Effects of Imidacloprid on the Survival and Recruitment of *Folsomia quadrioculata* in Soil Microcosms

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Master's thesis in Biosciences (Toxicology and Environmental Science)

60 Credits

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10.12.2021

ABSTRACT

As the global population increases and demand for resources grows, increasing demands have been placed on the agricultural industry. With limited space and arable land available, the drive to increase yield has resulted in increasing use of pesticides to reduce pests detrimental to crops. Amongst these, the neonicotinoids have been gaining increased popularity since their introduction. However, in addition to their target organisms, pesticides can have detrimental effects to other soil-dwelling organisms.

Folsomia quadrioculata is a widely distributed species of springtail. Springtails play an important role in soil communities, with various species filling a number of ecological roles. Previous studies have shown them to be susceptible to negative effects of the neonicotinoid imidacloprid.

This study looks at the effect of imidacloprid on *Folsomia quadrioculata* in defaunated soil cores in a laboratory setting. Microcosms were collected in the summer of 2019. They were frozen down to -80°C to remove any soil fauna. Each microcosm was subsequently spiked with imidacloprid representing either 0, 0.004, 0.02, 0.1 or 0.5 mg/kg imidacloprid per dry soil weight. 20 adult individuals were added to each microcosm, and placed at 15°C for either 28, 35, 42 or 84 days. *F. quadrioculata* were extracted from the microcosms at their respective final timepoints using a soil fauna extractor. After extraction, samples were counted by classifying individuals as either adults, intermediates or juveniles based on body length.

Results show that adult survival is high at 0, 0.004 and 0.02 mg/kg, before reducing at 0.1 and 0.5 mg/kg. Juvenile recruitment was seen to be affected by all concentrations above 0 mg/kg. The effects of duration were seen to be significant after analysis, with the greatest differences seen after 84 days of exposure to imidacloprid.

This study has shown that imidacloprid has a negative effect on both adult survival and juvenile recruitment over time in defaunated soil microcosms, and that the using defaunated soil microcosms in a laboratory setting produces insightful results.

Acknowledgements

This master's thesis is funded by the Research Council of Norway (RCN) as part of the Effects of climate change in a multiple stress multispecies perspective (MULTICLIM) project (RCN Project No. 280843, UiO Project No. 144612). The work for this thesis was undertaken at the Department of Biosciences (IBV) at the University of Oslo (UiO) under the supervision of Katrine Borgå (main supervisor, UiO) and Sagnik Sengupta (co-supervisor, UiO).
First and foremost, I would like to thank my supervisors for their invaluable insight and constant support throughout this thesis. This project has provided me with the greatest academic challenge I have undertaken, and it would not have been without your support that this thesis would have been possible. I would like to thank Sagnik Sengupta for all his work throughout all aspects of this project, from the experimental design, through practical execution, and onto the thesis writing that made this thesis possible. I would also like to thank Katrine Borgå, likewise for all her work throughout the entirety of all aspects of this project, and without whom it would never have been possible.
I would also like to thank Heidi Sjursen Konestabo for your invaluable insight, knowledge and moral support throughout this project, without which our experiment would not have been possible.
I'd also like to thank the other members of the MULTICLIM project, Silje Marie Kristiansen, Mia Drazkowski Teksum, Andreia Silva, and Gunvor Evenrud for all of your support throughout this thesis - you have all answered every question I have had and gone out of your way to help me.

Abbreviations

ANOVA	Analysis of Variance
F. quadrioculata	*Folsomia quadrioculata*
LC50	Lethal Concentration 50
LD50	Lethal Dose 50
MULTICLIM	Effects of climate change in a multiple stress multispecies perspective
NMBU	Norwegian University of Life Sciences
UiO	University of Oslo

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1. Introduction

1.1 Pesticides and Soil Ecosystems

With a growing world population, the global demand for agricultural produce is increasing. However, with finite land resources, only a modest increase in arable land has been seen on a global scale, from 9.7% in 1961 to 10.8% in 2018 (World Bank, 2021). In the same time frame, the global population has increased from 3.1 billion to 7.6 billion (World Bank, 2021). This has led to an increased demand for increased efficiency of arable land, with higher yields required from the same area to feed the world's growing population.

One method commonly used to achieve this is the deployment of pesticides. Pesticides are widely used across the world for a variety of purposes in both industrial settings and as a vital part of agriculture (Simon-Delso et al., 2015). In 2012, the total estimated global market for all pesticides was approximately 2.6 million tonnes (Atwood & Paisley-Jones, 2017). They are used to keep damaging organisms from deteriorating crops and to increase food production, keeping harmful parasites at bay, and are used in the forestry industry to protect yields. However, despite their uses, pesticides are not without their downsides. They are intended to be toxic to certain organisms and target species, however, whilst these target species may be pests, the harmful effects of pesticides can also affect other non-target organisms that are exposed to these compounds. This can have harmful impacts on various aspects of an ecosystem, such as biodiversity and ecosystem functioning (van der Sluijs et al., 2014).

Whilst the effects of pesticides is an area of interest gaining increasing attention, the effects of these chemicals on non-target organisms is still not fully understood.

Soil communities represent an important element of a local ecosystem, carrying out many vital functions, such as decomposition and playing a role in the nutrient cycle (Swift et al., 2004). They are vast, complex systems that can have both direct and indirect effects on land productivity (Barrios, 2007). Disruption to these communities thus has the potential to cause disruption to the agricultural process.

Whilst intended to increase productivity and protect crops, the application of pesticides can have unintended consequences. For example, Szczepaniec et al. (2011) found that the application of imidacloprid to elm trees in an urban setting led to an increase in spider mites by causing a reduction in their natural predators.

1.2 Springtails

Springtails (Collembola) are a large group of arthropods (Hexapoda), found distributed throughout the world and throughout Europe (Fiera and Ulrich, 2012). With over 6500 species known at present, they are found in a wide variety of habitats, and can be found as an important component of a large number of terrestrial ecosystems (Rusek, 1998). They can fulfil a variety of different ecological functions, and have a wide variety of feeding mechanisms and diets, including fungivores, predators, and a number of other feeding methods (Rusek, 1998; Ferlian et al., 2015). Several species have been suggested to be beneficial for plant growth by targeting non-mycorrhizal fungi, thus reducing competition for mycorrhizal fungi (Gange, 2000). They can also act as decomposers, increasing the amount of available nitrogen for plant uptake, also increasing plant productivity (Partsch et al., 2006). Species of springtail, including notably *Folsomia candida*, have been used as model organisms in order to determine the effects of various toxicants on soil-dwelling organisms (Rombke et al., 2009). There is increasing research suggesting that such soil-dwelling organisms are sensitive to exposure to neonicotinoid pesticides, with effects being seen on both survival and reproduction (van Gestel et al., 2017).

1.3 Neonicotinoids

Neonicotinoids are a group of widely used pesticides that now represent the largest group of insecticides used worldwide (Goulson, 2013). They have a variety of applications, and are amongst the widely used chemicals to protect agricultural crops, constituting one of the fastest growing classes of insecticides (Jeschke et al., 2011). Since the rise of resistance to organophosphates, the usage of neonicotinoids as a replacement has increased (Simon-Delso et al. 2015). They have also been shown to be highly selectively toxic to invertebrates, whilst having little effect on vertebrates such as humans (Matsuda et al., 2001; Tomizawa and Casida, 2003). This has made them favourable choices of pesticide.

Neonicotinoids are classified as systemic pesticides. As such, after their deployment, they are absorbed by and subsequently distributed throughout the entirety of a plant, rather than remaining localised at the point of application. As a result, all aspects of the plant, including roots, nectar and leaves contain amounts of pesticide and thus become toxic to target organisms that consume them (van Gestel et al. 2017; European Commission, 2021). They

function by binding irreversibly to nicotinergic acetylcholine receptors in neurons, causing extended action potentials due to greatly increased ion flow across the membrane. This ultimately causes paralysis, resulting in the death of the organism (Buckingham et al., 1997; Goulson, 2013; Tomizawa and Casida, 2003; van Gestel et al., 2017).
They have been the subject of increasing study during the past years, with a number of studies aimed at identifying their effects on non-target organisms, including a number of studies on model invertebrates (Pisa et al., 2015). In light of the effects they have and risks they pose to non-target organisms, three neonicotinoids, clothianidin, thiamethoxam, and imidacloprid were banned for outdoor use in the European Union in 2018 (European Commission, 2021).

1.4 Laboratory vs. Field studies

Studying the effects of pesticides presents a number of challenges. The toxicity of compounds is generally assessed under laboratory conditions in order to determine the response of one or several characteristic endpoints, such as the measurements of the "Lethal Dose" (LD50) or "Lethal Concentration" (LC50), the dose or concentration at which 50% mortality is seen in a population exposed to the compound, and "Effect Concentration" (EC50) (Arena & Sgolastra, 2014), the concentration at which 50% of the study population shows sub-lethal effect from exposure to the compound, such as growth, reproduction or reduced mobility. The Lowest Observed Effect Concentration (LOEC), and No Observed Effect Concentrations (NOEC) are also commonly used. These toxicity values are often used as estimates for a relative toxicity or potency of a compound, and are commonly used to compare compounds' toxicity (Forfait-Dubuc et al., 2012).
However, pesticides are rarely deployed under such controlled conditions as a laboratory in practice. Laboratory studies are an important part of the scientific process, as the creation of accepted protocols and methodologies, and controlling of variables that are not under the scope of the study question allows for the reproducibility needed to test validity of results. However, this somewhat restricts the ability of laboratory studies to reflect natural systems. Most pesticides are dispersed over a large area, such as an agricultural field or a forest, and are thus immediately exposed to environmental factors, affecting their distribution and their interactions with the surrounding ecosystem. This poses a number of challenges in studying them, as exact replication and prediction of these conditions is not possible.

In addition to laboratory studies, field studies form an important source of data when assessing the impacts of toxicants on the local ecosystem, as they provide insight into the workings of toxicants in the setting of their application, and can provide data from an environment more reflective of the one in which pesticides are likely to be used. However, they do not offer the same level of control over conditions as can be attained in laboratory studies.
This creates a situation in which two different types of study each present their own advantages and disadvantages when assessing the impacts of pesticides. As such, one of the larger challenges that arises is collaborating results from the two.

1.5 Relevance to the MULTICLIM project

This study is part of the MULTICLIM project, which seeks to assess the Effects of climate change in a multiple stress multispecies perspective. Throughout this project, a number of experiments have been carried out to assess the effects of multiple stressors on various species of springtails (MULTICLIM, 2018).
In several previous laboratory studies under the MULTICLIM project, *Folsomia quadrioculata* (Tullberg, 1871) and a second species of springtail, *Hypogastria viatica* , were exposed to imidacloprid using spiked tree bark as feed (Kristiansen et al., 2021; Sengupta et al., 2021), as well as through soil exposure (Kristiansen et al. 2021; Teksum, 2021). Exposure solely through food represents a different exposure pathway to that which might be expected in a real-world application of imidacloprid. Soil-dwelling organisms are often exposed to imidacloprid via a number of exposure pathways (Silva et al., 2017). As imidacloprid is commonly held in the soil for a number of months after its application (EFSA, 2015), soil-dwelling organisms are often exposed via contact with contaminated soil (Silva et al., 2017). Exposure via this soil contact is constant, rather than the periodic exposure experienced during eating.
Both laboratory and field studies have been carried out assessing the effects of the pesticide imidacloprid on the springtail species *Folsomia quadrioculata* (Sengupta et al., 2021; Teksum, 2021). This study will attempt to combine methodologies of laboratory and field studies, and create a link between the two.

1.6 Aims

Various species of springtail, including the widely used model organism *Folsomia candida* (Willem, 1902), have been shown to be sensitive to the effects of neoniconinoids, including those of imidacloprid (van Gestel et al., 2017). This project aims to build on this, and assess the effects of the imidacloprid on a common and widely distributed species of springtail, *Folsomia quadrioculata.*

The primary aim of this project was to analyse the concentration dependent long term effects of imidacloprid on the adult survival of *Folsomia quadrioculata* in defaunated soil microcosms obtained from field sampling. Their response to imidacloprid exposure is expected to vary depending on the duration of exposure. After longer periods of exposure, newly recruited juveniles will have had time to mature into new adults, and thus reliably assessing the survival of original adults will not be possible for shorter time points. However, in these shorter time points, adult survival will be assessed.

The secondary aim was to analyse the concentration dependent effects of imidacloprid on and juvenile recruitment in defaunated soil microcosms. As imidacloprid has been seen to affect egg production earlier than adult survival (Sengupta et al., 2021), these results were expected to differ slightly from those seen in adult survival.

Adult survival and juvenile recruitment are important elements of population development. In using soil samples taken from the same site that this *F. quadrioculata* population originates from, and where a similar study was conducted in situ (Teksum, 2021), the intention is to address the concentration dependent response in adult survival and juvenile recruitment over the course of a growth season (12 weeks) in defaunated soil samples to remove competitors and predators.

2. Materials & Methods

Figure 1 outlines the experimental design for this experiment. It was designed with other experiments within the MULTICLIM project in mind to allow for comparison of results. This project was also designed as a foundation for future work to attempt to address the disjoint that is seen between laboratory and field experiments, and design an experiment that can facilitate bridging the middle-ground between laboratory and field experiments by using field collected soil cores and transferring them to a laboratory setting. By transporting field collected soil cores to the laboratory, we aimed to control environmental factors that cannot be controlled in field experiments. In particular, temperature and water content/moisture can be stringently controlled, whilst in field experiments there is little that can be done to regulate these. By defaunating soil cores before use in our experiments, we are also able to reduce any uncertainty that may arise due to predation or competition in field experiments. Field sampling occurred at the campus of the Norwegian University of Life Sciences (NMBU) and laboratory work was carried out at the Department of Biosciences at the University of Oslo (UiO), Norway.

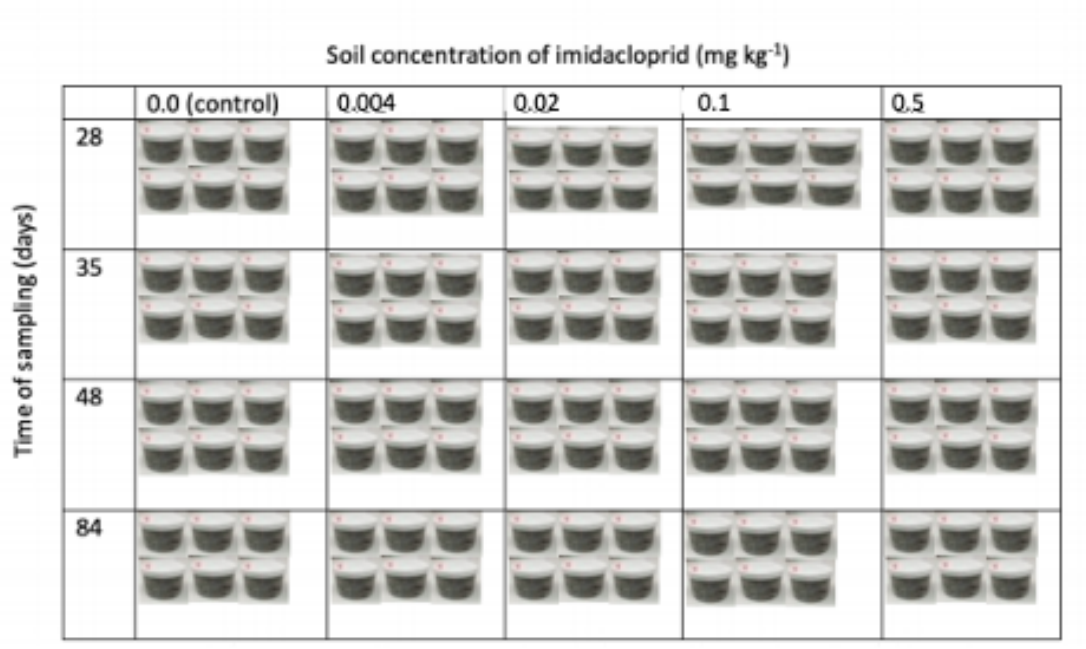

Figure 1. Experimental design of this study. 5 different concentrations of imidacloprid were used; 0, 0.004, 0.02, 0.1 and 0.5 mg/kg imidacloprid to dry soil weight. There were 4 different time-points of extraction at 28, 35, 48 and 84 days. There were 6 replicates for each time-point and concentration.

2.1 The study organism Folsomia quadrioculata

Folsomia quadrioculata (Figure 2) is an abundant species of springtail, found distributed throughout a wide range of habitats from the high arctic tundra (Birkemoe and Somme, 1998) to forests and grasslands in the temperate zone (Widenfalk et al., 2018). It is generally a litter dwelling species that can exhibit large variability in population due to its relative sensitivity to drought (Hertzberg & Lineaas, 1998). Their life-cycle is also highly variable, dependent on the climatic conditions in which a population lives, and can vary from a single generation every two years, to several generations a year (Hertzberg et al., 1994; Sengupta et al., 2016). They are generally regarded as primary or secondary decomposers (Ruess et al., 2007), and are generalists, capable of adapting their diet based on availability and habitat (Chahartaghi et al., 2005).

Folsomia quadrioculata have been used as an experimental organism in several other studies as part of the wider MULTICLIM project. The organisms used in this experiment are laboratory cultures that were originally harvested from Ås, Eastern Norway, and have passed through several generational cycles in climate chambers at the University of Oslo, having been kept in both soil cores and on plaster being fed tree bark.

A previous project (Teksum, 2021) has earlier assessed the impacts of imidacloprid on populations of *Folsomia quadrioculata* in field studies. Two of the time points in that project, 42 and 84 days, were chosen to coincide with two time points assessed in this project, with the aim of comparing results and discussing any potential similarities and future work.

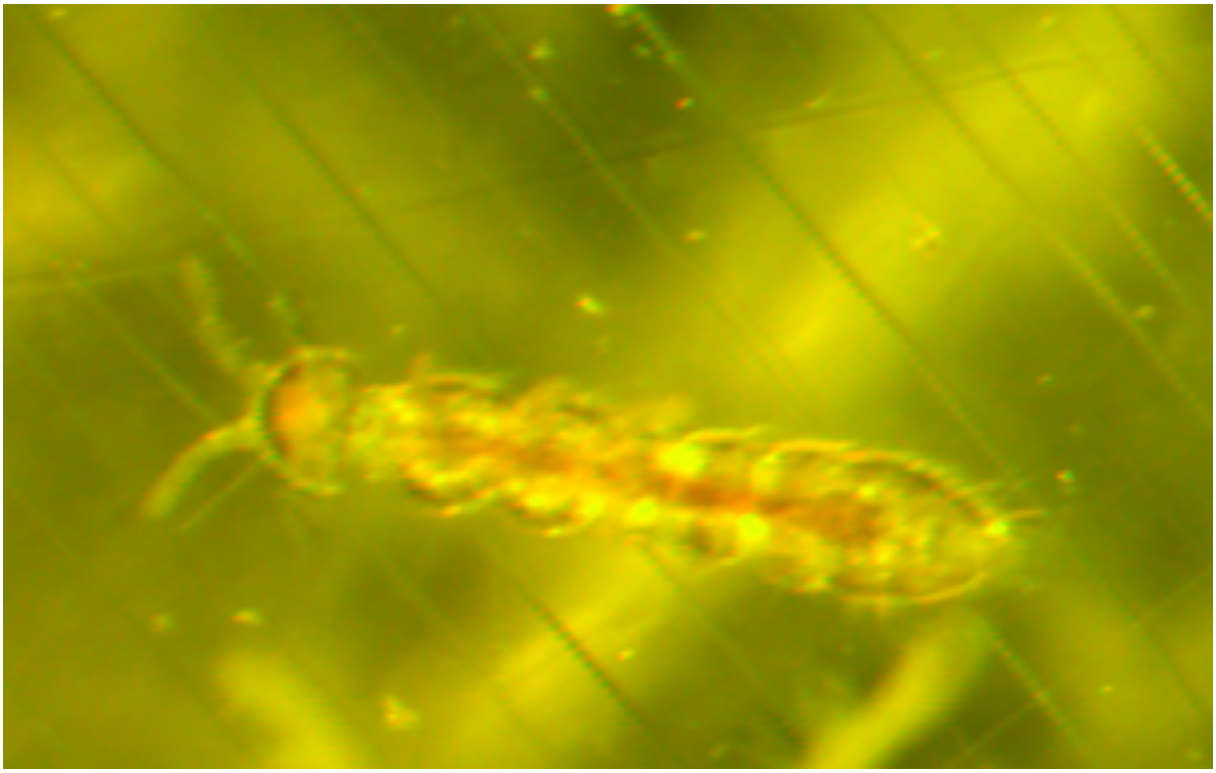

Figure 2. Individual *Folsomia quadrioculata* observed in the extraction of a control group after 84 days. Photo credit: Jack Sanderson

2.2 Imidacloprid

Imidacloprid (C9H10ClN5O2) (Figure 3) is a widely used neonicotinoid that forms a solid, white powder. Structurally related to nicotine, it is widely used as an insecticide (National Center for Biotechnology Information, 2021). Due to the low risk neonicotinoids pose to vertebrate life, it became widely used after it was first introduced in 1991(Jeschke and Nauen, 2008). It is highly stable in soil, with a half-life in field studies of 104 to 228 days, and a half-life in laboratory studies of 99 to 129 days at 20°C (EFSA, 2015).

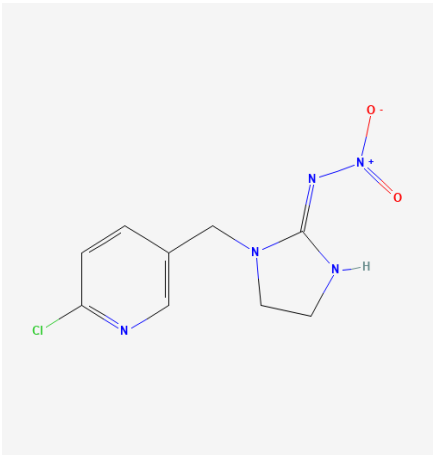

Figure 3. Chemical structure of imidacloprid. Photo credit: National Center for Biotechnology Information, 2021

2.3 Field Sampling

Field samples were collected from the edge of an agricultural field margin (Figure 4) in Ås, Eastern Norway (Figure 5). This location of sampling was selected, as:

1. The population of *Folsomia quadrioculata* to be used originated from this location.
2. The location was also used for field experiments with which this experiment will be compared.

90 soil cores were collected on 17th September, 2019 with a further 50 collected on 25th September 2019.

Intact soil cores were collected using a soil corer, cutting into the top layers of soil. Once extracted, the upper layers of foliage were removed, and samples placed into containers. After extraction, several samples were weighed, and all samples were stored at 3°C overnight. All samples were then weighed the following morning, and the water lost was calculated as an average of difference in weight from the samples weighed on the day of collection, which was calculated at 0.14g. This value was then added to all other samples to calculate the fresh weight of each sample.

Figure 4. The agricultural field margin in Ås, Eastern Norway, from which soil cores were sampled. Photo credit: Sagnik Sengupta

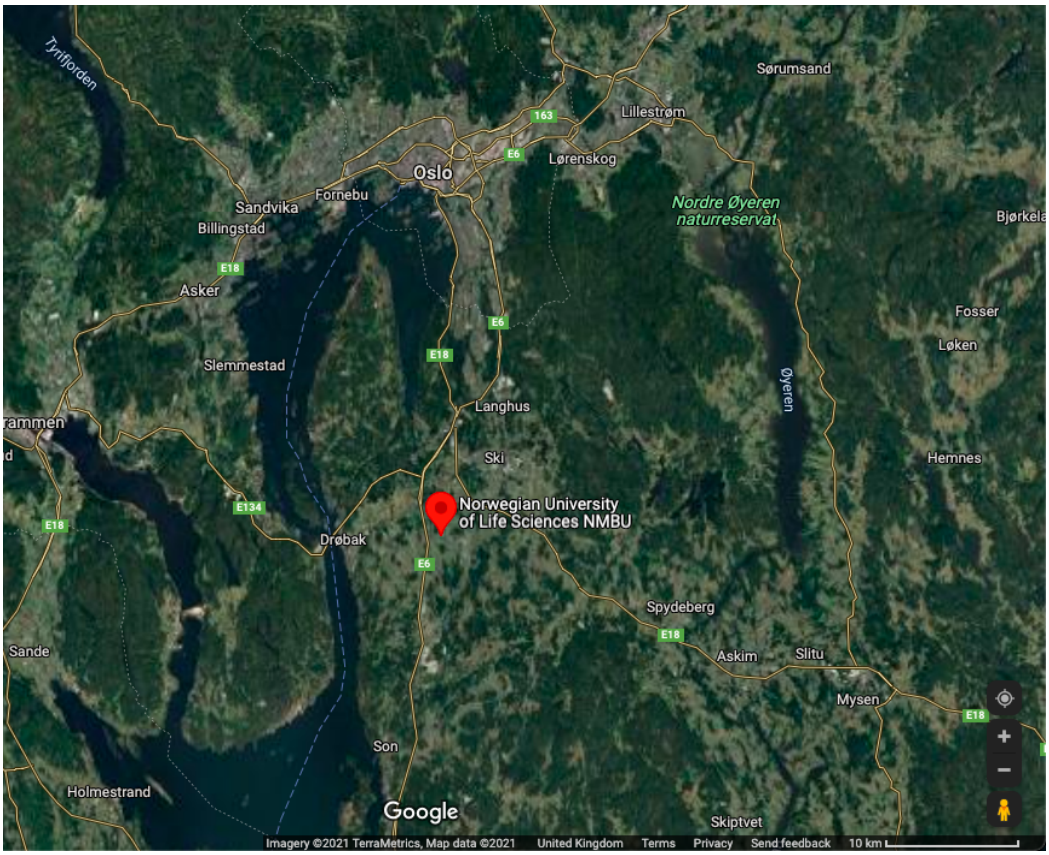

Figure 5. The location of Ås in Eastern Norway, from which field samples were collected. This is also the location of the original sampling of the population used in this experiment. Photo credit: Google Maps

Following this, all samples were frozen at -80°C for 5 days to remove any soil fauna. Once soil fauna were removed, the samples were placed in -15°C for storage.

2.4 Laboratory Preparations

This experiment was conducted using laboratory cultures of *Folsomia quadrioculata* that were originally sampled in Ås, Eastern Norway, and were maintained in laboratory cultures for several generations before being used for this experiment. This was found to have no impact on the characteristics of the population, which showed no evidence of adaptation to laboratory conditions (Sengupta et al. 2016). Groups of 10 individuals were moved from mass cultures into separate culture boxes, containing moistened . Each culture box was provided with bark as feed, and checked once weekly to replace the feed, remove any eggs, and remove and replace any dead individuals. Each culture box was stored at 15°C. In order to calculate dry weight, one sample was dried out until all moisture was removed. This value was

calculated to be 27%. Prior to spiking, samples were placed in a climate chamber at 15°C for 4 days to allow thawing and aeration of soil.

2.5 Spiking methods

The previously calculated value of water content was used in conjunction with fresh weight to calculate the dry weight of each soil core.

Prior to spiking, an imidacloprid stock solution was prepared with a concentration of 0.2 mg/mL. This was made by dissolving powdered imidacloprid in distilled water and placed on a magnetic stirrer in the dark until all particles were fully dissolved. The volume of 0.2 mg/mL imidacloprid stock solution required was then calculated for each microcosm and diluted with distilled water to attain a concentration of either 0, 0.004, 0.02, 0.1 or 0.5 mg/kg imidacloprid per dry soil weight (Appendix I). This was then added to distilled water to make a total volume of spiking solution of 0.2 mL. If total imidacloprid stock needed was higher than this, then that full volume and no additional distilled water was added. The spiking solution was applied with a pipette to the soil surface with drops being distributed as uniformly as possible across the surface. The values for fresh and dry weight, as well as imidacloprid stock added, can be found in Appendix I

Once spiked, microcosms were kept in aluminium foil covered boxes to reduce exposure to sunlight.

Once all microcosms were spiked, 20 adults were added to each microcosm. They were kept in the dark by covering the boxes with aluminium foil, and placed in climate chambers at 15°C.

2.6 Maintenance During Experiment

Throughout the duration of the experiment, each microcosm was watered once a week by calculating the average water lost across all microcosms, and applying this to each microcosm with a pipette distributing drops uniformly across the soil surface. The average water loss was calculated either by weighing all samples and calculating the average water loss, or by taking a sample of random microcosms and calculating their average water loss, depending on time constraints.

2.7 Extraction and Preservation

Each microcosm was terminated at its designated time point of 28, 35, 42 or 84 days. Termination at 42 days and 84 days was chosen as these were also the timepoints selected by Teksum 2021, and thus to allow comparison between the two studies. 28 and 35 days were thus selected to provide greater resolution at a shorter timescale. The shortest time point, 28 days, was chosen to allow sufficient time for egg development and hatching before the first extraction point. Egg development time in *Folsomia quadrioculata* from the Ås population was calculated to be 28 days at 15°C by Sengupta et al. 2016.

At time of extraction, microcosms were placed in a soil fauna extractor. Microcosms were inverted and placed on a mesh layer surrounded by plastic tubing over a pot of saturated benzoic acid. These pots were then submerged in water that was cooled with ethanol at 4°C. Heat was then applied from above, starting at 25°C for 2 days, then increasing by increments of 5°C per day until 60°C, which was maintained for 2 days. Once extraction was finished, samples in benzoic acid were sealed and stored in a cold room at approximately 4°C. Following this, samples were filtered to remove any soil that had been captured in the benzoic acid during extraction. This was done by rotating the sample to cause any animals to rise to the surface, and dirt particles to gather at the bottom. The fluid was then rapidly transferred to a new container, leaving the lower layers and sediment in the original container. Both containers were allowed to settle, and the upper layers of benzoic acid in the container containing animals were extracted and replaced in the original container. This was repeated until all animals had been extracted. All animals and remaining benzoic acid were then transferred into a marked petri-dish, and placed under a fume hood. Glycerol was then added to cover the bottom approximately 2 mm of the petri-dish, and samples were left in the fume hood for approximately 2 days, in order to allow all the benzoic acid to evapourate and the animals to sink into the glycerol. This was done in order to extend the time the samples can be safely stored for, and to assist in counting. As glycerol is more viscous than benzoic acid, animals do not move about as easily when a sample is moved, simplifying the counting process and reducing the likelihood of double-counting or failing to count individual animals.

2.8 Counting

During counting, individuals were categorised by size as Adults (>1.1 mm), intermediate (0.9 – 1.1 mm) and juveniles (<0.9 mm). In order to determine size in each sample, a sample photograph was taken and a representative adult, intermediate and juvenile was measured, and these were used for reference throughout the sample to determine category. Once counted, pictures were taken of 4 to 5 "representative" squares per sample, in order to capture as much information for future use. This was done as animals can be very unevenly distributed throughout the petri dish and highly concentrated in certain areas. As such, taking images of randomly assigned squares may result in no animals being imaged in a sample with a high number of individuals, as they may all be concentrated into one or two squares. These were taken for future reference and use.

2.9 Data and Statistics

Statistical analysis was carried out on all results following the protocol outlined in Zuur (2009) using R(). The primary packages used for statistical analysis were ggplot2 (v 3.3.3) (Wickham, 2016) and nlme (v3.1-142) (Pinheiro et al. 2019). Several linear and log-linear models were designed to fit to the data. These models were then run through the protocol in Zuur. Four models were designed, and various variance structures were attached to each model for all individuals extracted, all adults, all juveniles, and all intermediates. These variance structures were the *VarIdent* structure, the fixed variance structure, the *varPower* structure, the *varExp* variance structure, the *varConstPower* variance structure, and the varComb structure. The models were then ranked by Akaike Information Criterion (AIC) values using the *anova()* command, and the most appropriate models selected. Models were graphically assessed using residual plots for investigation.

3. Results

The results of this experiment show the effects of imidacloprid on both adult survival and juvenile recruitment across a 12-week time scale, in a concentration dependent manner. Exposure to imidacloprid was achieved by spiking defaunated soil cores from Ås, Eastern Norway, by uniformly applying various concentrations of imidacloprid spiking solution across the surface of each soil core. The method of application was chosen to mimic the methodology also used in the earlier field study by Teksum (2021). The extraction time points at 42 and 84 days were also chosen to match those of Teksum (2021), whilst the two shorter time points, 28 and 35 days, were chosen to give a greater resolution of the effects of imidacloprid before 42 days.

3.1 Effects on adult survival

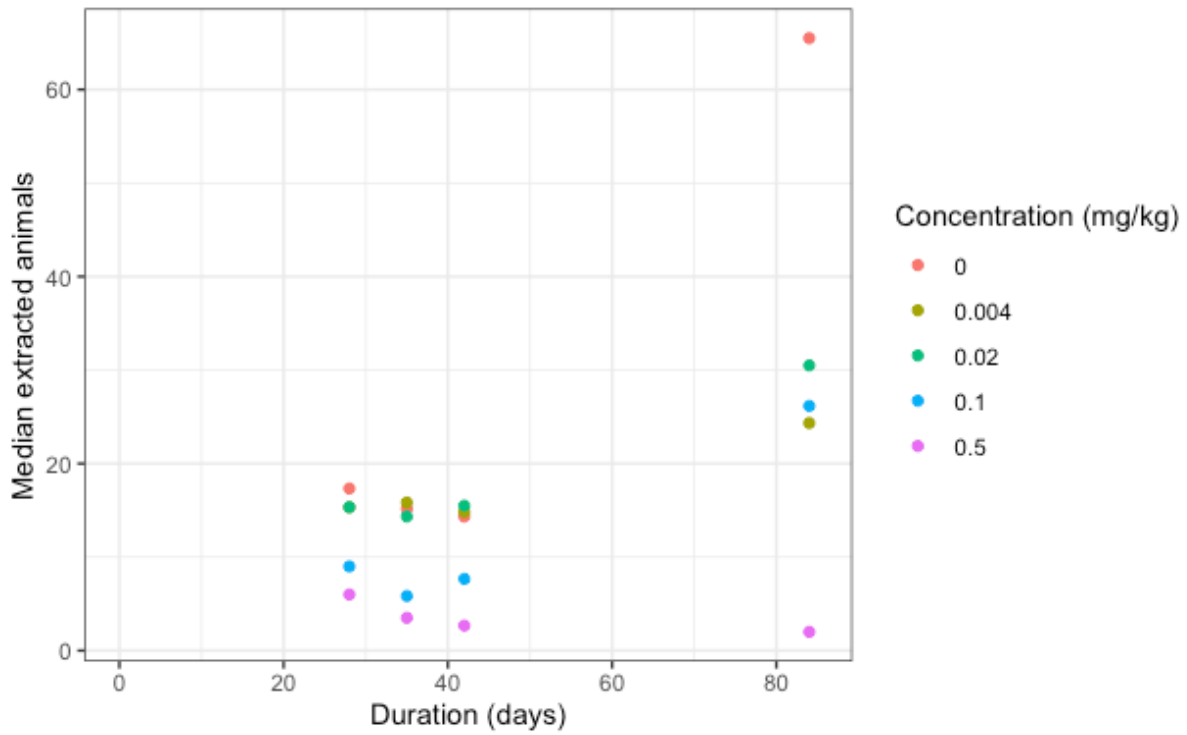

Figure 6. Scatterplot showing the median number of adult (>1.1 mm) *F. quadrioculata* extracted at 28, 35, 42 and 84 days. Each colour represents the concentration of imidacloprid used on each microcosm, and each point represents the median number of intermediate individuals extracted from all 6 replicates at the given concentration and time point. The number of adults at duration = 0 days is 20.

When looking at adult survival (Figure 6), the same general trends can be seen at 28, 35 and 42 days.. At 28 days, there is little significant change in adult survival at 0.004 mg/kg and 0.02 mg/kg when compared to control groups, with a median value of 17 adults extracted from the control group, and 15 each from 0.004 mg/kg and 0.02 mg/kg, representing the majority of the original 20 animals surviving. There is a level of mortality seen, however, it would be expected that a number of individuals would naturally die during the course of the experiment due to natural causes.Using the results from the control group at 28 days, there is an estimated mortality rate amongst adults in control groups of 13.3%. This is the only group that can be used to estimate adult survival rates, as at later time points there is a small possibility that new adults could have matured. However, by looking at the number of intermediates seen (Figure 8) the lack of intermediates suggests that no new adults will be seen at 35 days. Indeed at 15°C, this population of *F, quadrioculata* did not reach 1mm in length until over 42 days in age (Sengupta et al., 2017)

There is a reduction in the number of adults at both 0.1 and 0.5 mg/kg, with a more significant drop at 0.5 mg/kg. At 0.1 mg/kg at 28 days, a median number of 9 adults was extracted, with 6 extracted at 0.5 mg/kg.

A similar pattern is seen at 35 days, with medians of 15, 16, and 14 adults extracted from 0 mg/kg, 0.004 mg/kg and 0.02 mg/kg respectively. There is subsequently a reduction to 6 adults at 0.1 mg/kg, and down to 4 at 0.5 mg/kg.

The same is seen amongst adults at 42 days, with a median value of 14 adults extracted at 0 mg/kg, 15 at 0.004 mg/kg, and 16 at 0.02 mg/kg, before falling to 8 at 0.1 mg/kg, and down to 3 at 0.5 mg/kg.

The pattern seen at 84 days is somewhat different to those seen at shorter time points. The number of adults seen in the control group is substantially higher than those seen both at other time points and at other concentrations at this time point, with a median of 66 adults extracted. This is to be expected, as this is the first time-point in which there has been sufficient time for a significant number of eggs laid during the experiment to mature to adults. At shorter time points, there will have been insufficient time for either any or for a significant number of new adults to mature (Sengupta et al., 2017).

The number of adults drops significantly between 0 mg/kg and 0.004 mg/kg, with 24 adults extracted. There is then an increase at 0.02 mg/kg, with 31 animals extracted, before dropping

again to 26 at 0.1 mg/kg. The lowest number of adults extracted is at 0.5 mg/kg at 84 days, with a median of only 2 adults extracted.

Assessment solely of survival is only most reliable at 28 days due to the lifecycle and time scales involved. Beyond this, there is a possibility of new individuals maturing into adults, although this possibility remains small at 35 and 42 days.

At 28 days, the highest number of individuals can be seen in the control group. There is generally a high level of survival, with the least number of adults extracted from a single microcosm being 15. However, it should be noted that extraction from one microcosm yielded 21 adult individuals. This suggests an error in starting this replicate, as the experimental design outlines 20 adult individuals be released into each microcosm, and there has been insufficient time after 28 days for new adults to mature.

3.2 Effects on Recruitment

The effects of imidacloprid on recruitment can be measured by analysing the numbers of juveniles and intermediates extracted from microcosms. As only adult individuals were initially added to each microcosm, any juvenile or intermediate individual extracted at termination must result from eggs laid during the exposure period. The number of juveniles extracted is summarised in Figure 7, and the number of intermediates is summarised in Figure 8.

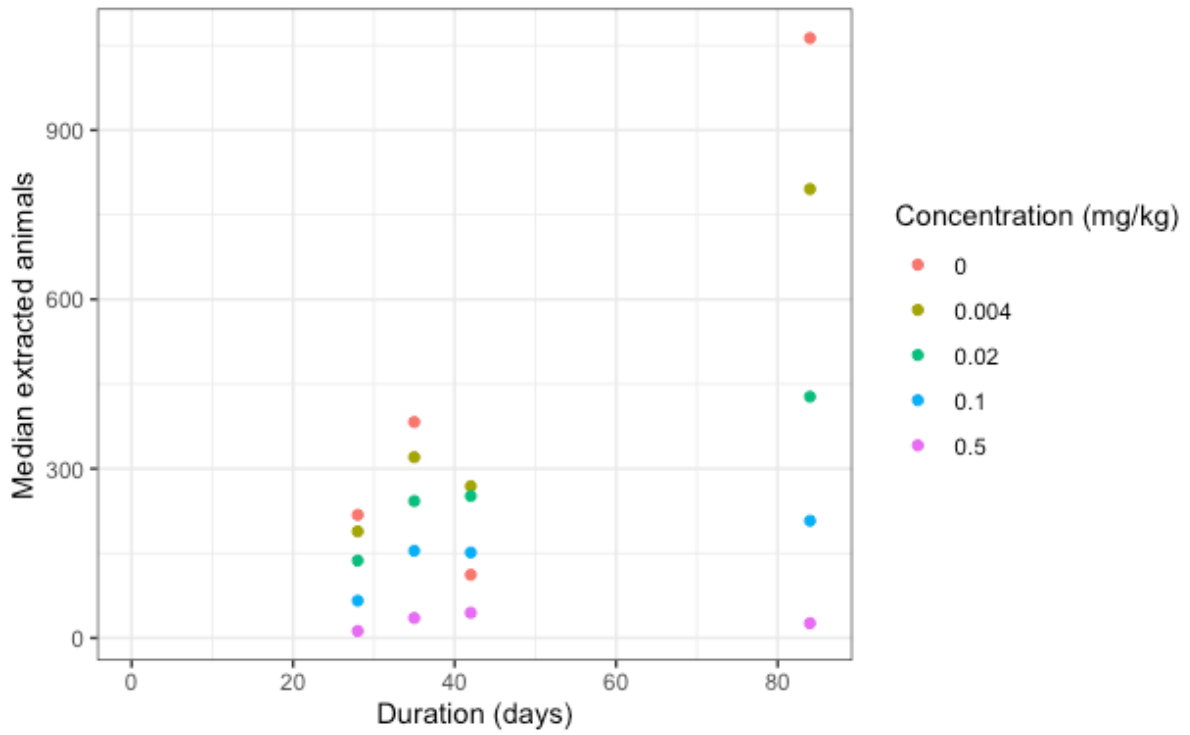

Figure 7. Scatterplot showing the median number of juvenile (>0.9 mm) *F.quadrioculata* extracted at 28, 35, 42 and 84 days. Each colour represents the concentration of imidacloprid used on each microcosm, and each point represents the median number of juvenile individuals extracted from all 6 replicates at the given concentration and time point. The number of juveniles at duration = 0 days is 0.

The number of juveniles extracted initially shows a differing pattern to that seen in adults. There is generally a reduction in the number of animals at each different concentration, with the population of juveniles decreasing linearly as concentration increases.

As duration increases, the populations begin to vary more dependent on the concentration of imidacloprid exposed to. 0 mg/kg, 0.004 mg/kg, and 0.02 mg/kg all show, with the exception of at 42 days, an increase in number as duration increases.

The greatest level of increase is seen in the control group microcosms, which increases steeply as duration increases. Amongst this group, 218 animals were extracted at 28 days, 383 at 35 days, and 1063 at 84 days, the largest number extracted at any time point or concentration. The exception to this trend is at 42 days, at which only 112 juveniles were extracted.

There is also a dramatic increase in 0.004 mg/kg at all time points (except 42 days), rising from 189 juveniles at 28 days to 320 at 35 days, and 796 at 84. Again, there is a reduction between 35 and 42 days, with 269 juveniles extracted after 42 days.

Microcosms exposed to 0.02 mg/kg imidacloprid initially show a similar steep increase from 137 at 28, to 243 at 35 days, but this begins to level off, and the increase between 35 and 84 days has a shallower gradient, with 251 juveniles extracted at 42 days, and 427 at 84.

This is also the case at 0.1 mg/kg, where population again increases between 35 and 84 days, but with a lower gradient than seen between 28 and 35 days. In this group, 66 juveniles were extracted at 28 days, 155 at 35 days, 152 at 42 days, and 208 at 84 days. Notably, compared with microcosms at lower concentrations, the reduction in juveniles between 35 and 42 days is lower, although it would still not be expected.

The gradient is shallowest at 0.5 mg/kg, which consistently has the lowest population extracted of all concentrations. Here, only 12 juveniles were extracted after 28 days, and 35 after 35. There is a slight increase up to 42 days, where 45 juveniles were extracted, before reducing at 84 days, at which point 26 juveniles were extracted.

In contrast to the results from 28, 35 and 84 days, the results at 42 days stand out. Here, the results for 0.5 mg/kg appear to fit the general pattern seen at other time points, but all other concentrations show results that would not be expected. Most notably, the control group, exposed to 0 mg/kg imidacloprid, has the second lowest number of juveniles extracted, compared to all other time points where replicates in this group are consistently those with the highest number of extracted juveniles. This represents a dramatic drop from the otherwise consistent increase that is seen at other time points at this concentration, and is dramatically different from what would be expected, as it would be expected that, in the absence of environmental stressors, the population would increase in a linear fashion as time increases.

This phenomenon is also seen at 0.004 mg/kg, which, while not showing such dramatic reductions as the one seen at 0 mg/kg, also shows a reduction in extracted juveniles that is inconsistent with the increase otherwise seen at other time points. Possible reasons for these trends are discussed in Section 4.3.

The results at 42 days for 0.02 mg/kg once again show a slight increase, which, whilst not as large as may be expected, represents a return to the pattern that might be expected based on other time points.

At 0.1 mg/kg, there is also a slight reduction at 42 days compared to 35 days, but this is very slight and not as significant as those seen at 0 and 0.004 mg/kg.

The results (with the exception of 42 days) beyond this seem to suggest that there is an immediate effect of imidacloprid on juvenile populations, even at the lowest concentrations, as reduction in population relative to the control group is seen at 0.004 mg/kg, something which was not seen in the numbers of adults extracted at shorter time points.

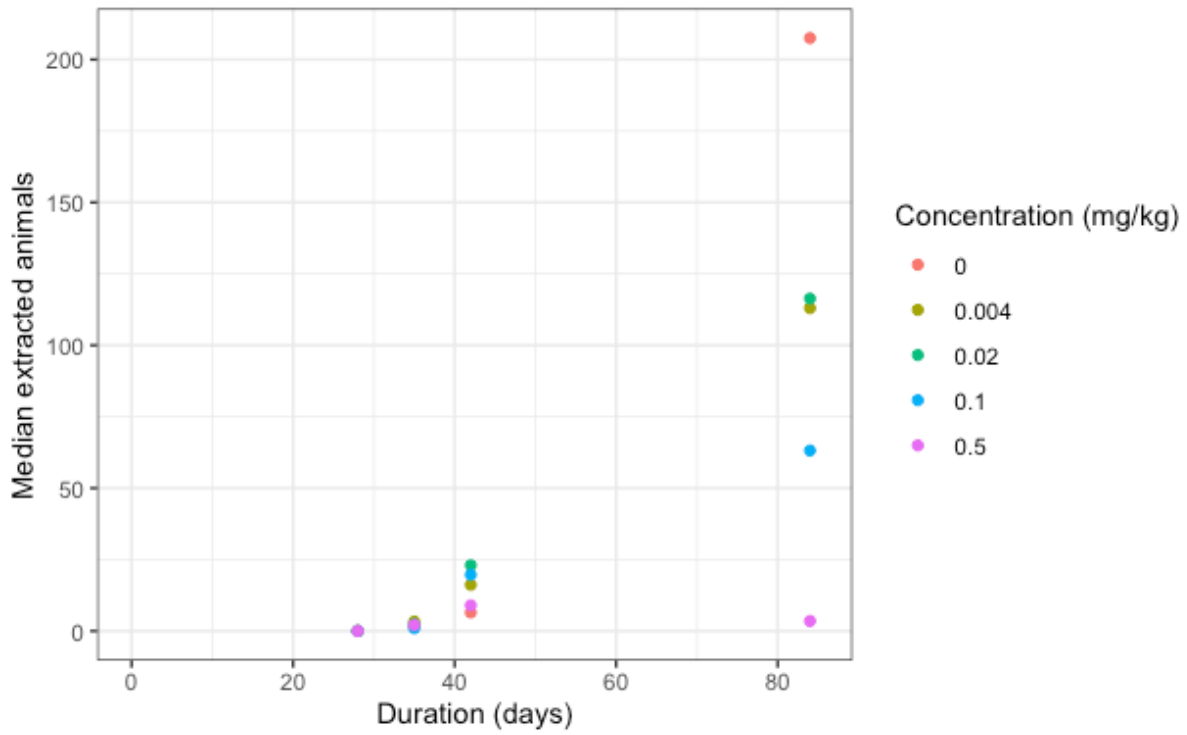

Figure 8. Scatterplot showing the median number of intermediate (0.9 - 1.1 mm length) *F.quadrioculata* extracted at 28, 35, 42 and 84 days. Each colour represents the concentration of imidacloprid used on each microcosm, and each point represents the median number of intermediate individuals extracted from all 6 replicates at the given concentration and time point. The number of intermediates at duration = 0 days is 0.

For this experiment, intermediates were defined as individuals that were approximately 0.9 - 1.1 mm in length, and thus were difficult to categorise as either adults or juveniles.

There were no intermediates observed at 28 days, and few were observed at 35 days. Here, a median value of 1 was observed at 0 mg/kg, 3 at 0.004 mg/kg, 2 at 0.02 mg/kg, 1 at 0.1mg/kg, and 2 at 0.5mg/kg.

There were also relatively few observed at 42 days, however, as was seen with juveniles, the number of intermediates extracted from control replicates was lower than would be expected, with 7 intermediates extracted, the fewest extracted from this time point. There were 16 intermediates observed at 0.004 mg/kg, rising to a maximum for this time point of 23 at 0.02 mg/kg. The number then begins to decrease, with 20 observed at 0.1 mg/kg, and 9 at 0.5 mg/kg.

At 84 days, the number of intermediates increases significantly. Here, the largest group is once again seen at 0 mg/kg, with a median value of 208 intermediates extracted. There is then a reduction to 13 at 0.004 mg/kg, and a slight increase up to 0.02 mg/kg, where 116 were extracted.. The numbers seen at these two concentrations are relatively similar, although a

slightly higher median number is seen in 0.02 mg/kg than 0.004 mg/kg, which would not be expected from the pattern seen in juveniles, in which 0.02 mg/kg has a much lower population of juveniles than 0.004 mg/kg. There is then another reduction to 0.1 mg/kg, with a median value of 63 intermediates extracted. The lowest number of intermediates is seen in 0.5 mg/kg, with a median value of 4. .

3.3 Statistical analysis

Statistical analysis was carried out in R following the protocol outlined in chapter 4 of Zuur (2004).

Table 1. Summary table of statistical modelling of all extracted individuals based on the most appropriate model according to Zuur 2010, with concentration set as a factor against a continuous variable of duration, as factor(concentration) * duration, with a response variable as log(Total). The most statistically significant results are seen at 0.5 mg/kg.

	Value	**Std.Error**	**t-value**	**p-value**
Intercept	4.343891	0.4153692	10.457903	0.0000
Concentration 0.004	0.460884	0.4702995	0.979979	0.3292
Concentration 0.02	0.259594	0.4644803	0.558892	0.5774
Concentration 0.1	-0.430796	0.5490420	-0.784633	0.4344
Concentration 0.5	-0.464490	0.7148267	-0.649795	0.5172
Duration	0.029396	0.0079831	3.682335	0.0004
Concentration 0.004:Duration	-0.007606	0.0090388	-0.841527	0.4019
Concentration 0.02:Duration	-0.008692	0.0089269	-0.973676	0.3324
Concentration 0.1:Duration	-0.008711	0.0105522	-0.825484	0.4109
Concentration 0.5:Duration	-0.046931	0.0137384	-3.416049	0.0009

Table 2. Summary table of statistical modelling of all extracted adults individuals based on the most appropriate model according to Zuur 2010, with gls(Adults ~ Duration + factor(Concentration).

	Value <chr>	Std.Error <chr>	t-value <chr>	p-value <chr>
(Intercept)	18.159871	0.9583142	18.949808	0.0000
Duration	-0.045872	0.0190210	-2.411672	0.0175
factor(Concentration)0.004	-1.068867	1.1262829	-0.949022	0.3446
factor(Concentration)0.02	-1.468676	1.1845229	-1.239888	0.2176
factor(Concentration)0.1	-8.469226	0.9128923	-9.277354	0.0000
factor(Concentration)0.5	-12.450733	0.9387884	-13.262555	0.0000

Table 3. Summary table of statistical modelling of all extracted juvenile individuals based on the most appropriate model according to Zuur 2010, with gls(Juveniles ~ Duration * factor(Concentration))

	Value <chr>	Std.Error <chr>	t-value <chr>	p-value <chr>
(Intercept)	-20.056321	2.5788537	-7.777223	0.0000
Duration	0.697132	0.0854538	8.157993	0.0000
factor(Concentration)0.004	0.522193	0.8716281	0.599101	0.5503
factor(Concentration)0.02	0.584595	0.8716281	0.670693	0.5038
factor(Concentration)0.1	0.314010	0.8716281	0.360257	0.7193
factor(Concentration)0.5	0.160990	0.8716281	0.184701	0.8538

Table 4. Summary table of statistical modelling of all extracted intermediate individuals based on the most appropriate model according to Zuur 2010, with gls(Intermediate ~ Duration + factor(Concentration)).

	Value <chr>	Std.Error <chr>	t-value <chr>	p-value <chr>
(Intercept)	-20.056321	2.5788537	-7.777223	0.0000
Duration	0.697132	0.0854538	8.157993	0.0000
factor(Concentration)0.004	0.522193	0.8716281	0.599101	0.5503
factor(Concentration)0.02	0.584595	0.8716281	0.670693	0.5038
factor(Concentration)0.1	0.314010	0.8716281	0.360257	0.7193
factor(Concentration)0.5	0.160990	0.8716281	0.184701	0.8538

Several models were initially designed. Following this, a number of variance structures were applied to the models in order to determine the most appropriate model. The Linear and Non-linear Mixed Effects Models (nlme) package was used to facilitate this. The variance structures tested were the *VarIdent* structure, the fixed variance structure, the *varPower* structure, the *varExp* variance structure, the *varConstPower* variance structure, and the

varComb structure. The models were then ranked by AIC values using the *anova()* command, the results of which can be seen in Appendix II. Following this, Model 4.4 (table 3.3.1) , constrained by the varIdent variance structure, was found to be most appropriate. This model was chosen as it had the most balanced AIC and BIC results of all models and variance structures assessed.
Following this protocol, the model selected, Model 4.4, shows that, for total number of individuals of all classifications over time, only 0.5 mg/kg shows statistical significance.

3.4 Comparison of defaunated soil cores and intact cores

The third aim of this study is to compare, where possible, the results of this experiment with Teksum (2021), which was undertaken as part of the MULTICLIM using in-tact soil cores in a field setting. Teksum (2021) released 20 adult *F. quadrioculata* into microcosms within the same agricultural field margin in Ås from which soil cores for this experiment were gathered, with extraction points at 42 and 84 days, counting the total number of *F. quadrioculata* extracted at these time points. The total number of animals extracted in this experiment are presented in Figure 9. They found that the greatest increase in animals occurred at 0.02 mg/kg and 0.1mg/kg. There was a modest increase at 42 days in 0 mg/kg, before falling again at 84 days. This was also seen in 0.5 mg/kg. They saw a modest increase in 0.02 mg/kg at 48 days, with a maximum number of animals extracted at slightly over 100 at 84 days in 0.02 mg/kg. The greatest increase seen in their project was at 0.1 mg/kg at 84 days, with slightly over 300 animals extracted. In this project, 0.1 mg/kg at 84 days yielded a similar result (Figure 9), with slightly under 300 animals extracted. However, unlike Teksum (2021), this was not the largest increase seen, with 0.02 mg/kg and 0 mg/kg all showing much larger increases (0.004 mg/kg was not used by Teksum (2021)). In general, this project saw larger increases in individuals extracted at lower concentrations over time, especially as duration increased.

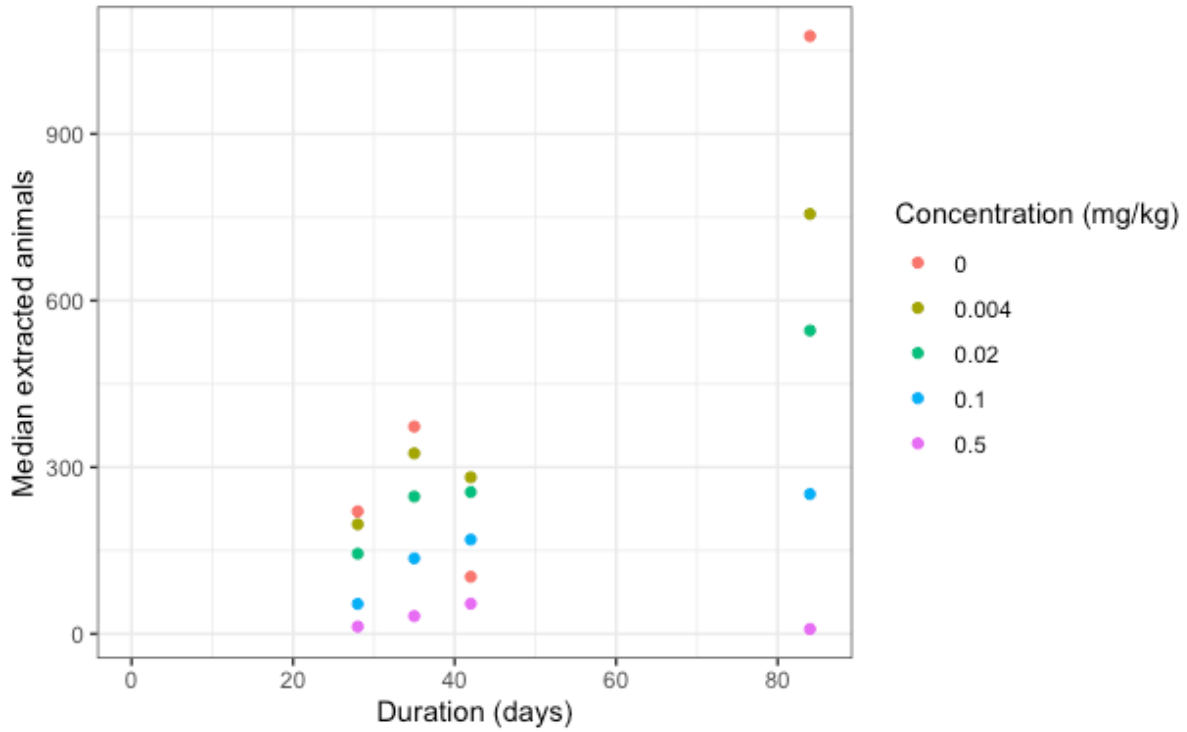

Figure 9. Scatterplot showing the median number of *F.quadrioculata* extracted at 28, 35, 42 and 84 days. Each colour represents the concentration of imidacloprid used on each microcosm, and each point represents the median number of individuals extracted from all 6 replicates at the given concentration and time point. The number of animals at duration = 0 days is 20.

4. Discussion

The primary focus of this study is to assess the effects of imidacloprid in defaunated soil microcosms on the survival and reproduction of *Folsomia quadrioculata,* and to compare with a field and laboratory situation. There are several trends that can be identified from the results of this study that can be broadly divided into the effects of imidacloprid on adult survival, and those on juvenile recruitment.

Sengupta et al. (2021) exposed *Folsomia quadrioculata* to imidacloprid through dietary means, spiking feed with various concentrations. This will not be the exposure pathway utilised in this experiment, which will expose *Folsomia quadrioculata* by adding a spiking solution to soil cores to calculate a concentration per dry soil weight. As such, the two experiments will not be directly comparable, however, comparisons in patterns seen will be possible. From this, it would thus be expected that adult survival will be largely unaffected by exposure to imidacloprid regardless of the studied concentration. However, it would be expected that the

number of juvenile individuals will be reduced with exposure to imidacloprid, as egg production is negatively affected by exposure.

Several laboratory and field experiments have been previously undertaken in the scope of the MULTICLIM project.. Laboratory studies consisted of exposing adults of *F. quadrioculata* to dietary imidacloprid for 14 days. Following this, they were given a 28-day recovery period. Results showed that adults survived well after being subjected to this procedure, with over 96% survival after 14 days of exposure (Sengupta et al. 2021). At the end of the experiment, exposure to imidacloprid was found to have no significant effect on survival regardless of concentration, with a maximum measured concentration of 290 mg/kg dry food (Sengupta et al. 2021). In contrast, egg production was found to be significantly impacted by imidacloprid exposure. Egg production was found to have been reduced during exposure at all concentrations (Sengupta et al. 2021).

We also aim to address some of the issues found in solely laboratory-based experiments. These typically have simplified set-ups, with systems that have few of the complexity found in the natural environment. By using intact soil cores, the soil structure used in this experiment is intended to closely represent the physical conditions in which *Folsomia quadrioculata* lives in nature. By replicating soil conditions, this experiment is designed to have similar distribution and exposure pathways to imidacloprid as would be seen in nature, as well as providing a similar habitat and feeding environment.

4.1. Adults

The patterns seen in adults generally follow those that would be expected from this study. The increase in adults at 84 days is expected, as this is the first time point to have had a significant duration to allow maturation of new adults As can be seen from the numbers of intermediates in Figure 8 observed at 28 and 35 days, juvenile maturation into adults is unlikely to have occured before 42 days. Indeed, Sengupta et al. (2016) found that age of first reproduction amongst the Ås population of *F. quadrioculata* to be over 42 days, and size of first reproduction to be between 1.1 and 1.2 mm in the same population. As such, it would be unlikely for new adults to have matured before 42 days. This is seen in the lack of increase in adult numbers before 84 days in this experiment. The similar numbers of adults seen at 0,

0.004 and 0.02 mg/kg up to 42 days suggests that exposure to these concentrations has little effect on adult survival. The slight reduction in adults in control groups is also not unexpected. As juveniles are unlikely to mature into adults is unlikely in this population before 42 days, it would be expected that a degree of mortality is seen as the original population of 20 begin to reach the end of their natural life span and are not replaced by maturing juveniles.

The pattern seen at 84 days is different to those seen at shorter time points, with a significant drop between the control group and all other concentrations. This may be indicative of the patterns seen in juveniles. As only 20 adults were added at the start of the experiment, any new adults must be the result of the maturation of juveniles into adults during the course of the experiment. As seen in the juvenile and intermediate results from this experiment, juveniles appear to be more sensitive to the effects of imidacloprid, with decreases in numbers at as low as 0.004 mg/kg. Thus, it would be expected that this reduction in juvenile numbers would begin to have a subsequent effect on the adult numbers seen as juveniles mature into adults. This may be the result of one of two things: an effect imidacloprid is having on egg laying, as seen by Sengupta et al. (2021), or that younger individuals are more sensitive to lower concentrations and have a higher mortality at lower concentrations. In previous experiments, it has been seen that even at low concentrations, imidacloprid has a negative impact on egg production, with an immediate reduction in egg laying upon exposure (Sengupta et al., 2021). However, interestingly, from the results of this experiment, the patterns of numbers of intermediates seen at 84 days more closely reflects those seen in adults at 84 days, rather than juveniles. This may suggest that mortality among juveniles is higher than in adults, and that juveniles are dying after they have hatched, but before they have matured into intermediates and thus adults.

4.2 Juveniles

Results in juveniles can be seen to broadly follow the same pattern across all time points, with the exception of at 42 days, which will be discussed in section 4.3.

At 28, 35 and 84 days, broadly the same pattern can be seen. At these time points, the highest number of juveniles is seen in the control group, as would be expected. The numbers of juveniles extracted then decreases as concentration of imidacloprid increases, with the lowest numbers seen at 0.5 mg/kg. The exception to this can be seen at 42 days. Here, the number of juveniles seen at 0 mg/kg is substantially lower than those seen at 0.004, 0.02 and 0.1 mg/kg.

This would not usually be expected, as adults and juveniles in the control group have been exposed to less pressure, due to the absence of imidacloprid.
The results from this study show a reduction in the number of juveniles at concentrations lower than those at which reductions are seen in adults, which typically have stable populations at up to 0.1 mg/kg before 84 days.
The consistent reduction in juveniles even at 84 days would be expected given imidacloprid's stability in soil, with a half-life of 99 to 129 days at 20°C (EFSA, 2015). Whilst this would suggest that, whilst the levels of imidacloprid in each microcosm are decreasing over time, it would be expected that imidacloprid was still present in significant quantities in the soil, even at 84 days. As such, animals have been constantly exposed to imidacloprid throughout this experiment, and there has been no opportunity for recovery.
There are potentially 2 explanations for why a reduction in the number of juveniles compared to the control is seen at lower concentrations than adults: either juveniles are more susceptible to mortality from imidacloprid, thus die at much lower concentrations than adults; or imidacloprid is affecting the reproduction and egg laying of adults, consequently leading to a reduction in the number of hatchlings and juveniles.
It might be expected that juveniles are more susceptible to toxicity from imidacloprid. Often, juvenile individuals (of most species) are more susceptible to contaminants for a number of reasons. Thus, it might be expected that juvenile *Folsomia quadrioculata* show a reduction in numbers at lower concentrations than adults. However, the numbers in this experiment begin to reduce at concentrations orders of magnitude lower than when reductions in adult numbers are seen at the same time point.
An alternative explanation is that the reduction in number of juveniles is the result of a reduction in reproduction caused by exposure to imidacloprid. Indeed, Sengupta et al. (2021) saw a reduction in egg production in *Folsomia quadrioculata* at sublethal concentrations of imidacloprid significantly lower than those at which mortality was seen. This may suggest that the reduction in juveniles seen here is a result of reduced reproduction, rather than increased mortality in juveniles.

4.3 42 Day Juvenile population

One unexpected outcome from this project is the results seen in juveniles and intermediates in 0 and 0.004 mg/kg at 42 days. It would generally be expected that the control group at each time point to have the highest (or at least roughly equal to other concentrations) number of

individuals. In juveniles, this is the case in control groups at 28, 35 and 84 days. However, we see in the 42 day control group that the control group has a significantly lower number of juveniles than 0.004 and 0.02 mg/kg, and there are fewer juveniles in 0.004 mg/kg than in 0.02 mg/kg. Generally, we would expect that increasing concentration to have a negative effect on the number of juveniles.. Indeed, juveniles from 28, 35 and 84 time-points appear to be sensitive to even the lowest concentration used in this project, with a reduction in their population at even 0.002 mg/kg.

The exact reasons for this are unclear.

One possible explanation could be that water stress has been introduced onto these microcosms, affecting the outcome. The microcosms for this termination were watered by calculating the average amount of water lost, and adding this amount once weekly, as with other time points. However, due to time constraints, only the first watering of this time point (after 1 week) had all microcosms measured individually. For each other watering, a random sample was taken, and the average water lost from this sample was calculated and applied to all microcosms.

However, the amounts calculated by this method do not seem to differ greatly from those calculated for other time points or other microcosms from this time point, and calculated loss from control microcosms also seems to align with those of other microcosms. In addition, the adult population appears to align with expectations from those of other time points. Had water stress been introduced, we might expect to see negative effects on the survival in adults, as well as a reduction in juveniles. This is not seen.

Notably, this time point was affected by the shutdown on university facilities in March of 2020 as a response to the Coronavirus pandemic. This time point was started on 11th February and had a scheduled termination on 24th March 2021. The Government of Norway closed all University facilities to all but essential work. Whilst this had the potential to cause disruption, this project was fortunate, and a co-supervisor was able to secure access to the laboratory on a limited schedule. This was sufficient to maintain the watering and extraction regimes as planned with only limited disruption. As such, this wouldn't be expected to have had a significant impact on results. Moreover, again, any effects seen as a consequence of this would be expected to be seen across all results from this time point. However, we do not see any change in adult populations compared with other time points that were not affected by government lockdowns, and juvenile populations at 0.02, 0.1 and 0.5 mg/kg do not seem to show significant deviation from what would be expected in comparison with other time points.

5. Conclusion

This study has shown that both adults and juveniles of *Folsomia quadrioculata* are negatively affected after exposure to imidacloprid in soil microcosms, and that a single application of imidacloprid has the potential to affect population growth, and cause population decline at 0.5 mg/kg. The effects are seen differently in adults compared to those in earlier life stages. The results of this study seem to suggest that adults are more resistant to the effects of imidacloprid, whilst juveniles are more susceptible. However, it is not possible from this study to say whether the reduction in juvevnile numbers at higher concentrations of imidacloprid are as a result of mortality amongst juveniles, or as a result of reduced egg laying by adults at higher concentrations.

Indeed, Sengupta et al. (2021) saw that increasing levels of imidacloprid led to a reduction in eggs produced per adult. This may suggest that rather than being more toxic to juveniles, imidacloprid is causing a reduction in egg laying, which in turn is leading to the reduction in juvenile populations seen as imidacloprid concentration increases. However, here we again see some of the difficulties in study design. This experiment was designed in such a way so as to combine certain benefits of both laboratory and field studies. In being unable to see the interactions and behaviours of animals during the course of the experiment, we see some of the disadvanges faced by field studies. However, this comes with the benefit of a more representative exposure pathway than has been achieved in the laboratory in other experiments under the MULTICLIM project, which have attempted to preserve the ability to observe animals during the course of the experiment whilst finding an appropriate exposure mechanism representative of real-world conditions.

6. Future perspectives

This study has shown the viability in using defaunated soil cores in laboratory based studies to assess the effects on neonicotinoids on non-target, soil dwelling springtails. However, there are several areas of interest that should be explored in further studies.

By defaunating soil cores, all elements of competition and predation have been removed. This is in contrast to both field studies and the natural environment, in which *F. quadrioculata* would face competition for resources from other soil-dwelling organisms, as well as potential predation. As such, future studies could be carried out in which *F. quadrioculata* are released

into soil microcosms that also contain other soil-dwelling organisms. This could be achieved in a number of ways. Teksum 2021 released 20 *F. quadrioculata* into microcosms in the field under natural conditions. This could be replicated by taking soil cores and transferring them directly to the laboratory, omitting the defaunation stage carried out in this study, and instead directly releasing *F. quadrioculata* into the microcosms with the soil ecosystem intact. This would allow the effects of natural competition and predation to be studied as factors affecting the population of *F. quadrioculata*, whilst also allowing the analysis of how imidacloprid affects the soil ecosystem.
It would also be possible to defaunate soil microcosms as in this experiment, and release a variety of soil-dwelling arthropods representative of a soil community into the microcosm to assess competition and interactions between different species.

This project also spiked microcosms by uniformly distributing imidacloprid spiking solution across the surface of each microcosm. Future studies may wish to analyse and assess different methods of spiking, and analyse the distribution of imidacloprid throughout the soil core.

Whilst not the aim of this study, the use of soil microcosms does not allow the observation of individuals throughout the duration of the experiment, and only individuals alive at the time of extraction can be observed after preservation. As such, sublethal effects of imidacloprid, such as mobility, cannot be assessed. Future studies may wish to assess this.

References

Arena, M., & Sgolastra, F. (2014). A meta-analysis comparing the sensitivity of bees to pesticides. *Ecotoxicology, 23*(3), 324-334. doi:10.1007/s10646-014-1190-1

Atwood, D. Paisley-Jones., C. (2017). Pesticides Industry Sales and Usage 2008 - 2012 Market Estimates. United States Environmental Protection Agency. Washington D.C.

Barrios, E. (2007). Soil biota, ecosystem services and land productivity. ECOLOGICAL ECONOMICS, 64(2), 269-285. doi:10.1016/j.ecolecon.2007.03.004

Birkemoe, T., & Somme, L. (1998). Population dynamics of two collembolan species in an Arctic tundra. PEDOBIOLOGIA, 42(2), 131-145.

Buckingham, S. D., Lapied, B., LeCorronc, H., Grolleau, F., & Sattelle, D. B. (1997). Imidacloprid actions on insect neuronal acetylcholine receptors. *JOURNAL OF EXPERIMENTAL BIOLOGY, 200*(21), 2685-2692.

European Commission, (2021). Neonicotinoids. Retrieved from https://ec.europa.eu/food/plants/pesticides/approval-active-substances/renewal-approval/neonicotinoids_en

European Food Safety Agency. (2015). Conclusion on the peer review of the pesticide risk assessment for bees for the active substance imidacloprid considering all uses other than seed treatments and granules. EFSA JOURNAL, 13(8). doi:10.2903/j.efsa.2015.4211

Ferlian, O., Klarner, B., Langeneckert, A. E., & Scheu, S. (2015). Trophic niche differentiation and utilisation of food resources in collembolans based on complementary analyses of fatty acids and stable isotopes. SOIL BIOLOGY & BIOCHEMISTRY, 82, 28-35. doi:10.1016/j.soilbio.2014.12.012

Fiera, C., & Ulrich, W. (2012). Spatial patterns in the distribution of European springtails (Hexapoda: Collembola). *BIOLOGICAL JOURNAL OF THE LINNEAN SOCIETY, 105*(3), 498-506. doi:10.1111/j.1095-8312.2011.01816.x

Forfait-Dubuc, C., Charles, S., Billoir, E., & Delignette-Muller, M. L. (2012). Survival data analyses in ecotoxicology: critical effect concentrations, methods and models. What should we use? Ecotoxicology, 21(4), 1072-1083. doi:10.1007/s10646-012-0860-0

Gange, A. (2000). Arbuscular mycorrhizal fungi, Collembola and plant growth. TRENDS IN ECOLOGY & EVOLUTION, 15(9), 369-372. doi:10.1016/S0169-5347(00)01940-6

Goulson, D. (2013). REVIEW: An overview of the environmental risks posed by neonicotinoid insecticides. *JOURNAL OF APPLIED ECOLOGY, 50*(4), 977-987. doi:10.1111/1365-2664.12111

Jeschke, P., & Nauen, R. (2008). Neonicotinoids - from zero to hero in insecticide chemistry. PEST MANAGEMENT SCIENCE, 64(11), 1084-1098. doi:10.1002/ps.1631

Jeschke, P., Nauen, R., Schindler, M., & Elbert, A. (2011). Overview of the Status and Global Strategy for Neonicotinoids. JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY, 59(7), 2897-2908. doi:10.1021/jf101303g

Kristiansen, S. M., Borga, K., Rundberget, J. T., & Leinaas, H. P. (2021). Effects on Life-History Traits of Hypogastrura viatica (Collembola) Exposed to Imidacloprid Through Soil or Diet. ENVIRONMENTAL TOXICOLOGY AND CHEMISTRY, 40(11), 3111-3122. doi:10.1002/etc.5187

Matsuda, K., Buckingham, S. D., Kleier, D., Rauh, J. J., Grauso, M., & Sattelle, D. B. (2001). Neonicotinoids: insecticides acting on insect nicotinic acetylcholine receptors. *TRENDS IN PHARMACOLOGICAL SCIENCES, 22*(11), 573-580. doi:10.1016/S0165-6147(00)01820-4

MULTICLIM. (2018, 17/04/2021). MULTICLIM: Effects of climate change in a multiple stress multispecies perspective. Retrieved from https://www.mn.uio.no/ibv/english/research/sections/aqua/research-projects/144612/

National Center for Biotechnology Information (2021). PubChem Compound Summary for CID 86287518, Imidacloprid. Retrieved December 7, 2021 from https://pubchem.ncbi.nlm.nih.gov/compound/Imidacloprid.

Partsch, S., Milcu, A., & Scheu, S. (2006). Decomposers (Lumbricidae, Collembola) affect plant performance in model grasslands of different diversity. ECOLOGY, 87(10), 2548-2558. doi:10.1890/0012-9658(2006)87[2548:DLCAPP]2.0.CO;2

Pinheiro J, Bates D, DebRoy S, Sarkar D, R Core Team (2019). nlme: Linear and Nonlinear Mixed Effects Models. R package version 3.1-153, https://CRAN.R-project.org/package=nlme.

Pisa, L. W., Amaral-Rogers, V., Belzunces, L. P., Bonmatin, J. M., Downs, C. A., Goulson, D., . . . Wiemers, M. (2015). Effects of neonicotinoids and fipronil on non-target invertebrates. Environmental Science and Pollution Research, 22(1), 68-102. doi:10.1007/s11356-014-3471-x

Rombke, J., Jansch, S., Junker, T., Pohl, B., Scheffczyk, A., & Schallnass, H. J. (2006). Improvement of the applicability of ecotoxicological tests with earthworms, springtails, and plants for the assessment of metals in natural soils. *ENVIRONMENTAL TOXICOLOGY AND CHEMISTRY, 25*(3), 776-787. doi:10.1897/04-584R.1

Rusek, J. (1998). Biodiversity of Collembola and their functional role in the ecosystem. *BIODIVERSITY AND CONSERVATION, 7*(9), 1207-1219. doi:10.1023/A:1008887817883

Sengupta, S., Ergon, T., & Leinaas, H. P. (2017). Thermal plasticity in postembryonic life history traits of a widely distributed Collembola: Effects of macroclimate and microhabitat on genotypic differences. ECOLOGY AND EVOLUTION, 7(19), 8100-8112. doi:10.1002/ece3.3333

Sengupta, S., Ergon, T., & Leinass, P. H. (2016). Genotypic differences in embryonic life history traits of Folsomia quadrioculata (Collembola: Isotomidae) across a wide geographical range. *Ecological Entomology, 41*(1), 72-84. doi:10.1111/een.12270

Sengupta, S., Leinaas, H. P., van Gestel, C. A. M., Rundberget, J. T., & Borga, K. (2021). A Multiple Life-History Trait-Based and Time-Resolved Assessment of Imidacloprid Effects and Recovery in the Widely Distributed Collembolan Folsomia quadrioculata. *ENVIRONMENTAL TOXICOLOGY AND CHEMISTRY, 40*(1), 139-147. doi:10.1002/etc.4897

Silva, C. D. E., Brennan, N., Brouwer, J. M., Commandeur, D., Verweij, R. A., & van Gestel, C. A. M. (2017). Comparative toxicity of imidacloprid and thiacloprid to different species of soil invertebrates. Ecotoxicology, 26(4), 555-564. doi:10.1007/s10646-017-1790-7

Simon-Delso, N., Amaral-Rogers, V., Belzunces, L. P., Bonmatin, J. M., Chagnon, M., Downs, C., . . . Wiemers, M. (2015). Systemic insecticides (neonicotinoids and fipronil): trends, uses, mode of action and metabolites. *Environmental Science and Pollution Research, 22*(1), 5-34. doi:10.1007/s11356-014-3470-y

Szczepaniec, A., Creary, S. F., Laskowski, K. L., Nyrop, J. P., & Raupp, M. J. (2011). Neonicotinoid Insecticide Imidacloprid Causes Outbreaks of Spider Mites on Elm Trees in Urban Landscapes. PLoS ONE, 6(5). doi:10.1371/journal.pone.0020018

Teksum, M. (2021). Assessing the exposure and effects of imidacloprid on the abundance of microarthropods in an agricultural soil community. Master's Thesis. University of Oslo. Norway

Tomizawa, M., & Casida, J. E. (2003). Selective toxicity of neonicotinoids attributable to specificity of insect and mammalian nicotinic receptors. ANNUAL REVIEW OF ENTOMOLOGY, 48, 339-364. doi:10.1146/annurev.ento.48.091801.112731

van der Sluijs, J. P., Amaral-Rogers, V., Belzunces, L. P., Bijleveld van Lexmond, M. F. I. J., Bonmatin, J. M., Chagnon, M., . . . Wiemers, M. (2015). Conclusions of the Worldwide Integrated Assessment on the risks of neonicotinoids and fipronil to biodiversity and ecosystem functioning. *Environmental Science and Pollution Research, 22*(1), 148-154. doi:10.1007/s11356-014-3229-5

van Gestel, C. A. M., de Lima e Silva, C., Lam, T., Koekkoek, J. C., Lamoree, M. H., & Verweij, R. A. (2017). Multigeneration toxicity of imidacloprid and thiacloprid to Folsomia candida. *Ecotoxicology, 26*(3), 320-328. doi:10.1007/s10646-017-1765-8

Wickham H (2016). ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag New York. ISBN 978-3-319-24277-4, https://ggplot2.tidyverse.org.

World Bank. (2021). Arable Land (% of land area) Retrieved from https://data.worldbank.org/indicator/AG.LND.ARBL.ZS?end=2018&start=1961&view=chart

World Bank. (2021). Population, Total. Retrieved from https://data.worldbank.org/indicator/SP.POP.TOTL

Zuur, A. F. (2009). Mixed effects models and extensions in ecology with R. New York: Springer.

Appendices

Appendix I

Table A.1. Overview of the fresh weight, calculated dry weight, and imidacloprid stock solutions added to each microcosm. Imidacloprid stock was added to distilled water to make a total spiking volume of 200 µL. Where the amount of imidacloprid stock required exceeded this, no distilled water was added, and the full amount of imidacloprid stock added.

Sample ID	Fresh weight (g)	Dry weight	Imidacloprid dose (mg kg-1)	Imidacloprid weight (mg)	Imidacloprid stock to be added (µL)	Distilled water to be added (µL)
JP6	100,87	73,58	0	0	0,00	200,00
JP7	101,69	74,18	0	0	0,00	200,00
JP8	106,64	77,79	0	0	0,00	200,00
JP9	106,17	77,45	0	0	0,00	200,00
JP10	98,83	72,10	0	0	0,00	200,00
JP11	101,29	73,89	0	0	0,00	200,00
JP12	105,79	77,17	0,004	0,0003	1,54	198,46
JP13	97,81	71,35	0,004	0,0003	1,43	198,57
JP14	88,12	64,28	0,004	0,0003	1,29	198,71
JP15	111,16	81,09	0,004	0,0003	1,62	198,38
JP16	113,77	83,00	0,004	0,0003	1,66	198,34
JP17	89,16	65,04	0,004	0,0003	1,30	198,70
JP18	112,28	81,91	0,02	0,0016	8,19	191,81
JP19	112,76	82,26	0,02	0,0016	8,23	191,77
JP20	113,78	83,00	0,02	0,0017	8,30	191,70
JP21	117,18	85,48	0,02	0,0017	8,55	191,45
JP22	108,40	79,08	0,02	0,0016	7,91	192,09
JP23	97,59	71,19	0,02	0,0014	7,12	192,88
JP24	106,37	77,60	0,1	0,0078	38,80	161,20
JP25	105,57	77,01	0,1	0,0077	38,51	161,49
JP26	106,15	77,44	0,1	0,0077	38,72	161,28
JP27	104,11	75,95	0,1	0,0076	37,97	162,03
JP28	100,71	73,47	0,1	0,0073	36,73	163,27
JP29	91,76	66,94	0,1	0,0067	33,47	166,53
JP30	98,83	72,10	0,5	0,0360	180,24	19,76
JP31	110,79	80,82	0,5	0,0404	202,05	x
JP32	103,31	75,36	0,5	0,0377	188,41	11,59
JP33	98,36	71,75	0,5	0,0359	179,38	20,62

JP34	98,59	71,92	0,5	0,0360	179,80	20,20
JP35	101,91	74,34	0,5	0,0372	185,86	14,14
JP36	84,17	61,40	0	0	0,00	200,00
Jp37	94,46	68,91	0	0	0,00	200,00
JP38	97,18	70,89	0	0	0,00	200,00
JP39	98,35	71,75	0	0	0,00	200,00
JP40	96,09	70,10	0	0	0,00	200,00
JP41	95,17	69,43	0	0	0,00	200,00
JP42	86,87	63,37	0,004	0,00025349	1,27	198,73
JP43	107,70	78,57	0,004	0,00031427	1,57	198,43
JP44	96,18	70,16	0,004	0,00028065	1,40	198,60
JP45	77,17	56,30	0,004	0,00022518	1,13	198,87
JP46	115,90	84,55	0,004	0,0003382	1,69	198,31
JP47	97,47	71,10	0,004	0,00028442	1,42	198,58
JP48	97,39	71,05	0,02	0,00142092	7,10	192,90
JP49	106,79	77,90	0,02	0,00155807	7,79	192,21
JP50	110,08	80,30	0,02	0,00160607	8,03	191,97
JP51	94,12	68,66	0,02	0,00137321	6,87	193,13
JP52	89,26	65,12	0,02	0,0013023	6,51	193,49
JP53	103,49	75,50	0,02	0,00150992	7,55	192,45
JP54	97,53	71,15	0,1	0,00711481	35,57	164,43
JP55	95,91	69,97	0,1	0,00699663	34,98	165,02
JP56	86,91	63,40	0,1	0,00634008	31,70	168,30
JP57	95,45	69,63	0,1	0,00696308	34,82	165,18
JP58	94,93	69,25	0,1	0,00692514	34,63	165,37
JP59	107,75	78,60	0,1	0,00786036	39,30	160,70
JP60	95,37	69,57	0,5	0,03478621	173,93	26,07
JP61	100,80	73,53	0,5	0,0367668	183,83	16,17
JP62	118,12	86,17	0,5	0,04308427	215,42	x
JP63	98,81	72,08	0,5	0,03604095	180,20	19,80
JP64	88,85	64,82	0,5	0,03240804	162,04	37,96
JP65	93,88	68,49	0,5	0,03424273	171,21	28,79
JP66	114,05	83,20	0	0	0,00	200,00
JP67	99,42	72,53	0	0	0,00	200,00
JP68	105,59	77,03	0	0	0,00	200,00
JP69	101,31	73,91	0	0	0,00	200,00
JP70	106,40	77,62	0	0	0,00	200,00
JP71	101,30	73,90	0	0	0,00	200,00
JP72	115,08	83,95	0,004	0,0003	1,68	198,32
JP73	115,61	84,34	0,004	0,0003	1,69	198,31
JP74	91,10	66,46	0,004	0,0003	1,33	198,67
JP75	97,03	70,78	0,004	0,0003	1,42	198,58
JP76	89,32	65,16	0,004	0,0003	1,30	198,70
JP77	109,45	79,84	0,004	0,0003	1,60	198,40

JP78	104,22	76,03	0,02	0,0015	7,60	192,40
JP79	104,05	75,90	0,02	0,0015	7,59	192,41
JP80	101,87	74,31	0,02	0,0015	7,43	192,57
JP81	105,22	76,76	0,02	0,0015	7,68	192,32
JP82	94,86	69,20	0,02	0,0014	6,92	193,08
JP83	105,01	76,60	0,02	0,0015	7,66	192,34
JP84	102,55	74,81	0,1	0,0075	37,41	162,59
JP85	98,71	72,01	0,1	0,0072	36,00	164,00
JP86	100,36	73,21	0,1	0,0073	36,61	163,39
JP87	92,24	67,29	0,1	0,0067	33,64	166,36
JP88	110,47	80,59	0,1	0,0081	40,29	159,71
JP89	121,29	88,48	0,1	0,0088	44,24	155,76
JP90	105,48	76,95	0,5	0,0385	192,37	7,63
JP91	105,64	77,06	0,5	0,0385	192,66	7,34
JP92	118,43	86,39	0,5	0,0432	215,99	x
JP93	118,49	86,44	0,5	0,0432	216,10	x
JP94	103,18	75,27	0,5	0,0376	188,17	11,83
JP95	113,01	82,44	0,5	0,0412	206,10	x
JP96	97,47	71,10	0	0	0,00	200,00
JP97	105,41	76,90	0	0	0,00	200,00
JP98	95,41	69,60	0	0	0,00	200,00
JP99	128,60	93,81	0	0	0,00	200,00
JP100	115,59	84,32	0	0	0,00	200,00
JP101	110,76	80,80	0	0	0,00	200,00
JP102	106,74	77,87	0,004	0,0003	1,56	198,44
JP103	106,83	77,93	0,004	0,0003	1,56	198,44
JP104	109,15	79,62	0,004	0,0003	1,59	198,41
JP105	95,30	69,52	0,004	0,0003	1,39	198,61
JP106	110,12	80,33	0,004	0,0003	1,61	198,39
JP107	111,41	81,27	0,004	0,0003	1,63	198,37
JP108	98,98	72,21	0,02	0,0014	7,22	192,78
JP109	101,93	74,36	0,02	0,0015	7,44	192,56
JP110	95,38	69,58	0,02	0,0014	6,96	193,04
JP111	109,31	79,74	0,02	0,0016	7,97	192,03
JP112	92,78	67,68	0,02	0,0014	6,77	193,23
JP113	109,02	79,53	0,02	0,0016	7,95	192,05
JP114	98,18	71,62	0,1	0,0072	35,81	164,19
JP115	99,73	72,75	0,1	0,0073	36,38	163,62
JP116	98,00	71,49	0,1	0,0071	35,74	164,26
JP117	120,87	88,17	0,1	0,0088	44,09	155,91
JP118	101,39	73,97	0,1	0,0074	36,98	163,02
JP119	95,48	69,65	0,1	0,0070	34,83	165,17
JP120	118,12	86,17	0,5	0,0431	215,42	x
JP121	108,99	79,51	0,5	0,0398	198,77	1,23

JP122	120,72	88,07	0,5	0,0440	220,16	x
JP123	106,94	78,01	0,5	0,0390	195,03	4,97
JP124	114,19	83,30	0,5	0,0417	208,25	x
JP125	106,96	78,03	0,5	0,0390	195,07	4,93

Appendix II

Table A.2 Results of ANOVA analysis for total individuals based on the model gls(Total ~ Duration + Concentration). Model 1.2 uses no variance structure. Model 1.3 uses *varFixed*, Model 1.4 uses *varIdent*, Model 1.5 uses *varPower*, Model 1.6 uses *varPower*, Model 1.7 uses *varExp*, Model 1.8 uses *varConstPower*, Model 1.9 uses *varConstPower*, and Model 1.10 uses *varComb* of *varIdent* and *varExp*.

	Model	df	AIC	BIC	logLik	Test	L.Ratio	p-value
Mod1.2	1	4	1704.870	1715.919	-848.4350			
Mod1.3	2	4	1643.057	1654.105	-817.5284			
Mod1.4	3	8	1608.039	1630.137	-796.0197	2 vs 3	43.01750	<.0001
Mod1.5	4	5	1558.257	1572.068	-774.1285	3 vs 4	43.78223	<.0001
Mod1.6	5	9	1509.023	1533.882	-745.5113	4 vs 5	57.23442	<.0001
Mod1.7	6	5	1557.452	1571.263	-773.7259	5 vs 6	56.42921	<.0001
Mod1.8	7	6	1560.257	1576.830	-774.1284	6 vs 7	0.80487	0.3696
Mod1.9	8	14	1500.072	1538.743	-736.0361	7 vs 8	76.18452	<.0001
Mod1.10	9	9	1507.011	1531.870	-744.5054	8 vs 9	16.93857	0.0046

Table A.3 Results of ANOVA analysis for total individuals based on the model gls(Total ~ Concentration * Duration). Model 2.2 uses no variance structure. Model 2.3 uses *varFixed*, Model 2.4 uses *varIdent*, Model 2.5 uses *varPower*, Model 2.6 uses *varPower*, Model 2.7 uses *varExp*, Model 2.8 uses *varConstPower*, Model 2.9 uses *varConstPower*, and Model 2.10 uses *varComb* of *varIdent* and *varExp*.

	Model	df	AIC	BIC	logLik	Test	L.Ratio	p-value
Mod2.2	1	5	1684.959	1698.727	-837.4796			
Mod2.3	2	5	1623.385	1637.153	-806.6924			
Mod2.4	3	9	1568.694	1593.476	-775.3469	2 vs 3	62.69086	<.0001
Mod2.5	4	6	1548.745	1565.266	-768.3725	3 vs 4	13.94899	0.0030
Mod2.6	5	10	1483.889	1511.424	-731.9443	4 vs 5	72.85637	<.0001
Mod2.7	6	6	1549.237	1565.759	-768.6186	5 vs 6	73.34861	<.0001
Mod2.8	7	7	1550.745	1570.020	-768.3724	6 vs 7	0.49240	0.4829
Mod2.9	8	15	1486.459	1527.762	-728.2293	7 vs 8	80.28615	<.0001
Mod2.10	9	10	1486.556	1514.092	-733.2779	8 vs 9	10.09713	0.0725

Table A.4 Results of ANOVA analysis for total individuals based on the model gls(Total ~ + factor(Concentration) : Duration). Model 3.2 uses no variance structure. Model 3.3 uses *varFixed*, Model 3.4 uses *varIdent*, Model 3.5 uses *varPower*, Model 3.6 uses *varPower*, Model 3.7 uses *varExp*, Model 3.8 uses *varConstPower*, Model 3.9 uses *varConstPower*, and Model 3.10 uses *varComb* of *varIdent* and *varExp*.

	Model	df	AIC	BIC	logLik	Test	L.Ratio
Mod3.2	1	7	1673.400	1692.553	-829.7001		
Mod3.3	2	7	1618.007	1637.160	-802.0035		
Mod3.4	3	7	1673.400	1692.553	-829.7001		
Mod3.5	4	8	1544.222	1566.112	-764.1110	3 vs 4	131.17811
Mod3.6	5	12	1478.228	1511.062	-727.1138	4 vs 5	73.99439
Mod3.7	6	8	1548.285	1570.174	-766.1423	5 vs 6	78.05694
Mod3.8	7	9	1546.222	1570.848	-764.1112	6 vs 7	4.06217
Mod3.9	8	17	1479.443	1525.958	-722.7215	7 vs 8	82.77947
Mod3.10	9	8	1548.285	1570.174	-766.1423	8 vs 9	86.84164

Table A.5 Results of ANOVA analysis for total individuals based on the model gls(log(Total) ~ factor(Concentration) * Duration). Model 4.2 uses no variance structure. Model 4.3 uses *varFixed*, Model 4.4 uses *varIdent*, Model 4.5 uses *varPower*, Model 4.6 uses *varPower*, Model 4.7 uses *varExp*, Model 4.8 uses *varConstPower*, Model 4.9 uses *varConstPower*, and Model 4.10 uses *varComb* of *varIdent* and *varExp*.

	Model	df	AIC	BIC	logLik	Test	L.Ratio	p-value
Mod4.2	1	11	343.4402	373.1455	-160.7201			
Mod4.3	2	11	343.4402	373.1455	-160.7201			
Mod4.4	3	15	319.6460	360.1532	-144.8230	2 vs 3	31.79425	<.0001
Mod4.5	4	12	341.4615	373.8673	-158.7308	3 vs 4	27.81553	<.0001
Mod4.6	5	16	319.2320	362.4397	-143.6160	4 vs 5	30.22952	<.0001
Mod4.7	6	12	341.2353	373.6411	-158.6176	5 vs 6	30.00330	<.0001
Mod4.8	7	13	343.1525	378.2587	-158.5762	6 vs 7	0.08279	0.7736
Mod4.9	8	21	324.5182	381.2283	-141.2591	7 vs 8	34.63429	<.0001
Mod4.10	9	16	318.9617	362.1694	-143.4808	8 vs 9	4.44346	0.4875

Table A.6 Results of ANOVA analysis for total adults based on the model gls(Adults ~ Duration + factor(Concentration)).Model 1.2 uses no variance structure. Model 1.3 uses *varFixed*, Model 1.4 uses *varIdent*, Model 1.5 uses *varPower*, Model 1.6 uses *varPower*, Model 1.7 uses *varExp*, Model 1.8 uses *varConstPower*, Model 1.9 uses *varConstPower*, and Model 1.10 uses *varComb* of *varIdent* and *varExp*.

	Model	df	AIC	BIC	logLik	Test
AdultsMod1.2	1	7	1037.3781	1056.5315	-511.6891	
AdultsMod1.3	2	7	969.7412	988.8946	-477.8706	
AdultsMod1.4	3	11	898.5141	928.6123	-438.2571	2 vs 3
AdultsMod1.5	4	8	794.7496	816.6391	-389.3748	3 vs 4
AdultsMod1.6	5	12	776.7859	809.6203	-376.3930	4 vs 5
AdultsMod1.7	6	8	783.7683	805.6579	-383.8842	5 vs 6
AdultsMod1.8	7	9	796.7494	821.3751	-389.3747	6 vs 7
AdultsMod1.9	8	17	751.3064	797.8218	-358.6532	7 vs 8
AdultsMod1.10	9	12	774.7172	807.5516	-375.3586	8 vs 9

Table A.7 Results of ANOVA analysis for total adults based on the model gls(Adults ~ factor(Concentration) * Duration).Model 2.2 uses no variance structure. Model 2.3 uses *varFixed*, Model 2.4 uses *varIdent*, Model 2.5 uses *varPower*, Model 2.6 uses *varPower*, Model 2.7 uses *varExp*, Model 2.8 uses *varConstPower*, Model 2.9 uses *varConstPower*, and Model 2.10 uses *varComb* of *varIdent* and *varExp*.

	Model	df	AIC	BIC	logLik	Test
AdultsMod2.2	1	11	1031.8192	1061.5245	-504.9096	
AdultsMod2.3	2	11	964.2994	994.0046	-471.1497	
AdultsMod2.4	3	15	870.9971	911.5043	-420.4985	2 vs 3
AdultsMod2.5	4	12	806.8442	839.2500	-391.4221	3 vs 4
AdultsMod2.6	5	16	784.0779	827.2856	-376.0390	4 vs 5
AdultsMod2.7	6	12	796.5259	828.9317	-386.2630	5 vs 6
AdultsMod2.8	7	13	808.8441	843.9503	-391.4220	6 vs 7
AdultsMod2.9	8	21	762.6484	819.3584	-360.3242	7 vs 8
AdultsMod2.10	9	16	784.0905	827.2982	-376.0453	8 vs 9

Table A.8 Results of ANOVA analysis for total adults based on the model gls(Adults ~ + factor(Concentration) : Duration). Model 3.2 uses no variance structure. Model 3.3 uses *varFixed*, Model 3.4 uses *varIdent*, Model 3.5 uses *varPower*, Model 3.6 uses *varPower*, Model 3.7 uses *varExp*, Model 3.8 uses *varConstPower*, Model 3.9 uses *varConstPower*, and Model 3.10 uses *varComb* of *varIdent* and *varExp*.

	Model	df	AIC	BIC	logLik	Test
AdultsMod3.2	1	7	1056.1118	1075.2652	-521.0559	
AdultsMod3.3	2	7	988.3975	1007.5509	-487.1987	
AdultsMod3.4	3	7	1056.1118	1075.2652	-521.0559	
AdultsMod3.5	4	8	820.3386	842.2282	-402.1693	3 vs 4
AdultsMod3.6	5	12	798.4480	831.2823	-387.2240	4 vs 5
AdultsMod3.7	6	8	810.5492	832.4388	-397.2746	5 vs 6
AdultsMod3.8	7	9	822.3384	846.9642	-402.1692	6 vs 7
AdultsMod3.9	8	17	782.1090	828.6244	-374.0545	7 vs 8
AdultsMod3.10	9	8	810.5492	832.4388	-397.2746	8 vs 9

Table A.9 Results of ANOVA analysis for total juveniles based on the model gls(Juveniles ~ Duration + factor(Concentration)). Model 1.2 uses no variance structure. Model 1.3 uses *varFixed*, Model 1.4 uses *varIdent*, Model 1.5 uses *varPower*, Model 1.6 uses *varPower*, Model 1.7 uses *varExp*, Model 1.8 uses *varConstPower*, Model 1.9 uses *varConstPower*, and Model 1.10 uses *varComb* of *varIdent* and *varExp*.

	Model	df	AIC	BIC	logLik	Test
JuvenilesMod1.2	1	7	1634.711	1653.865	-810.3556	
JuvenilesMod1.3	2	7	1579.082	1598.236	-782.5411	
JuvenilesMod1.4	3	11	1529.779	1559.877	-753.8896	2 vs 3
JuvenilesMod1.5	4	8	1508.101	1529.991	-746.0504	3 vs 4
JuvenilesMod1.6	5	12	1454.489	1487.323	-715.2445	4 vs 5
JuvenilesMod1.7	6	8	1510.049	1531.939	-747.0245	5 vs 6
JuvenilesMod1.8	7	9	1510.101	1534.727	-746.0505	6 vs 7
JuvenilesMod1.9	8	17	1450.972	1497.487	-708.4860	7 vs 8
JuvenilesMod1.10	9	12	1453.954	1486.788	-714.9769	8 vs 9

Table A.10 Results of ANOVA analysis for total juveniles based on the gls(Juveniles ~ factor(Concentration) * Duration).Model 2.2 uses no variance structure. Model 2.3 uses *varFixed*, Model 2.4 uses *varIdent*, Model 2.5 uses *varPower*, Model 2.6 uses *varPower*, Model 2.7 uses *varExp*, Model 2.8 uses *varConstPower*, Model 2.9 uses *varConstPower*, and Model 2.10 uses *varComb* of *varIdent* and *varExp*.

	Model	df	AIC	BIC	logLik	Test
JuvenilesMod2.2	1	11	1596.248	1625.953	-787.1239	
JuvenilesMod2.3	2	11	1545.010	1574.715	-761.5049	
JuvenilesMod2.4	3	15	1485.187	1525.694	-727.5935	2 vs 3
JuvenilesMod2.5	4	12	1493.048	1525.453	-734.5238	3 vs 4
JuvenilesMod2.6	5	16	1426.776	1469.984	-697.3880	4 vs 5
JuvenilesMod2.7	6	12	1496.135	1528.541	-736.0676	5 vs 6
JuvenilesMod2.8	7	13	1495.048	1530.154	-734.5241	6 vs 7
JuvenilesMod2.9	8	21	1429.024	1485.734	-693.5119	7 vs 8
JuvenilesMod2.10	9	16	1430.473	1473.681	-699.2364	8 vs 9

Table A.11 Results of ANOVA analysis for total juveniles based on the model gls(Juveniles ~ + factor(Concentration) : Duration). Model 3.2 uses no variance structure. Model 3.3 uses *varFixed*, Model 3.4 uses *varIdent*, Model 3.5 uses *varPower*, Model 3.6 uses *varPower*, Model 3.7 uses *varExp*, Model 3.8 uses *varConstPower*, Model 3.9 uses *varConstPower*, and Model 3.10 uses *varComb* of *varIdent* and *varExp*.

	Model	df	AIC	BIC	logLik	Test
JuvenilesMod3.2	1	7	1641.881	1661.035	-813.9408	
JuvenilesMod3.3	2	7	1588.502	1607.655	-787.2509	
JuvenilesMod3.4	3	7	1641.881	1661.035	-813.9408	
JuvenilesMod3.5	4	8	1530.512	1552.401	-757.2559	3 vs 4
JuvenilesMod3.6	5	12	1462.880	1495.715	-719.4402	4 vs 5
JuvenilesMod3.7	6	8	1534.149	1556.038	-759.0744	5 vs 6
JuvenilesMod3.8	7	9	1532.512	1557.138	-757.2559	6 vs 7
JuvenilesMod3.9	8	17	1465.761	1512.276	-715.8803	7 vs 8
JuvenilesMod3.10	9	8	1534.149	1556.038	-759.0744	8 vs 9

Table A.12 Results of ANOVA analysis for total intermediates based on the model gls(Intermediate ~ Duration + factor(Concentration)Model 1.2 uses no variance structure. Model 1.3 uses *varFixed*, Model 1.4 uses *varIdent*, Model 1.5 uses *varPower*, Model 1.6 uses *varPower*, Model 1.7 uses *varExp*, Model 1.8 uses *varConstPower*, Model 1.9 uses *varConstPower*, and Model 1.10 uses *varComb* of *varIdent* and *varExp*.

	Model	df	AIC	BIC	logLik	Test
IntermediateMod1.2	1	7	1251.6992	1270.8526	-618.8496	
IntermediateMod1.3	2	7	1186.3055	1205.4588	-586.1527	
IntermediateMod1.4	3	11	1204.5572	1234.6553	-591.2786	2 vs 3
IntermediateMod1.5	4	8	903.5436	925.4332	-443.7718	3 vs 4
IntermediateMod1.6	5	12	895.1966	928.0310	-435.5983	4 vs 5
IntermediateMod1.7	6	8	940.5666	962.4561	-462.2833	5 vs 6
IntermediateMod1.8	7	9	905.5436	930.1694	-443.7718	6 vs 7
IntermediateMod1.9	8	17	905.1966	951.7120	-435.5983	7 vs 8
IntermediateMod1.10	9	12	934.0203	966.8547	-455.0101	8 vs 9

Table A.13 Results of ANOVA analysis for total intermediates based on the gls(Intermediate ~ Concentration * Duration).Model 2.2 uses no variance structure. Model 2.3 uses *varFixed*, Model 2.4 uses *varIdent*, Model 2.5 uses *varPower*, Model 2.6 uses *varPower*, Model 2.7 uses *varExp*, Model 2.8 uses *varConstPower*, Model 2.9 uses *varConstPower*, and Model 2.10 uses *varComb* of *varIdent* and *varExp*.

	Model	df	AIC	BIC	logLik	Test
IntermediateMod2.2	1	11	1208.6177	1238.3230	-593.3089	
IntermediateMod2.3	2	11	1143.2551	1172.9603	-560.6275	
IntermediateMod2.4	3	15	1116.4891	1156.9963	-543.2445	2 vs 3
IntermediateMod2.5	4	12	908.7051	941.1108	-442.3525	3 vs 4
IntermediateMod2.6	5	16	900.8655	944.0732	-434.4327	4 vs 5
IntermediateMod2.7	6	12	942.9469	975.3527	-459.4735	5 vs 6
IntermediateMod2.8	7	13	910.7051	945.8113	-442.3525	6 vs 7
IntermediateMod2.9	8	21	910.8655	967.5756	-434.4327	7 vs 8
IntermediateMod2.10	9	16	935.9051	979.1128	-451.9526	8 vs 9

Table A.14 Results of ANOVA analysis for total intermediates based on the model gls(Intermediate ~ + factor(Concentration) : Duration). Model 3.2 uses no variance structure. Model 3.3 uses *varFixed*, Model 3.4 uses *varIdent*, Model 3.5 uses *varPower*, Model 3.6 uses *varPower*, Model 3.7 uses *varExp*, Model 3.8 uses *varConstPower*, Model 3.9 uses *varConstPower*, and Model 3.10 uses *varComb* of *varIdent* and *varExp*.

	Model	df	AIC	BIC	logLik	Test
IntermediateMod3.2	1	7	1258.1851	1277.3385	-622.0926	
IntermediateMod3.3	2	7	1198.7746	1217.9280	-592.3873	
IntermediateMod3.4	3	7	1258.1851	1277.3385	-622.0926	
IntermediateMod3.5	4	8	930.0937	951.9833	-457.0469	3 vs 4
IntermediateMod3.6	5	12	921.7265	954.5608	-448.8632	4 vs 5
IntermediateMod3.7	6	8	966.8589	988.7485	-475.4295	5 vs 6
IntermediateMod3.8	7	9	932.0937	956.7195	-457.0469	6 vs 7
IntermediateMod3.9	8	17	931.7265	978.2418	-448.8632	7 vs 8
IntermediateMod3.10	9	8	966.8589	988.7485	-475.4295	8 vs 9