strict regulations in the EU, neonicotinoids, has been in focus of scientific investigations the last years as concern and evidence of harmful effects on non-target species are emerging. This study investigates the effect of exposure to the neonicotinoid imidacloprid at two temperatures on the life-history traits survival and onset of first reproduction in an arctic population of the Collembola *Hypogastrura vitica*. In two laboratory experiments, newly hatched juveniles were exposed to imidacloprid in natural LUFA 2.2 standard soil at six concentrations in the span of 0.01 – 10 mg/kg at 15ºC and 20ºC. The survival was highest at 20º at all treatments when juveniles were exposed for a timespan equal to the same physiological age at the two temperatures. This was not expected as 15ºC is closer to the temperatures the arctic population are exposed to in its natural arctic environment, with 20ºC expected to induce temperature-dependent stress. One of the possible explanations for this difference in survival is the difference in duration of exposure, as the time exposed was shorter at 20ºC. The survival was low and no reproduction occurred at the highest imidacloprid concentrations of this study. When converted to Dº there is no difference in the age at first reproduction for the treatments between the two temperatures, except at 0.1 mg/kg, where first reproduction occurs later at 20Cº than at 15Cº.

Sammendrag

Klimaendringer, med økende temperaturer, kan påvirke hvordan arter takler effektene av eksponering for pesticider. Neonikotinoider, en gruppe innsektsmidler med streng regulering for bruk i EU, har vært i fokus de siste årene med bakgrunn i bekymring for og bevis på skadelige effekter hos arter som ikke er målgruppe-organismer for disse innsektsmidlene. Denne studien undersøker effekten på livshistoriestrategiene overlevelse og alder ved første reproduksjon eksponering for neonikotinoidet imidacloprid har på en arktisk populasjon av spretthalearten *Hypogastrura viatica* ved to temperaturer. I to laboratorieeksperimenter ble nylig klekkede juvenile spretthaler av denne arten eksponert for imidacloprid gjennom naturlig LUFA 2.2 standard jord i seks konsentrasjoner i spennet 0.01 – 10 mg/kg jord ved 15ºC og 20ºC. Overlevelsen var høyest ved 20ºC i alle konsentrasjoner etter juvenil eksponering av spretthalene i et tidsintervall tilsvarende samme fysiologiske alder. Dette resultatet var ikke forventet, siden 15ºC ligger nærmere temperaturer den arktiske populasjonen utsettes for i sitt naturlige miljø, og 20ºC var antatt å være en høy nok temperatur til indusere temperaturstress i denne populasjonen. En av de mulige forklaringene for forskjellen i overlevelse mellom temperaturene kan være forskjellen i eksponeringstid, siden eksponeringen var kortere ved 20ºC. I de høyeste konsentrasjonene i denne studien var det lav overlevelse og ingen reproduksjon. Etter konvertering til Dº var det svært liten forskjell i alder ved første reproduksjon mellom de to temperaturene, bortsett fra ved 0.1 mg/kg jord, der første reproduksjon forekommer senere ved 20ºC enn ved 15ºC.

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Abbreviations

ANOVA	analysis of variance
appx.	appendix
C	carbon
$C_9H_{10}ClN_5O_2$	imidacloprid
CITS	climate-induced toxicant sensitivity
cm	centimeter
conc.	concentration
D^o	day-degrees / degree days
e.g.	exempli gratia / for example
fig.	figure
g/mol	molar mass
H. viatica	*Hypogastrura viatica*
i.e.	id es / in other words
ind.	individuals
kg	kilo gram
K_{ow}	octanol-water partition coefficient
L	litre
LC	lethal concentration
mg	milligram
mL	millilitre
nAChR	nicotinic acetylcholine receptors
no.	number
OECD	Organization for Economic Co-operation and Development
pic.	picture
SE	standard error
temp.	temperature
TICS	toxicant-induced climate susceptibility
UiO	University of Oslo

Contents

1 Introduction

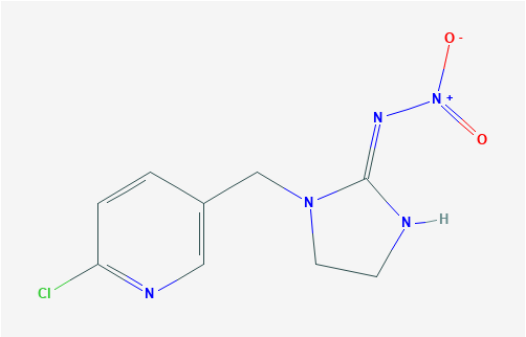
1.1 Pesticides

In our world today, the environment is exposed to an increasing amount of human-made stressors (e.g., climate change, utilization of resources, increased production and release of chemicals, modification of landscape, extinction of species, and spread of alien species), at expense of quality of air, soil and water, biodiversity, habitat loss, and shifts in communities (Díaz et al., 2019; Field et al., 2014; Johnson et al., 2017; Nielsen, 2019; Parker et al., 1999; Sala et al., 2000; Scheffers et al., 2016). When organisms are exposed to several of these stressors in combination, and in combination with natural occurring drivers (e.g., shifts in season and competition) the resulting effects on the organisms can be both complex and hard to predict (Breitburg et al., 1998; Holmstrup et al., 2010). In the last decade, there has been an increased awareness of the need for a better understanding of the interactions between toxic chemical substances and climate, especially with the increase in the presence of chemicals in environments far from emission points (e.g., in the arctic) (AMAP, 2018; Borgå, 2019; Meltofte, 2013).

A group of chemicals that is intentionally released to the environment are pesticides, substances used in agriculture to protect crops and livestock. As the human population is growing and environmental change renders areas unsuitable for food production, the efficiency and output of agriculture are increasingly important (FAO, 2017, 2018; UN, 2019). To protect crops from being damaged or eaten by insects or other pests we use pesticides designed to be toxic to their specific target species, but they can also affect non-target species. One group of pesticides that have been used in large quantities worldwide is the group of insecticides called neonicotinoids. These are manmade agricultural pesticides made to protect crops from insects, i.e., insecticides. The neonicotinoids are, per now, the most widely used insecticide-group globally, registered for use in more than 120 countries (Jeschke et al., 2011; Simon-Delso et al., 2015). They provide effective pest control, being a group of synthetic broad-spectrum pesticides with high specific toxicity to arthropods by targeting the nicotinic acetylcholine receptors (nAChR) in the insect central nervous system, and systemic distribution in plant tissue, i.e., protects all the tissues in the whole plant (Elbert et al., 2008; Jeschke et al., 2011; Sheets, 2010; Tomizawa, M & Casida, 2005). The term neonicotinoid is used to distinguish this group of chemicals from the natural occurring nicotinoids, as

neonicotinoids are synthetic, more effective as insecticides, and less toxic to mammals (Tomizawa, M & Casida, 2005; Tomizawa, M. & Yamamoto, 1993). However, evidence of toxicity in mammals and other vertebrates are emerging (Berheim et al., 2019; Burke et al., 2018; Eng et al., 2019; Hallmann et al., 2014). Neonicotinoids have to a large degree replaced pyrethroids, organophosphates and metylcarbamates, as resistant strains of their target species has reduced the effectiveness of these insecticides (Elbert et al., 2008; Tomizawa, M & Casida, 2005). Strict regulations have been imposed on the usage of neonicotinoids and imidacloprid in the EU both in 2013 and 2018 (EFSA, 2013; European Commission Directorate-General for Health and Food Safety, 2013, 2018), as there is evidence of toxicity in non-target organisms (Alexander et al., 2007; Berheim et al., 2019; Burke et al., 2018; Eng et al., 2019; Hallmann et al., 2014), especially bees (Blacquière et al., 2012; Henry et al., 2012; Krupke et al., 2012; Palmer et al., 2013; Whitehorn et al., 2012).

The first neonicotinoid developed and made commercially available was imidacloprid (Kollmeyer et al., 1999; Sheets, 2010). It was patented in 1985 (Tomizawa, M & Casida, 2005) and made available on the market in 1991 and has thus been the neonicotinoid most widely used in both time and quantity (Jeschke et al., 2011). The main use of imidacloprid is to protect crops against sucking insects, termites, and chewing insects, as well as flea control in domestic animals. Imidacloprid has chemical formula $C_9H_{10}ClN_5O_2$ (pic. 1), a molecular weight of 255.66 g/mol, a water solubility of $6.1x10^2$ mg/L at 20°C, and a octanol-water partition coefficient, i.e., log K_{ow}, of 0.57 at 21 °C (PubChem).



Picture 1: Chemical structure of imidacloprid $C_9H_{10}ClN_5O_2$ (PubChem)

In the imidacloprid molecule, the electronegative 2-nitroimino-imidazolidine group is the key to binding it to the post-synaptic nicotinic acetylcholine receptors in the central nervous system of insects (Greenslade & Whalley, 1986; Kagabu, 1997; Tomizawa, M. & Yamamoto, 1993). It interacts as an agonist with the alpha-BGTX site of the nicotinic acetylcoline

receptor, as it is partially positive, and binds strongly (Bai et al., 1991; Buckingham et al., 1995; Greenslade & Whalley, 1986; Jeschke et al., 2011; Tomizawa, M. & Yamamoto, 1992). The strong binding results in disrupting and blocking of the action potential transmission with paralyzation and ultimately, if at a high enough dose, the death of the organism exposed (Simon-Delso et al., 2015). The degradation of imidacloprid in soil is reported to be between 28 and 1250 days dependent on the characteristics of the soil, i.e., content of organic matter, pH, exposure to UV-light, temperature, moisture and texture (Bonmatin et al., 2015; Goulson, 2013). Due to the high water solubility and low K_{ow} value of imidacloprid it is not expected to bind strongly in soils, and has the potential of being washed out with water (Goulson, 2013).

1.2 Climate-induced toxicant sensitivity

Noyes et al. (2009) highlight that since higher temperatures and other effects of global warming may reduce concentrations of pesticides in the soil through increased degradation and volatilization, this may increase the load of pesticides used to maintain the effect. Climate change (e.g., temperature change), has the potential to alter the toxicity of pesticides, so-called climate-induced toxicant sensitivity (Hooper et al., 2013; Laskowski et al., 2010; Noyes et al., 2009). Higher temperatures can alter physiological mechanisms (e.g. homeostasis), and the toxicokinetic properties of the pesticides in organisms (Hooper et al., 2013; Noyes et al., 2009). As increasing temperature may give increased rates of uptake and elimination of pesticides, the effect depends on if there are changes in metabolism, and if these changes are in the direction of increased detoxification or bio-activation (Noyes et al., 2009). Populations of organisms living close to their upper or lower physiological tolerance range may be especially vulnerable as they are already living in a stressful environment, especially if climate change induces changes in habitats and food webs (AMAP, 2002; Noyes et al., 2009).

Pesticides as an additional stressor may also alter the upper/lower tolerances of organisms (Noyes et al., 2009) As toxic stress from pesticides may affect how an organism respond to climatic change, i.e., toxicant-induced climate susceptibility (TICS) (Hooper et al., 2013), while climatic stress, on the other hand, may affect the tolerance of the organism to the toxicants in pesticides, i.e., climate-induced toxicant sensitivity (CITS) (Barmentlo et al., 2017; González-Alcaraz et al., 2015; Hooper et al., 2013; Sjursen & Holmstrup, 2004).

The outcomes of multiple stressors can lead to effects we do not expect and give additive, synergistic or antagonistic responses in populations (Crain et al., 2008; Folt et al., 1999; Gessner & Tlili, 2016; Holmstrup et al., 2010; Laskowski et al., 2010; Paine et al., 1998; Piggott et al., 2015; Todgham & Stillman, 2013). Additive responses are when the overall effect of the stressors together is equivalent to the sum of the effects of the stressors separately. Synergistic responses are when the overall effect of the stressors together is higher than the sum of the effects of the stressors separately. Antagonistic responses are when the overall effect of the stressors is less than the sum of the effects of the stressors separately. The more stressors affecting an organism/population, the more complex the composition of effects and outcomes may be, as combinations of stressors might have additive, synergistic and antagonistic effects at the same time (e.g., different effects in different types of tissues or on internal processes in an organism) (Crain et al., 2008; Folt et al., 1999; Gessner & Tlili, 2016; Piggott et al., 2015). To explore the complex relationships between stressors experiments are performed in laboratories or in the field. In a laboratory, we can simulate perfect conditions and expose organisms to a few stressors at a time. Laboratory experiments are thus fruitful for finding the isolated effect of specific stressors as it does not mirror the complexity of mixed influences in the real world (Todgham & Stillman, 2013).

Climatic induced changes in abiotic factors, i.e., air temperature, sea temperature, moisture, weather patterns, has the potential to make alterations and induce stress at different biological levels (e.g., changes in habitat quality and quantity, alternation of species distribution in areas, species composition and interactions in communities, reduce genetic diversity in the species present in the communities, reduce the biomass present and biomass productivity, affect biological rates, and shift the timing of biological events (e.g., flowering, reproduction, spawning, and hibernation)) (Sala et al., 2000; Scheffers et al., 2016). The half-life of a chemical (e.g., pesticide) in the environment is influenced by temperature, UV-radiation, pH, types of internal chemical bindings in the molecule, hydrophobic/hydrophilic properties, and the presence of water and organic materials (Gessner & Tlili, 2016). Chemicals with hydrophobic properties will thus bind to the organic matter in sediment/soil, while chemicals with hydrophilic properties will follow the water and wash out. Organisms exposure to a chemical are dependent on the presence of the chemical in the environment, exposure time, exposure pathway (e.g., ingestion, inhalation or dermal), behavior, habitat use, ability to biotransform the chemical, and capacity of elimination together with elimination rates (Borgå, 2008; Jørgensen & Fath, 2010). If the chemical is hydrophobic, i.e., lipophilic, it

will have the potential to accumulate in the organism by storage in fatty tissues (Borgå, 2008). The sum of the chemical properties, environmental factors and properties of exposed organisms will affect how pesticides inflict both target species and non-target species in means of physical damage, behavioral changes, and by species interaction (Gessner & Tlili, 2016). This can again alter their ability to fill their role in the ecosystem and affect how they perform their ecosystem services (Chagnon et al., 2015; Palmer et al., 2013). Chemicals can thus alter the biodiversity of soil communities by inflicting sublethal and lethal damage to a higher degree in some species than others (Borgå, 2008; Gessner & Tlili, 2016; Thompson et al., 2016). As organisms can be exposed to several pesticides simultaneously by living in soil treated with several pesticides at the same time, multiple treatments spread throughout the year, and the potential of previously used pesticides remaining in the soil, the effect and outcome can be complex and hard to predict, especially if metabolites of the pesticides are taken into account. In an evaluation of topsoil samples from Europe collected in 2015 58% of the samples had traces of two or more pesticides, with presence of imidacloprid in 7% of the total number of samples (Silva et al., 2019).

1.3 Collembola – affected non-target organisms

Collembola are important members of most soil communities owning to their role in the decomposition of dead organic matter, and thereby recycling of plant nutrients. They are abundant in agricultural areas and their surroundings, and thus exposed to pesticides in use. Collembola (common name: springtails) is a subclass of the subphylum Hexapoda. They are among the oldest known terrestrial animals, with the 400 million years old fossil Collembola, *Rhyniella praecursor*, being the oldest known species (Greenslade & Whalley, 1986; Stanley & Maulik, 1926). Currently, over 6000 species are known worldwide, with an assumption that the number of unknown species is high (Hopkin, 1997; Rusek, 1998). Collembola has a global distribution and are found in most terrestrial habitats, including the extreme (e.g., deserts and polar regions). They occupy a wide variation of niches, as they have proven to be very adaptable in terms of temperature, dryness, substrate, and feed (Hopkin, 1997). Collembola normally have a low local dispersal ability, being wingless and with most species living in the soil or layer of litter. This makes them vulnerable to sudden changes in their environment (e.g., spraying of insecticides), as they have few possibilities of quick escape.

The role of Collembola in decomposition is mainly indirect by feeding on bacteria and fungi, and thereby regulating the activity of these primary decomposers (Hanlon & Anderson, 1979; Hopkin, 1997; Petersen, 1994; Rusek, 1998). Collembola also plays a role in the ecosystem as food for predatory insects, spiders, mites, birds and parasites (Hopkin, 1997; Leinaas & Ambrose, 1992; Petersen, 1994). They are especially important in areas at high altitude and high latitude were fewer other taxa of soil-dwelling fauna are present (Seastedt, 1984). Collembola are useful as model organisms in studies of eco-toxicology (de Lima e Silva et al., 2017; de Lima e Silva et al., 2019; OECD, 2016; Ogungbemi & van Gestel, 2018; Schnug et al., 2014a; Schnug et al., 2014b; Sjursen & Holmstrup, 2004; Stam et al., 1996; van Gestel, 2012; van Gestel et al., 2017) and international guidelines for acute and chronic toxicity are developed (OECD, 2016). The species of *Folsomia candida* and *Folsomia fimetaria* are recommended test species for testing of chemicals (OECD, 2016), but other species are also suggested and used; *Proisotoma minuta*, *Isotoma viridis*, *Isotoma anglicana*, *Orchesella cincta*, *Sinella curviseta*, *Paronychiurus kimi*, *Orthonychiurus folsomi*, and *Mesaphorura macrochaeta* (OECD, 2016). Collembola are also useful as model organisms for general studies on life-history traits (Birkemoe & Leinaas, 2000, 2001; Sengupta et al., 2016, 2017). As Collembola is suitable model organisms for experiments with a life-history approach, this approach in combination with toxicological studies can give us a better understanding of underlaying primary and secondary effects of chemicals.

Most Collembola do not have a tracheal system, and gas exchange occurs through diffusion across the body surface (OECD, 2016). Along with the gas exchange other compounds, including toxic substances will diffuse through the cuticle. The diffusion rate is affected by the humidity and temperature of the air and soil, combined with the volume surface ratio and metabolic rate (Hopkin, 1997). The cuticle is assumed to be the main exposure route of imidacloprid in the present experiment, due to the water-soluble properties of the insecticide and by contact with the moistened contaminated soil.

1.4 Life-history traits

Survival, growth, development rates, age at maturity, size at maturity, fecundity, number of offspring, and size of offspring are all important life-history traits (Stearns, 1992). Temperature is considered one of the major drivers of the evolution of life-history traits in ectotherms due to dependence between life-history traits and physiological rate (Angilletta et

al., 2003; Huey & Kingsolver, 1989). An individual organism will, at any point in life, have a limited amount of internal resources and energy (Stearns, 1992). These resources and energy are allocated to different processes, both during everyday life and at different stages in the lifetime of an organism. A certain amount of energy will be required to high priority needs to maintain the organism (e.g., digestion, excretion, and basal metabolism). The rest can be allocated to (e.g., growth, reproduction, foraging and eating, finding shelter or competition). Collembola have indeterminate growth, i.e., they continue to grow after maturation (Hopkin, 1997). This means that they allocate energy and resources to growth also when they are reproducing (Stearns, 1992). Thus Collembola is a group of hexapods that can be used as model organisms for investigations of trade-offs with energy and resources allocated to growth. How the resources and energy are allocated will affect the fitness of the individual, i.e., ability to survive and reproduce. An individual has to balance its internal resources between several processes and stressors might disrupt this balance at the cost of reproduction, self-maintenance, growth, or other processes (Jensen et al., 2006). The allocation of resources and energy will be a result of the influence of stressors combined with the life-history strategy and phenotypic plasticity of the species/population in question.

The combination of raised temperature and imidacloprid are suspected to affect the survival and basic life-history parameters of Collembola (de Lima e Silva et al., 2017; Ogungbemi & van Gestel, 2018; van Gestel et al., 2017). Differences in phenotypes, i.e., the ability to change gene expression (switch between alternative phenotypes of the genotype) as a response to stimuli from the environment, can be a sign of adaption to variation and changes in the environment and may have a great impact on fitness (Fusco & Minelli, 2010; West-Eberhard, 2008). This can appear as different phenotypes in different species and populations of the same species of Collembola, as phenotypic plasticity is influenced by adaption to different environments (Sengupta et al., 2017). Thus different populations of the same species can respond differently to exposure to the same stressors due to differences in phenotypic plasticity. Plastic responses in life-history traits are a reflection of how a species or population cope with changes in the environment (Stearns, 1992) and are useful parameters in studies of ecotoxicology, as sublethal doses of chemicals can have huge effects on organism and population level long before lethal doses are reached (Eng et al., 2019; Saaristo et al., 2018; Tappert et al., 2017). If life-history traits of the Collembola are affected by changes in temperature and the presence of imidacloprid, this will not only have effects on the individual and population level, but also have potential effects on their ecosystem services.

Juvenile survival until maturation and first reproduction is essential for the fitness of an individual and the population (Stearns, 1992). Any stressors affecting during this period may, if sufficiently severe, affect the development and survival of the juveniles (pic. 2), and thus affecting the number, age and size of individuals maturing.

Picture 2: Juvenile *H. viatica*. The light individuals are newly hatched.

At maturation, the resource and energy budget and allocation of an organism change from growth and development to also include the costs of reproduction (Stearns, 1992). If compared with other life-history traits, a change in the juvenile survival and age at maturity have a large impact as it is the life-history traits closest linked to fitness (Martin & Bize, 2018; Stearns, 1992). A stressor might delay the timing of maturation, as organisms use energy more energy on self-maintenance. A delay in the timing of maturation will affect the fitness through a delay in the reproduction of the next generation (Stearns, 1992)

The investment of resources in each reproductive event might also affect future reproductive events, and thus fitness (Stearns, 1992). In Collembola the development time of eggs is relatively long compared with time from hatching to maturation. It can often be as much as half of the juvenile stage (H. P. Leinaas, pers. comm.), and a delay in development time or reduction of the quality of eggs may have consequences for fitness through prolongation of the generation time with a longer period at risk of not surviving to reproduction, and impaired population growth (Stearns, 1992). Exposure to imidacloprid has the potential of affecting both the survival of the juvenile stage and the age at first reproduction, together with other aspects of the life history (e.g., the quality of eggs, hatching success and the size of the offspring). In a life-history approach, these are important parameters to understand sublethal effects of the pesticide.

1.5 Aim and objectives

The aim of the present study is to investigate how changes in temperature affect the effect of imidacloprid on the life-history traits survival and age at first reproduction in an arctic population of the Collembola *Hypogastrura viatica*, i.e., temperature-dependent toxicant sensitivity.

The northern arctic population from Svalbard is chosen for this master thesis as a similar study is carried out on a southern temperate population from Norway as a part of the "Effects of climate change in a multiple stress multispecies perspective"- project (from now on MULTICLIM). A similar experiment is carried out on a southern population from Norway, and the results from the populations of Svalbard and Norway will be combined in future work.

The life-history traits examined in this thesis are; survival at end of exposure to different concentrations of imidacloprid through soil, survival at end of the experiment after a post-exposure period in an environment without exposure to imidacloprid, i.e., clean environment, and timespan from hatching to maturity, i.e., age at first reproduction. Survival was chosen as one of the life-history traits for this master thesis as survival is essential for investigation of all the other life-history traits, it is closely linked to fitness, and it indicates at which concentrations the population reach sub-lethal doses. Age at first reproduction was chosen as it is one of the most important life-history traits, it is closely related to fitness, it is interesting in terms of potential trade-offs with other life-history traits, and useful in investigating sub-lethal effects.

The experiments also included data on other life-history traits (size at end of exposure and end of experiment, fecundity, hatching success and size of offspring) that were outside the scope of the present master thesis. This data will be examined in future work as a part of the MULTICLIM project.

Objective i) Survival

To assess if there is a temperature dependent effect of exposure to imidacloprid, i.e., climate-induced toxicant sensitivity, in terms of survival after exposure of juvenile *H. viatica* to different concentrations of imidacloprid through soil, and survival after a post-exposure period in a clean environment.

Hypothesis and expectations:

H0 i.1: The effect of imidacloprid on survival is not dependent on temperature.

Prior to the experiments, it is assumed that the survival rate will decrease with higher concentrations of imidacloprid at both 15ºC and 20ºC. It is assumed that the two different temperatures will result in different lethal concentration (LC) values for survival. It is also assumed and that increased doses of imidacloprid will give lower LC values. It is assumed that temperature will affect the sensitivity to imidacloprid with a higher sensitivity at 20ºC than 15ºC. This is assumed due to the arctic origins of the population with 20ºC being an unrealistic high temperature, and thus assumed to induce a higher level of temperature-induced stress than 15ºC. It is assumed that the survival rate will be higher in the post- exposure period from end of exposure to the end of the experiment, as the organisms are not at exposure, i.e., in a clean environment, in this period.

Objective ii) Development time to first reproduction

To assess if there is a temperature dependent effect of exposure to imidacloprid, i.e., climate-induced toxicant sensitivity, in terms of the development time from hatching to first reproduction, i.e., age at first reproduction.

H0 ii.1: The effect of imidacloprid on the development time to first reproduction is not dependent on temperature.

Prior to the experiments, it is assumed that the time from hatching to first reproduction will be delayed with increasing concentrations of imidacloprid due to slower development. The age at first reproduction is expected to be the same at the different treatments at both temperatures when converted to day-degrees (Dº). The age at first reproduction in days is expected to be earlier at 20ºC than 15ºC due to faster development in ectotherms at higher temperature.

2 Materials and methods

2.1 Model organism: *Hypogastrura viatica*

2.1.1 Population: site and sampling

The population of *H. viatical* was sampled at the "Fjortende Julibukta" (fig. 1) in Krossfjorden (79º 7' 26'' N, 11º 53' 47'' E) during fieldwork in 2016 (Kristiansen, 2017). The Collembola were collected from moss and cyanobacteria on the soil surface in a nutrient-rich area beneath a sea bird cliff at the northern side of the bay.

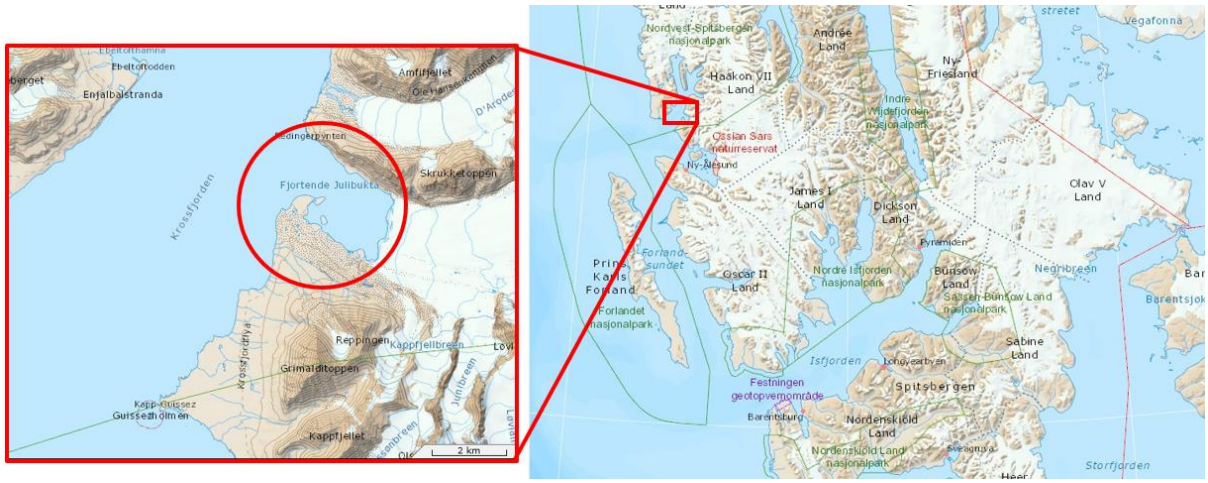


Figure 1: Map showing the location of the sampling site - Fjortende Julibukta, Svalbard. (Toposvalbard, 2018)

2.1.2 *Hypogastrura viatica* (Tullberg 1872)

H. viatica (Collembola: Hypogastruridae) is an active soil-surface dwelling species widely distributed in the Holarctic. It is known to have high ability to invade new environments, and thus have successfully been introduced to many areas in the southern hemisphere (Greenslade, 1995; Hertzberg et al., 2000). The color is dark grey/blue (pic. 3), due to their surface living lifestyle with the need for protection from UV radiation (Hertzberg et al., 1994; Hertzberg et al., 2000; Leinaas, 2002; Mertens et al., 1983). In the Svalbard

environment with sparse shading vegetation and low temperatures, the pigmentation may also help the animals to absorb heat from solar energy (Hopkin, 1997).

Picture 3: *H. viatica* at soil surface. Longyearbyen May 2018.

On western Svalbard, *H. viatica* usually has a two-year life cycle with eggs hatching the first summer season, overwintering as juveniles, growth through the second summer season, a second overwintering, and reproduction the third summer season (Hertzberg et al., 2000; Serbezov, 2002). After reaching maturity, they have the potential to reproduce every second molt (Mertens et al., 1983). They have a flexible, opportunistic life cycle with constant reproductive effort under good conditions, while arresting reproduction under restricting conditions (e.g., food shortage) (H. P. Leinaas, pers. comm.)(Serbezov, 2002).

In an earlier study on a population from Bjørndalen in Svalbard, temperature was shown to affected the timespan from hatching to reaching maturity in *H. viatica* at day 51 at 15ºC and day 42 at 20ºC (Serbezov, 2002).

2.1.3 Pre-experimental conditions

The laboratory population was kept in large culture boxes with the bottom coated in solidified plaster of Paris mixed with charcoal (Leinaas, 2002; Sengupta et al., 2017; Serbezov, 2002). To maintain close to saturated humidity in the boxes I added some droplets of distilled water to the plaster of Paris at each feeding event. As feed, the Collembola were given small pieces of bark (pic. 4 and 5) with cyanobacteria obtained from trees at the campus

of the University of Oslo (Jensen et al., 2006; Kristiansen, 2017; Sengupta et al., 2017; Serbezov, 2002).

Picture 2: Culture boxes with plaster of Paris and cultures of *H. viatica* together with pieces of bark with cyanobacteria.

Food was renewed every seventh day. At the same time, molted skin, feces, and dead individuals were removed to hinder fungal growth. The culture boxes were kept at 15ºC in climate chambers (Sanyo MIR 553, Osaka, Japan; accurate to +/- 0.5ºC) with constant light as photoperiod, to simulate arctic summer, and thereby stimulate activity and reproduction.

Picture 3: *H. viatica*, from a culture box of mixed-age, feeding on bark with cyanobacteria.

One and a half months prior to the start of the experiment individuals from the culture boxes were transferred to smaller boxes (diameter = 3.4 cm, height = 3 cm) with the same bottom as described above. 20-30 individuals were transferred to each box to provide a lower density of individuals and the amount of food was increased to promote reproduction and egg-laying. Removal of molted skins, and feces, as well as feeding and watering, were done every fourth day. At the same time eggs were collected and transferred to similar new plastic boxes. Both newly laid eggs with unbroken chorion (pic. 6) and eggs developed to a stage of broken chorion (pic. 7) were collected. They were placed in separate boxes based on stage of development, to ensure synchronized hatching of the eggs in each box. Both the boxes containing reproducing Collembola and those containing eggs were kept together in a larger rectangular plastic box to ensure an as stable as possible moisture and temperature condition in the smaller boxes. The boxes were kept in the same climate chamber and under the same conditions as previously described.

Picture 5: Newly laid eggs of H. viatica with unbroken chorion.

Picture 4: Eggs of H. viatica with broken chorion and visible eyes together with juveniles.

2.2 Experimental design

2.2.1 Overall experimental design

The experimental design consists of six concentrations of imidacloprid mixed in soil to simulate a toxic environment, and two temperatures as abiotic factors. The two temperatures of 15ºC and 20ºC were chosen as the two temperatures for this experiment, as 10ºC has been shown to delay development and reduce reproduction, and 25ºC have been showed to cause some stress reactions in this species in previous experiments (Serbezov, 2002). The initial concentrations were 0.00, 0.01, 0.1, 1.0 and 10 mg/kg soil. As the data showed high mortality at 1.0 and 10 mg/kg, and the sub-lethal concentration area the area seemed to be around 0.1 mg/kg, the concentrations of 0.05 and 0.5 mg/kg soil were included in a second round of the experiment with the concentrations 0.00, 0.05, 0.1 and 0.5 mg/kg soil. As a result of this, the control and 0.1 mg/kg have double the number of replicates as the rest of the concentrations (table 1). The total number of animals per temperature was 1800, with total 3600 for both temperatures.

Table 1: The experimental design of the experiment with concentrations of imidacloprid (mg/kg soil), number of replicas per concentration, and number of *H. viatica* per replica, per concentration and per temperature (15ºC and 20ºC).

Imidacloprid mg/kg soil	**0.00**	**0.01**	**0.05**	**0.1**	**0.5**	**1.0**	**10**
No. of boxes (replicates)	16	8	8	16	8	8	8
Individuals per box	25	25	25	25	25	25	25
Total no. of ind. per conc.	400	200	200	400	200	200	200

2.2.2 Soil

LUFA Speyer 2.2 standard soil (Speyer, 2017) was chosen as a substrate for pesticide exposure to keep the experiments as close to natural conditions as possible. Although the OECD guidelines (OECD, 2016) recommend using artificial soil, a natural soil was chosen since the MULTICLIM project will compare field and laboratory experiments. Usage of a standard soil secure the content (e.g., organic matter content, pH-value and particle size),

sampling procedure, sampling site and treatment of the soil prior to the experiment. The LUFA Speyer standard soils are guaranteed no usage of pesticides for the last 5 years prior to sampling. In a long-term perspective, standard natural soils have the advantage of being available and comparable, and enables comparison with the work of other scientists using the same soil (Barmentlo et al., 2017; de Lima e Silva et al., 2017; de Lima e Silva et al., 2019; González-Alcaraz et al., 2015; Ogungbemi & van Gestel, 2018; Szabó et al., 2019).

2.2.3 Calculation of mg imidacloprid/kg dry soil

The calculations for the imidacloprid concentrations were based on the calculations for the pre-experiment (Kristiansen, in prep.). Due to different batches of LUFA Speyer 2.2 soil and different storing prior to the experiments, the amount of water per kg of dry soil was re-tested and recalculated for this experiment. To find the right amount ratio of water per kg soil different weights of soil were tested with 6 mL water. A range of 28 – 36 g soil per 6 mL water was tested, and 32 g soil was estimated to give suitable humidity for the Collembola (H. P. Leinaas and H. S. Konestabo, pers. comm.). This gave 1 mL water per 5.3 g soil. 5.299 mg imidacloprid per 100 mL water was calculated to give a concentration equivalent to 10 mg imidacloprid /kg dry soil (the highest concentration of the treatments and the starting concentration for the dilutions).

2.2.4 Preparation of soil

The soil was pre-moistened 2 days before the mixing with the imidacloprid solutions to ensure an even mixing of the compound, as described with artificial soil in OECD Test No. 232 (OECD, 2016). Each batch of 307.4 g soil was moistened with 29 mL distilled water and stored packed in aluminum foil and a zip lock bag (to prevent light and loss of moisture) at 4ºC.

The imidacloprid dilutions for the concentrations of 0.01, 0.05, 0.1, 0.5, 1.0 and 10 mg/kg soil were made through dilutions from the 10 mg/kg concentration. As half of the distilled water was already mixed in the soil the mixing of the dilutions was balanced to give the final concentration in the soil. As imidacloprid is broken down by light the whole procedure was carried out in containers packed in aluminum foil and with dimmed lighting in the room. Each of the dilutions was mixed thoroughly with the premoistened soil and packed light- and airtight and stored at 4ºC.

Eight boxes of 5.5 g soil per concentration per temperature were weighed out (pic. 8). To avoid subjective bias, the experiment was randomized and blinded to make the concentration of each box unknown during the experiment (pic. 9). The randomized boxes were kept in the larger boxes covered in aluminum foil in the climate chamber for two days to acclimate the soil before adding of newly hatched Collembolas.

Picture 8: Example of plastic box (without lid) with 5.5 g moisturised soil.

Picture 6: Blinding and randomization of the boxes with soil. The larger containers are packed in aluminium foil to prevent any degradation of imidacloprid by light.

2.2.5 Exposure of Collembola in soil

Collembola were transferred to boxes with test soil the same day they hatched (pic. 10) . Each replicate had 25 individuals added randomly. As the juveniles are white and easy to see and count against a dark surface, the individuals were counted in a separate plastic box over a black surface to ensure exactly 25 individuals, before added to the boxes with soil. The boxes with soil and Collembolas were placed randomly in two larger rectangular plastic boxes, per temperature, to ensure an as stable as possible moisture and temperature condition in the smaller boxes (pic. 11).

Picture 10: Newly hatched juvenile of *H. viatica*, together with an egg and eggshells.

Picture 7: Larger rectangular boxes that contain the smaller randomized boxes at 15ºC.

The boxes were kept in the climate chambers at 15ºC and 20ºC, for 35 days and 26 days, respectively. The difference in duration was based on a calculation of physiological age, expressed as day-degrees Dº (can also be referred to as heat sum). 26 days at 20ºC equals 35 days at 15ºC in Dº when the theoretical lower threshold value (**t**) for development is set to **t** = degrees above 0ºC. The estimation of 35 days as the juvenile life stage at 15ºC were chosen based on the estimated age of maturation for *H. viatica* in the population from the southerner part of Norway, as this experiment is to be compared to the work of the Ph.D on the MULTICLIM project, Silje Marie Kristiansen (see aims and objectives). The southern population of *H. viatica* is known to have a faster development time to first reproduction than the arctic population (H. P. Leinaas, pers. comm.), and 35 days were set to ensure that there would be no reproduction in the soil in any of the populations (as age at first reproduction is one of the traits addressed in this master project). Imidacloprid exposure for the same length of time in day-degrees is done to ease the comparison of the data from the two experiments. The photoperiod was set to 20 hours of light and 4 hours of darkness each day due to the adaption of the set-up of this experiment to the southerner population (the arctic population in an experiment alone would have had a photoperiod of 24 hours of light).

The boxes were observed every 3rd day, and fungi were cleaned out. Crumbled bark with cyanobacteria was added as feed every 6th day. Moisture was balanced by adding a spray of distilled water when needed, with weighing before and after.

2.2.6 Extracting of Collembola from soil

After the exposure part of the experiment (35 and 26 days at 15ºC and 20ºC), the Collembola in each box were extracted from the soil by flotation. Prior to the flotation, any individuals observed on the soil surface were carefully picked up with a moist brush. When excess of water was added, the structure of the soil dissolved and Collembola floated to the surface due to the superhydrophobic properties of their cuticle (Gundersen et al., 2014). The number of surviving individuals were counted per box.

Four of the eight boxes per treatment (concentration and temperature) were transferred to a clean environment for 60 and 45 days, at 15ºC and 20ºC, respectively. The individuals of each box were transferred together to a new box of clean environment. As *H. viatica* is a social species of Collembola, the individuals of boxes of the same treatment with few surviving individuals (less than 9) were merged to ensure a large enough number of

individuals for well-being and reproduction. The remaining four boxes per treatment were concluded. The surviving individuals were killed and stretched by heating to 70ºC in 70% ethanol. The ethanol with the dead individuals were cooled and stored in glass vials for measurement of length (pic. 12). Additional data collected on fecundity, hatching, length of offspring, and juvenile and adult length will not be addressed as a part of this master thesis, but will be treated in further studies as a part of the MULTICLIM project (from now on referred to as "a further study").

High survival, notably in the controls, with several boxes having 25 individuals, confirm the efficiency of the flotation method, and thus acceptable for our purpose.

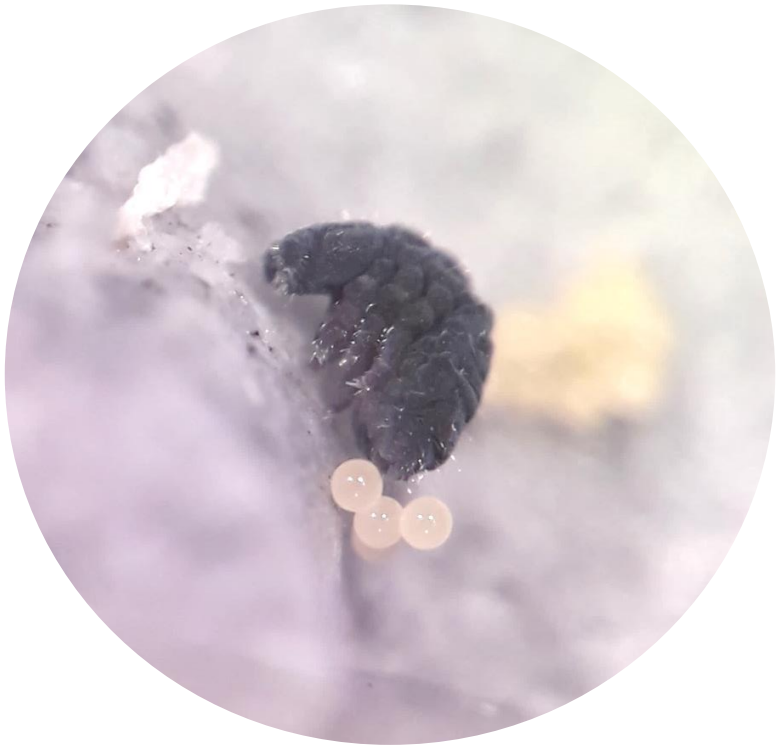
The culture boxes with living individuals were randomized and blinded to avoid subjective bias in the following collection of data.

Picture 8: An individual of *H. viatica* preserved in 70% ethanol after flotation from the soil.

2.2.7 Post-exposure

The culture boxes with pre-exposed individuals were observed every day with feeding and watering every 4th day. Dead individuals were registered and removed to prevent fungal growth, and molted skins were counted and removed for the same reason. Batches of eggs

(pic 13) were collected, counted and taken pictures of before they were put aside in separate boxes for monitoring of hatching. The boxes with eggs were inspected daily, and the number of eggs hatched was recorded for each batch. The hatchlings were killed and stretched in 70 % ethanol heated to 70ºC the day they hatched and stored in glass vials, to be photographed for measuring of length. All the data on the number of eggs, hatching success, and size of offspring was stored for a further study.



Picture 9: Mature H. viatica laying a batch of eggs

The developmental time to first reproduction is set as the period from the start of the exposure in each box to the first reproductive event. It is measured in days from the day of transferal of newly hatched Collembola to the contaminated soil, until the first batch of eggs was observed in each box of clean environment.

At 60 days (day 95 of the experiment) at 15ºC and 45 days (day 71 of the experiment) at 20ºC (same physiological age) after transfer to the clean environment, any remaining individuals were killed and stretched in 70% alcohol heated to 70ºC. The individuals of each

box were then counted for data on survival and photographed for measurement of length for a further study.

2.2.8 Statistical analyses

All statistical analyses for this thesis were carried out using the statistical software R 3.5.2 (R Development Core Team, 2018).

Survival

In the treatment of this data the drc package in R was used (Ritz et al., 2015). To find the best-fitted model the mselect() command and anova() were used. The data showed different optimal models for the different temperatures when treated seperately. As both temperatures should be treated in the same model the temperatures were run together and a model selection was run to find the best model. The model selection resulted in different optimal models (e.g., two-parameter log-logistic model, three-parameter log-logistic model and four-parameter log-logistic model), with negligible differences. Similar performance of the three-parameter log-logistic model and four-parameter log-logistic model is shown by overlapping dose-response curves for the survival at the end of exposure in soil at 20ºC (fig. 2). The three-parameter log-logistic model was chosen (eq. 1) as it gave the best Akaike's information criterion (AIC) (Bozdogan, 1987) values. The main difference between a four-parameter log-logistic model and the three-parameter log-logistic model is that the lower limit of survival is set to zero in the three-parameter log-logistic model. This limit fits the data as there can be no lower survival than zero individuals per box in the experiment, this determination of one of the parameters affects the survival curves minimally compared to the four-parameter log-logistic model.

Equation 1: Three-parameter log -logistic function with lower limit set to 0.

$$f(x) = 0 + \frac{d - 0}{1 + \exp(b(\log(x) - \log(e)))}$$

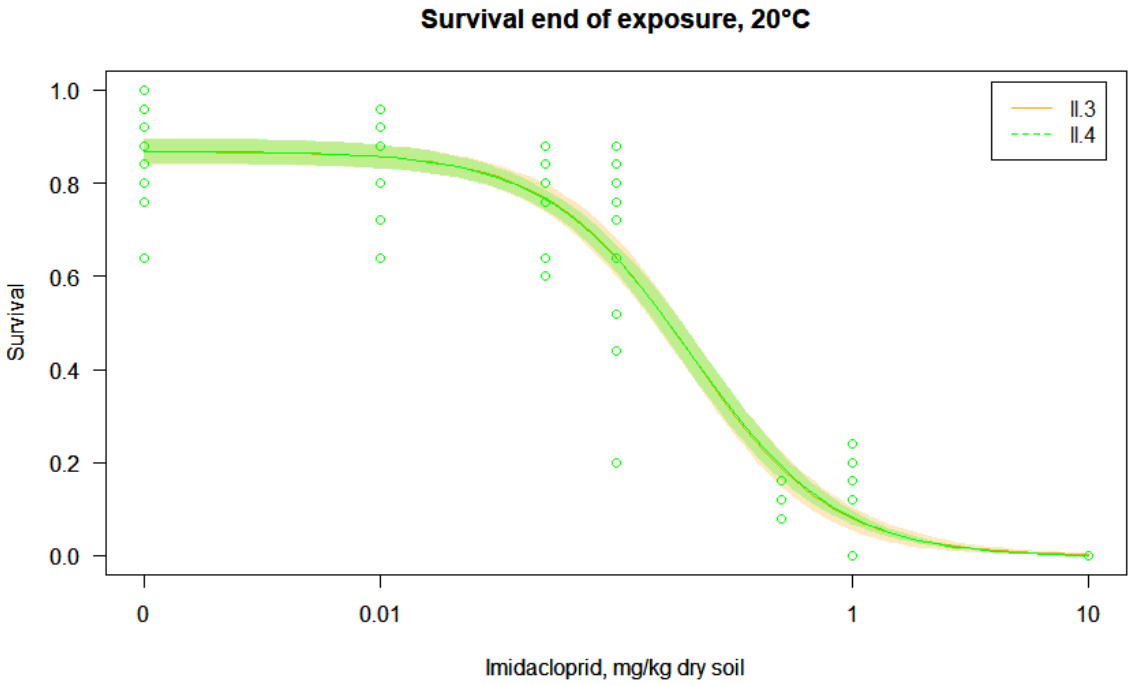


Figure 2: Survival of hatchlings at the end of exposure (after flotation) with confidence intervals. Three-parameter log-logistic model (orange) and four-parameter log-logistic model (green) for 20ºC. The results are shown as a proportion of survivors with 1.0 on the y axis equivalent to 25 individuals. This figure is included as an example of the similar performance of the three-parameter log-logistic model and the four-parameter log logistic model.

Lethal dose (LC) is the statistically derived concentration of the test substance at which given amount of the population are expected to die, i.e., LC_{50} is the statistically derived dose expected to have the effect of a mortality of 50% of the population (Jørgensen & Fath, 2010).

First reproduction

Dose response models with the drm() command from the drc package was tested, but did not fit the data well (Appx. 1). A negative binomial generalised linear model approach was tested and shown to fit the data well (Appx. 2).

The age at first reproduction was estimated as the age at first reproduction, i.e., first observed batch of eggs, in each of the boxes of clean environment. To treat this data a fitted negative binomial generalized linear model was used with estimated values per treatment through a random intercept model. The R-packages dplyr v0.78, ggplot2 v3.2.1, and mass

v7.3-51.5 were used. To fit the model the glm.nb() command was used. This command is a modification of the glm() with the inclusion of an additional parameter, as glm() is used for generalized linear models. The output was visualized using the ggplot() command.

The treatment 0.5 mg/kg soil from 15ºC was removed from the analysis due to data from only one box, which was considered not be representative for the whole treatment. There was no observed reproduction from boxes from concentrations higher than 0.5 mg/kg soil.

3 Results

3.1 Survival

3.1.1 Survival at the end of the exposure

In toxicology it is common to analyse mortality curves instead of survival curves. Here, I have chosen to present the data as survival, since this reflects the actual data of counted surviving individuals per box after flotation. Dead individuals could be observed if they died at the soil surface, but dead individuals in the soil were not necessarily extracted by flotation. Figure 9 show the survival at both test temperatures, expressed as a proportion of survivors: 1.0 on the y axis is equivalent to 25 individuals.

In both temperatures (Fig. 9), the survival of juvenile after exposure was high in the control, but decreased with increasing concentrations in the soil. At both 15ºC and 20ºC, the steepest decline in survival was observed between 0.05 mg/kg and 0.5 mg/kg soil, and the largest spread in survival occurred at 0.1 mg/kg soil. Moreover both 0.5 mg/kg and 1.0 mg/kg soil exposure, resulted in high mortality, and most so at 15ºC, and at 10 mg/kg soil there was no survival (only one moribund individual surviving for five days at 15ºC). The survival of juveniles was overall lower at 15ºC compared to 20ºC, with the difference in survival between the temperatures becoming larger from 0.05 mg/kg soil and towards higher concentrations. The trend of lower survival at 15ºC than 20ºC may be as prominent at 1 mg/kg and 10 mg/kg soil as at 0.1 and 0.5 mg/kg soil, but was masked by the low survival for these treatments at both of the temperatures at the end of the exposure.

The dose-response toxicity curves for survival of juveniles exposed to imidacloprid differed between the two temperatures (fig. 3) with different lethal concentrations (LC) for the temperatures (table 2). In accordance with the higher survival at 20ºC, the LC_{50} is higher at 20ºC than 15ºC. The LC_{10} and standard error (SE) has overlapping confidence intervals between the temperatures, with the LC_{50} value and SE showing a larger gap without overlapping between the temperatures. Thus there are no statistical difference between the LC_{10} of the temperatures, but there is a statistical difference between the temperatures at increased doses when the 95% confidence interwall of both temperatures does not overlap

with the mean of the other temperature. The difference between the temperatures increase with dose and a statistical difference is reached before the LC_{50} of both temperatures.

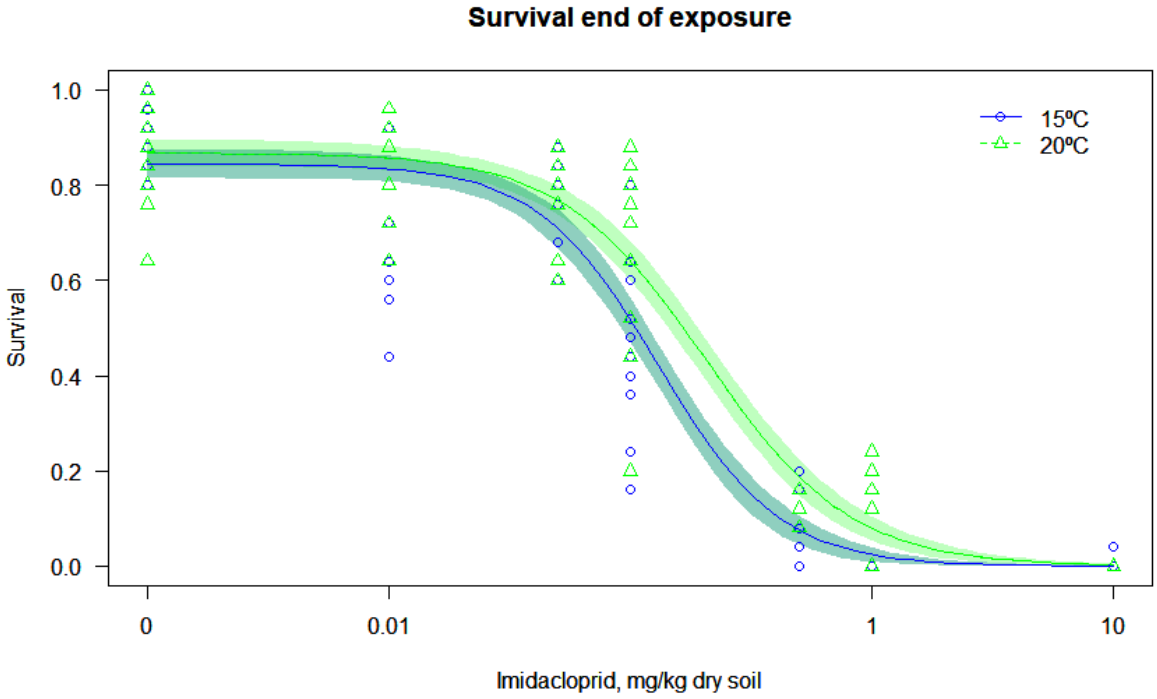


Figure 3: Graph based on a three-parameter log-logistic function of survival at end of exposure for 15ºC at 35 days and 20ºC at 26 days (same physiological age) with confidence intervals. The results are shown as a proportion of survivors with 1.0 on the y axis equivalent to 25 individuals (100% survival).

3.1.2 Survival at the end of the experiment

The survival at the end of the experiment is the survival throughout the whole experiment, i.e., the days exposed + the days in the clean environment (fig. 4). The end of the experiment was at day 95 at 15ºC, and at day 71 at 20ºC (same physiological age).

The survival of the Collembola were high after subsequent time in a clean environment with low mortality in the period after exposure at all treatments (table 2). This indicates little effect of the imidacloprid exposure on survival when moved from the contaminated environment to a clean environment. This high survival is reflected in the confidence intervals for the LC_{10} and LC_{50} values overlapping for the survival at the end of the experiment compared to the survival at the end of the exposure for both temperatures (table 3).

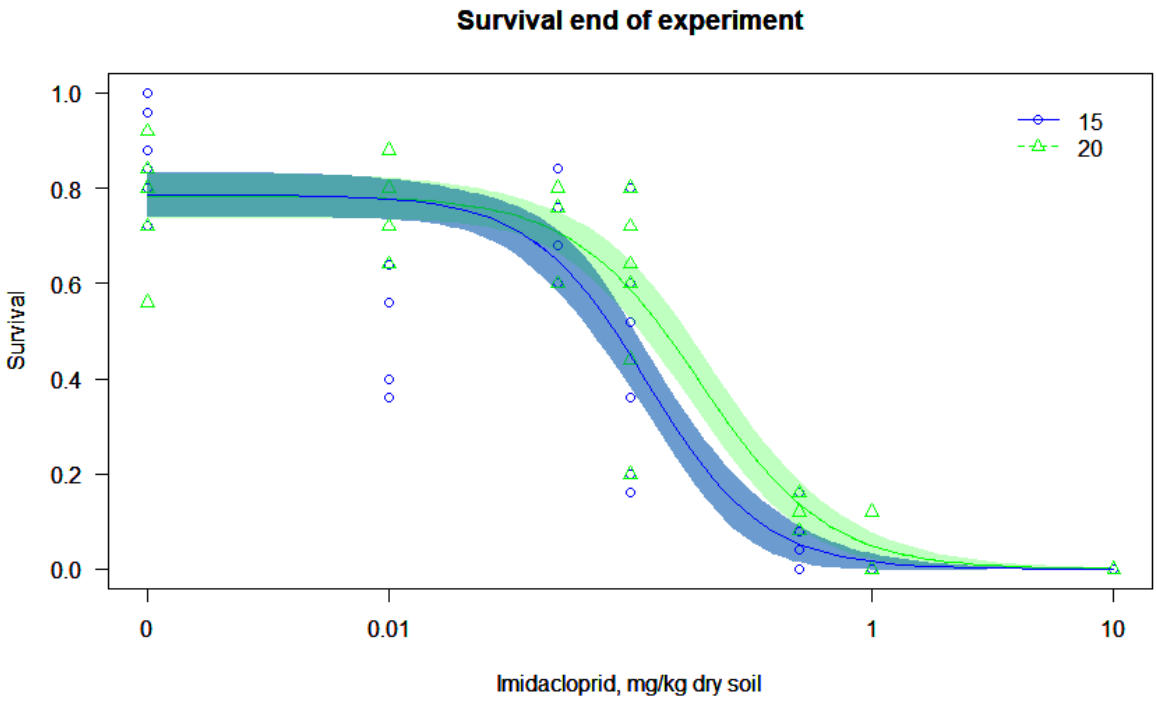


Figure 4: Graph based on a three-parameter log-logistic function of survival at the end of the experiment for 15ºC at 95 days and 20ºC at 71 days (same physiological age) with confidence intervals. The results are shown as a proportion of survivors with 1.0 on the y axis equivalent to 25 individuals (100% survival).

Table 2: Survival in clean environment from end of exposure to end of experiment expressed as % of total surviving individuals for each treatment. At 15ºC the time in clean environment was 60 days. At 20ºC the time in clean environment was 45 days.

	15ºC (60 days)	**20ºC** (45 days)
0.00	98.05 %	98.15%
0.01	96.08 %	100 %
0.05	100 %	100 %
0.1	98.46 %	99.17 %
0.5	100 %	100 %
1.0	No surviving individuals	100 %

Table 3: Estimated lethal concentrations for 10% and 50% of the population at the end of the exposure and at the end of the experiment, with standard error for survival at 15ºC and 20ºC. The LC_{10} shows no statistical difference with overlapping 2x SE (as a proxy for 95% confidence intervals) for both periods. The LC_{50} shows significant differences with non-overlapping 2x SE (as a proxy for 95% confidence intervals) for both periods.

	End of the exposure		**End of the experiment**	
	15ºC	20ºC	15ºC	20ºC
LC_{10}	0.04 ± 0.01 SE	0.05 ± 0.01 SE	0.03 ± 0.01 SE	0.05 ± 0.01 SE
LC_{50}	0.13 ± 0.01 SE	0.21 ± 0.02 SE	0.12 ± 0.01 SE	0.19 ± 0.02 SE

There seems to be an effect of temperature on survival, with higher survival at the highest temperature of 20ºC when exposed for the same amount of time in physiological age. The effect of imidacloprid on survival seems to be highest during the exposure, with clear effects of the insecticide on survival in the different treatments. There is no clear difference in survival at the different treatments after the exposure is ended and the surviving individuals are moved to a clean environment as the survival is high in all treatments.

3.2 Age at first reproduction

No individuals survived to reproductive age in the 10 mg/kg soil treatment. The 1.0 mg/kg soil treatment had surviving individuals, but no reproduction occurred during the post-exposure period in a clean environment. As there were only one of the boxes that reproduced from the 0.5 mg/kg soil treatment, this treatment was removed due to low replication. Thus there are no results on first reproduction from the treatments of three highest concentrations.

The effect of concentration is clearest seen at 15ºC (fig. 5 and table 3). Unexpectedly, at both temperatures, the youngest age at first reproduction was seen in the 0.05 mg/kg soil treatment, while the highest age was seen in the 0.01 mg/kg soil treatment. At 20ºC age at first reproduction was the same in the 0.01 mg/kg soil and 0.1 mg/kg soil treatments. The Collembola at 20ºC reproduced at an earlier date than those at 15ºC in all treatments.

Within each temperature the confidence intervals of all the treatments are overlapping, but there seems to be a difference between the 0.01 mg/kg soil treatment and the 0.05 mg/kg soil treatment at 15ºC. For both temperatures the pattern in earlier and later age at first

reproduction follow the same pattern for the treatments with the 0.05 mg/kg soil treatment reproducing before the control, and the 0.01 mg/kg soil treatment reproducing after.

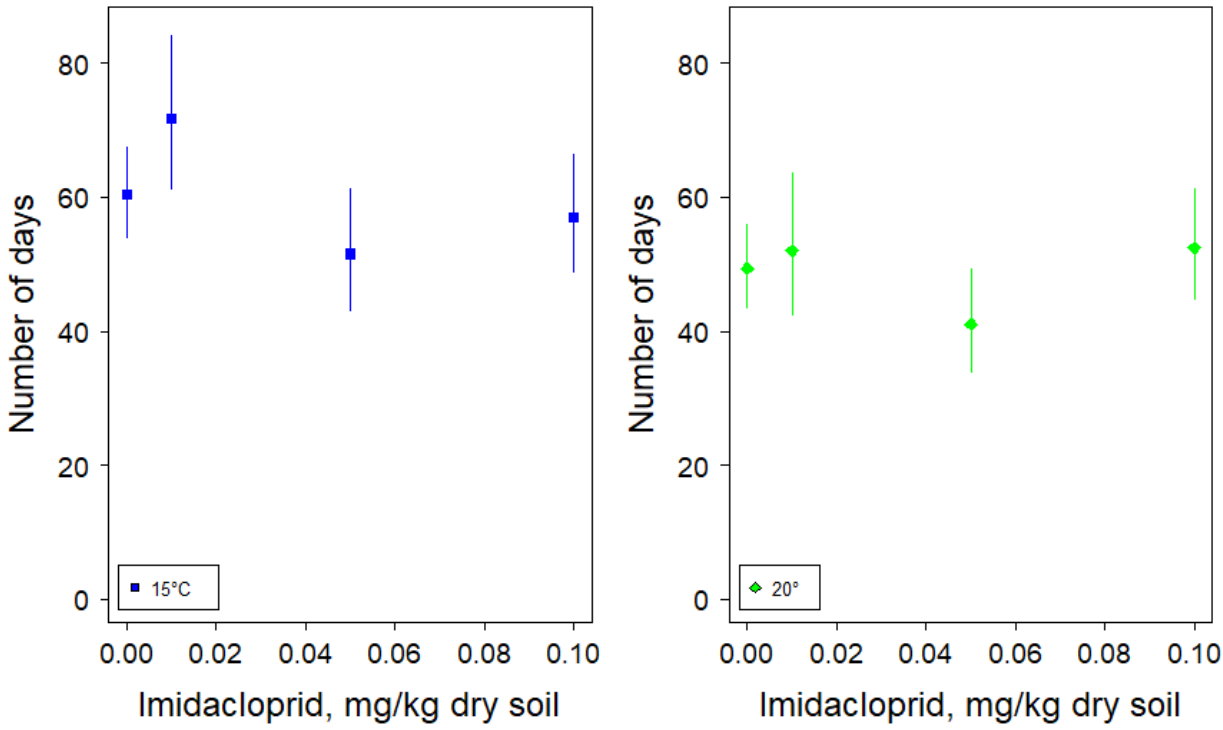


Figure 5: Developmental time, i.e., days from hatching to the first reproduction, with confidence intervals. 15ºC to the left (blue) and 20ºC to the right (green).

The age at first reproduction were converted to day-degrees (Dº) to investigate if there is a statistical difference between the temperatures when converted to the same estimated physiological age (fig. 6 and table 3). The result shows that there was reproduction at an younger age at 20ºC only at 0.01 mg/kg soil and that the first reproduction occurred at an older age at 20ºC than 15ºC for the rest of the doses. At 0.1 mg/kg soil the confidence intervals of the point estimate (average day of first reproduction) for 15ºC and 20ºC does not overlap with each other. For the rest of the treatments there are an overlap of confidence intervals in each treatment for the two temperatures.

Thus there seems to be no effect of temperature on age at first maturity except at 1.0 mg/kg soil, but a clear pattern in both temperatures of effects on age at first reproduction of the different treatments.

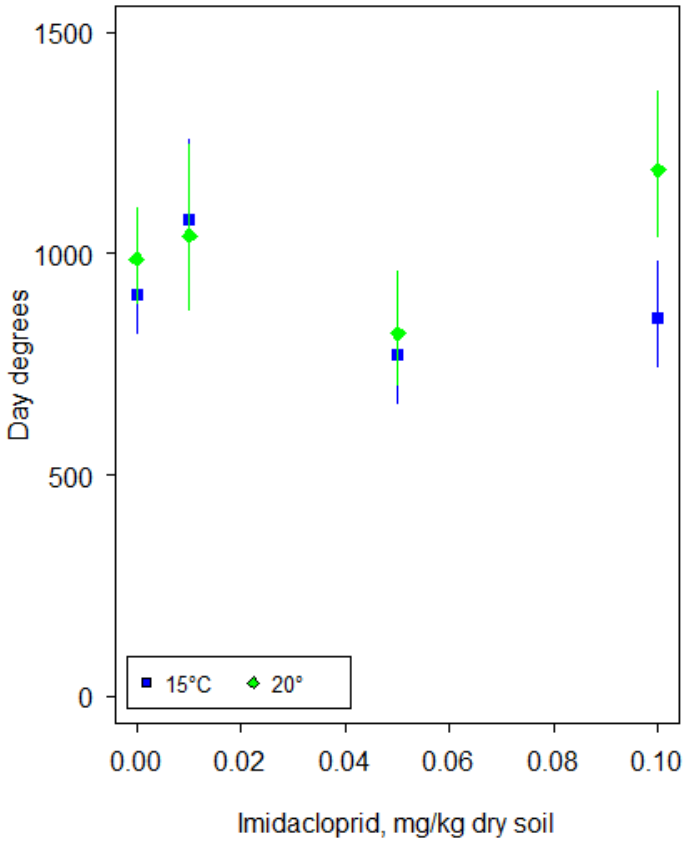


Figure 6: Developmental time, i.e., time from hatching to age at first reproduction in Dº, with confidence intervals. 15ºC in blue and 20ºC in green.

Table 3: Point estimate (average age at first reproduction) for first reproduction at the different treatments at 15ºC and 20ºC. Point estimate presented both in days and day-degrees (Dº). The point estimate for days and day-degrees are rounded to whole days without decimals, as the data was collected once a day (for estimated with decimals see appx. 3). Estimated upper and lower confidence intervals are stated in [,].

Conc. mg/kg	Point estimate days		Point estimate day-degrees (Dº)	
	15ºC	20ºC	15ºC	20ºC
0.00	60 [54,67]	49 [43,55]	906 [819,1006]	987 [887,1103]
0.01	72 [61,84]	52 [42,63]	1076 [926,1259]	1040 [874,1248]
0.05	51 [43,61]	41 [33,49]	772 [664,905]	820 [705,960]
0.1	57 [48,66]	52 [44,61]	855 [764,984]	1188 (1039,1367]

4 Discussion

The present study was conducted with an overall aim of understanding how temperature alters the effect of imidacloprid on selected life-history traits in an arctic population of the Collembola *Hypogastrua viatica*, exposed through soil, i.e., temperature-dependent toxicant sensitivity.

4.1 Survival

The lethal concentration resulting in mortality of 10% of the test population is estimated to be at 0.04 mg/kg soil at 15ºC and 0.05 mg/kg soil at 20ºC in the data of this thesis. A recent study of pesticide residuals in European topsoil (Silva et al., 2019) found imidacloprid residuals in 7% of the samples, with the highest concentration of 0.06 mg/kg soil. Thus, the results of present thesis show mortality at field realistic concentrations.

In two tests of the toxicity of imidacloprid to *Folsomia candida*, the LC_{50} was estimated to be 0.55 mg/kg soil (de Lima e Silva et al., 2019) and 0.47 mg/kg soil (de Lima e Silva et al., 2017). These experiments used the same LUFA Speyer 2.2 soil as in the present study, but they exposed 10-12 days old juveniles at 20ºC for 28 days, in accordance with the OECD guideline 232 (OECD, 2016). The LC_{50} of the present study with *H. viatica* at 20ºC was at 0.21 mg/kg soil. Comparison of the experiments is challenging to interpret as

It is challenging to compare these data due to different experimental design and different species with different life-histories and sensitivity are used. *F. candida* is parthenogenetic and *H. viatica* are sexually reproducing, the age of the test animals was different at the start of exposure, and the exposure was for a different number of days. Still, the lower LC_{50} of the present study might indicate either a higher sensitivity to imidacloprid in *H. viatica* than *F. candida*, or that exposure of *H. viatica* from the day of hatching increases the sensitivity as the individuals do not have any time to develop prior to exposure.

The higher average survival rate in all of the treatments at 20ºC was not expected. An explanation can be that the individuals were exposed to imidacloprid for a shorter timespan at 20ºC than those at 15ºC due to the experimental setup with exposure for the same estimated physiological age. Higher survival at 20ºC is more likely to be a result of time exposed than a higher degradation of imidacloprid as a result of the higher temperature. The reported half-life of imidacloprid shows a large variation dependent on the type of soil and other factors (e.g.

UV, temperature, moisture). In LUFA Speyer 2.2 soil the half-life is estimated to be < 125 days (van Gestel et al., 2017), which indicate that a higher rate of degradation is not likely to be the explanation of the higher survival at 20ºC as the exposure time was 26 days. To investigate if there was a lower survival rate at these concentrations this would have to be investigated at an earlier stage of the exposure.

The single moribund but surviving individual at 10 mg/kg at 15ºC was unexpected, as there was no survival in the 1.0 mg/kg soil treatment at this temperature, and no surviving individuals in this treatment at 20ºC. This may be an example of natural variation in the sensitivity of individuals in the population, with this individual being particularly robust. Differences in sensitivity may also be indicated by the wide spread in the numbers of surviving individuals per box at the 0.1 mg/kg treatment at both temperatures. The area around 0.1 mg/kg seems to be an area of interest with focus on sublethal effects, as there are boxes with both high survival and high mortality within this treatment (survival of 4-20 individuals per box at 15ºC and 5-22 individuals per box at 20ºC). This is in contrast to both lower and higher treatments than 0.1 mg/kg soil, with more limited variation in the effect within the treatments, with relative high survival at 0.05 mg/kg soil (survival of 15-21 individuals per box at 15ºC and 15-22 individuals per box at 20ºC) and low survival at 0.5 mg/kg soil (survival of 0-5 individuals per box at 15ºC and 2-4 individuals per box at 20ºC). As the original experimental design of the present study did not include the 0.05 mg/kg soil and 0.5 mg/kg soil treatments, the evaluation of 0.1 mg/kg being an area of interest, with the need of running the experiment again with these two concentrations seems to have given a more detailed perspective of the sub-lethal effects around the 0.1 mg/kg soil treatment.

4.2 Development time to first reproduction

When converted to the same physiological age, both temperatures reached first reproduction at almost the same time, with the same pattern, and with overlapping confidence intervals for all treatments but 0.1 mg/kg soil. Thus, there seems to be little effect of temperature on age at first reproduction when converted to the estimated same physiological age. The results were as expected with reproduction at an earlier day at 20ºC than 15ºC due to faster development at 20ºC, and quite similar age at first reproduction in the treatments when converted to day-degrees (except for 0.1 mg/kg soil). The two temperatures follow the same distinct pattern in the onset of the first reproduction for the different treatments with the 0.01

mg/kg soil treatment reproducing later than the control treatment and the 0.05 mg/kg soil reproducing earlier than the control treatment. This was not expected as the expectation was that the age at first reproduction would occur later with increased concentration in the treatments. The 0.05 mg/kg soil treatment reproducing at an earlier date than the control group may be induced by stress, making the individuals allocate energy and resources to mature and reproduce earlier, at cost other functions in the body. If this is the explanation it can indicate a trade-off between using energy and resources for self-maintenance and growth, and reproduction. The later reproductive day at 0.01 mg/kg soil may be due to an allocation of resources to growth and maintenance due to stress of imidacloprid exposure at a level high enough to delay reproduction, but too low to induce earlier reproduction at possible cost of self-maintenance and growth. The delay in age at reproduction at 0.1 mg/kg soil compared to 0.05 mg/kg soil might be due to a combination of stress to reproduce early and a need for self-maintenance and growth to reach the physiological requirements needed for reproduction. The pattern may also be due to

In an earlier study on an arctic population of *H. viatica* from Bjørndalen in Svalbard, temperature was shown to affected the timespan from hatching to reaching maturity day 51 (771 Dº) at 15ºC and day 42 (858 Dº) at 20ºC (Serbezov, 2002). This is earlier than the results for the age at first reproduction, respectively at day 60 (906 Dº) at 15ºC and day 49 (987 Dº) at 20ºC, for the control treatments of this thesis. This difference can be due to a difference in the different arctic populations or may be a result of unknown induced stress from living in soil (the whole study of Serbezov were on plaster of Paris), or the transfer of the collembola from the soil to the clean environment.

5 Conclusive remarks

The insecticide imidacloprid has been shown induce both lethal and sublethal effects on the arctic population of *Hypogastrura viatica* at both temperatures within the studied concentration range.

As assumed in objective i) the survival rate decreased with higher concentrations of imidacloprid at both temperatures. Increasing concentrations of imidacloprid gave lower LC values and the LC values were different for the two temperatures. The survival was highest at 20ºC compared to 15ºC. This was not expected as 15ºC is closer to the temperatures the population is exposed to in its natural environment, and 20ºC were expected to be an unrealistic high temperature for this population and thus expected to induce a higher level of temperature-induced stress. These results are assumed to be due to the shorter time at exposure in days, as the treatments were exposed for the same timespan in estimated physiological age (Dº). At the highest concentrations of this experiment the survival were low at both temperatures with no individuals surviving the highest concentration of 10 mg/kg soil. Survival during the post exposure period in a clean environment showed low to no mortality in all treatments.

Age at first reproduction occurred earlier at 20ºC than 15ºC as expected due to faster development in ectotherms at higher temperature. When converted to Dº the age at first reproduction was similar with overlapping confidence intervals, except for the 1.0 mg/kg soil treatment where the age at first reproduction was distinctly later than at 15ºC with no overlapping confidence intervals. There was no reproduction at 1.0 mg/kg and 10 mg/kg soil and only one box reproducing at 0.5 mg/kg soil. The expectation of age at reproduction increasing with increasing concentration of the treatments were not met. At both temperatures the 0.05 mg/kg soil treatment reproduced at a lower age than both the control and the 0.01 mg/kg treatment. This might be as a result of stress, making the individuals reproduce at an earlier age at the cost of self-maintenance and growth or future reproduction. There is no effect on post-reproductive survival of the earlier reproducing 0.05 mg/kg treatment compared to the other treatments. As development and age at first reproduction are complex aspects of the life history of an organism, and closely linked to fitness the pattern t is hard to say the exact cause of the pattern.

As the LC_{50} at 20ºC is lower in this experiment than in some other experiments following the OECD guideline with *F. candida* as test species, it is believed that either the test

population of this experiment, an arctic population of *H. viatica*, is more sensitive to imidacloprid than *F. candida*, or that life stage sensitivity might be the reason, as the experiment of this thesis exposed juveniles at the day they hatched while experiments following the OECD guideline exposed 10-12 days old juveniles.

6 Future perspectives

The MULTICLIM project:

- As all of the data from the experiment of this thesis has not been investigated it is expected that the results from these untreated data combined with the results of the data treated in this thesis will provide more nuanced insight of how the combined effects of imidacloprid and temperature affects the life-history parameters of the arctic population of *H. viatica*. Especially a comparison between age at fist reproduction and length after exposure, as a comparison between these parameters will provide further insight in the assumption proposed in this thesis about a possible trade-off between growth and age at first reproduction in the 0.05 mg/kg soil treatment.
- It is expected that the comparison of these results with the data on the more southern population from Norway provide insight to an even greater extent both in terms of the sensitivity of the species to imidacloprid and if there are any differences in effects between the populations.

For future studies outside the MULTICLIM project or as new experiments in the project it would be interesting to:

- Do the same experimental design with exposure for the same physiological age and for the same number of days (35 days) for both temperatures, to investigate if the time of exposure is the major reason for the difference in survival between the temperatures. Preferably with an extra harvests of boxes during the exposure time, to get more nuanced data on the highest concentrations. Also preferably with a control treatment going parallel on plaster of Paris, to investigate if there are any stress form the transferal from soil to plaster of Paris inducing a delay in age at first reproduction.
- The same experiment with different species of Collembola. Especially species that are part of the soil community of farmed land, to make a clearer link to the environment where imidacloprid is in use. As different species shows different adaptability to variables in the environment, the same setup for several species will give an understanding of the variation of sensitivity and responses to exposure with a link to their life-history adaptions.

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7 Appendix

Appendix 1)

Test of dose response model for the data on age at first reproduction

	logLik	IC	Lack of fit	Res var
Quad	-88.54609	185.0922	NA	148.6427
Lin	-89.72201	185.4440	NA	156.8059
Cubic	-88.37030	186.7406	NA	154.0924
LL.4	-88.48925	186.9785	3.943555e-02	155.6945
LL.3	-105.14485	218.2897	2.469018e-07	629.4931
LL.3	-105.14485	218.2897	2.469018e-07	629.4931
LL.2	-127.19153	260.3831	2.759946e-14	4077.4542

Anova for testing of the negative binomial general linear model for the data on age at first reproduction

Analysis of Deviance Table

Model: Negative Binomial(50.1674), link: log

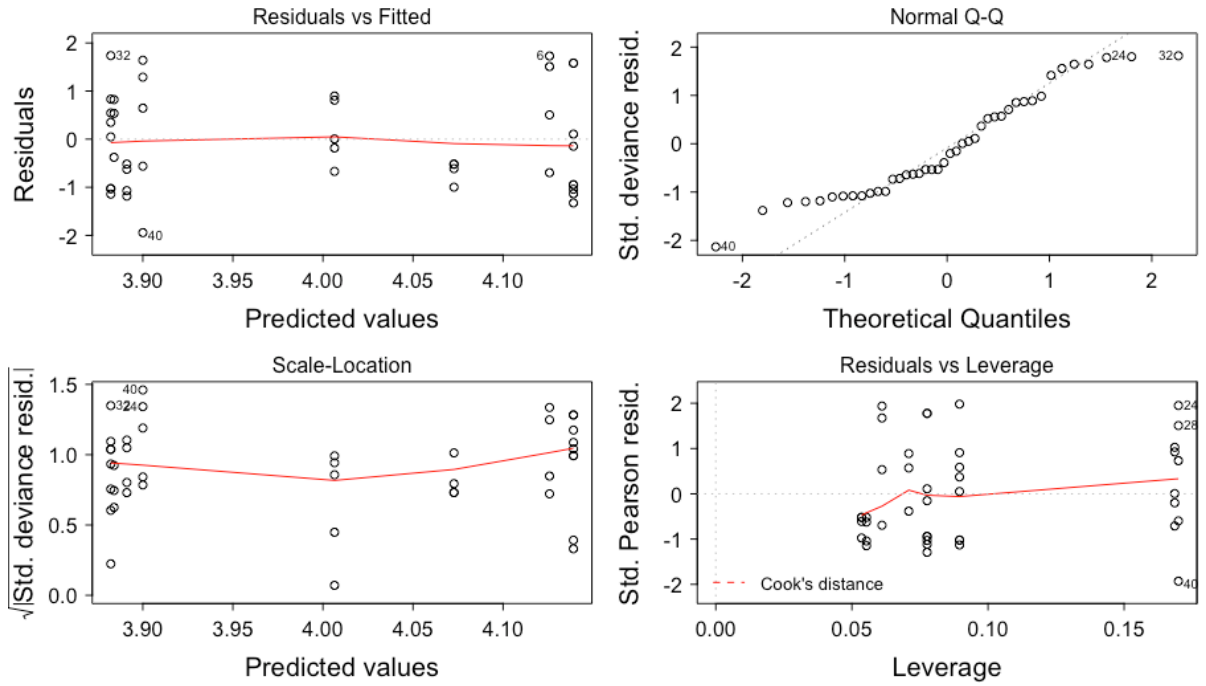
Response: Numberofdays

Terms added sequentially (first to last)

	Df	Deviance	Resid. Df	Resid. Dev	Pr(>Chi)
NULL			41	54.912	
Imidacloprid	1	1.0097	40	53.902	0.314982
Temperature	1	11.3437	39	42.558	0.000757
Imidacloprid:Temperature	1	1.0218	38	41.536	0.312089

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residuals for testing the negative binomial general linear model for the data on age at first reproduction



Appendix 2

Estimated lethal concentrations – end of exposure

	Estimate	Std. Error	Lower	Upper
e:15:10	0.0408411	0.0054632	0.0301334	0.0515489
e:15:50	0.1323649	0.0088618	0.1149961	0.1497337
e:20:10	0.0456810	0.0066901	0.0325688	0.0587933
e:20:50	0.2056446	0.0162561	0.1737833	0.2375060

Estimated lethal concentrations - end of experiment

	Estimate	Std. Error	Lower	Upper
e:15:10	0.0346163	0.0082323	0.0184814	0.0507513
e:15:50	0.1162792	0.0120469	0.0926677	0.1398906
e:20:10	0.0505882	0.0109270	0.0291717	0.0720047
e:20:50	0.1928723	0.0232580	0.1472875	0.2384572

‘

Appendix 3

Point estimate (pest) with upper (pupr) and lower (plwr) confidence intervals in days per treatment at 15ºC and 20ºC

pest	plwr	pupr	dose	temp
60.44444	54.07292	67.52643	0.00	15
71.75000	61.18767	84.09294	0.01	15
51.50000	43.19198	61.26717	0.05	15
57.00000	48.94754	66.28891	0.10	15

pest	plwr	pupr	dose	temp
49.375	43.53813	55.92354	0.00	20
52.000	42.45691	63.50111	0.01	20
41.000	33.90779	49.38466	0.05	20
52.400	44.81515	61.16291	0.10	20

Point estimate (pest) with upper (pupr) and lower (plwr) confidence intervals in day-degrees (D⁰) per treatment at 15ºC and 20ºC

pest	plwr	pupr	dose	temp
906.6667	819.3720	1006.4909	0.00	15
1076.2500	926.2154	1259.7360	0.01	15
772.5000	664.0145	905.1774	0.05	15
855.0000	746.7952	984.5454	0.10	15

pest	plwr	pupr	dose	temp
987.5	887.2305	1103.0983	0.00	20
1040.0	874.9011	1248.3131	0.01	20
820.0	705.0165	960.6233	0.05	20
1188.8	1039.3474	1367.7239	0.10	20